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# Insect bioactive capabilities of *Epichloë festucae var lolii* AR48 infected *Lolium perenne*

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#### ABSTRACT

As the modern world expands and develops, new innovative methodologies for more efficient and environmentally friendly agricultural practices are required. Loss of crops through abiotic (e.g. drought) and biotic (e.g. herbivory) stresses has a major effect on the success of an agricultural industry. For animal production pasture crops are a key aspect of animal husbandry and directly affects yield and health. Symbiotic fungi belonging to the genus *Epichloë* form associations with cool season forage grasses and have been exploited as a new innovative method for insect pest management. Ryegrass infected with the asexual *E. festucae* var *lolii* strain AR48 has insect bioactivity against both the stem boring fly (SBF-Ceradontha australis) and cutworm moth caterpillar (CC -Agrotis ipsilion). The bioactive/s targeting both insects is currently unknown. The aim of this thesis was to identify the gene/s and/or bioactive/s present in AR48 infected ryegrass that have bioactivity against the SBF and/or CC. Two approaches were taken; the known insect bioactive secondary metabolite pathways in *Epichloë* were investigated in AR48 through bioinformatics and mass spectrometry, and the gene 'makes caterpillars floppy' (mcf), encoding an insect toxin like protein, was investigated through reverse genetics and insect bioactivity trials.

A new indole diterpene compound (IDT) was identified in AR48 infected plant material and this compound was absent in other *Epichloë* strains that do not have SBF and CC bioactivity. The same *mcf* gene allele as that present in the *E. typhina mcf* model, previously identified as having CC bioactivity, is present and predicted to be functional in AR48. The other *Epichloë* strains also have *mcf* genes predicted to be functional, however the *mcf* allele is different to the bioactive *E. typhina mcf* model. Overall, this project was able to identify a new IDT compound with potential insect bioactivity as well as identify two *Epichloë mcf* gene alleles that potentially have differing insect bioactivities.

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## **ABBREVIATIONS**

%	Percentage
°C	degrees Celsius
3-GGI	3-geranylgeranyl indole
A600	Absorbance at 600 $\lambda$
	Amino acid
aa ACP	Acyl carrier protein domain
ACP	Actificial diet no choice
	Ampicillin
Amp AmpB	Ampicillin resistance
AmpR ASW	Argentine stem weevil
ASV	-
	Acyltransferase domain
Bcl-2	B-cell lymphoma 2
BH3	Bcl-2 domain three
BLAST	Basic Local Alignment Search Tool
BLAST	BLAST search against a nucleotide database with a nucleotide sequence
BLASTP	BLAST search against a protein database with a protein sequence
BLASTx	BLAST search against a protein database with a translated nucleotide sequence
Вр	base pairs
BS	Blocking solution
С	Condensation domain
CADN	Cutworm moth caterpillar artificial diet no choice
CC	Cutworm moth caterpillar
cDNA	Complementary DNA
CDTN	Cutworm moth caterpillar detached tiller no choice
CDYE	Czapek Dox yeast extract
CHEF	Contour-clamped homogeneous electric field electrophoresis
CM	Cutworm moth
cm2	centimetres squared
CNF	Cytotoxic necrotizing factors
СоА	Coenzyme A
COMP	Gene complementation
CPD	Cysteine protease domain
CPD 2	Cysteine protease domain two
CPD1	Cysteine protease domain one
СТ	Common toxic
CWPC	Cutworm moth caterpillar whole plant choice
CY	Cyclisation domain
CZ	Cell division zone
DH	Dehydratase domains
DMAPP	Dimethylallyl pyrophosphate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease

	De su uib su de stide trick soch sta
dNTP	Deoxyribonucleotide triphosphate
DSIR	Department of Scientific and Industrial Research
DTN	Detached tiller no choice
E	Epimerization domain
EAS	Ergot alkaloid
EDTA	Ethylenediaminetetraacetic acid
EZ	Expansion zone
FAD	Flavin adenine dinucleotide
FASTA	Fast-all
Fit	Fluorescens Insect Toxin
FPP	Farnesyl pyrophosphate
g	Gram
g	Acceleration due to gravity
Gen	Geneticin
GenR	Geneticin resistance
GGPP	Geranylgeranyl pyrophosphate
HGT	Horizontal gene transfer
hph	Hygromycin resistance conferring gene
Hyg	Hygromycin
HygR	Hygromycin resistance
IDT	Indole-diterpene
IGP	Indole-3-glycerol phosphate
IPP kb	Isopentenyl diphosphate
kb	Kilo base pairs
KR	Ketoreductase domains
KS	Keto-synthase domain
L	Litre
LADN	Light brown apple moth caterpillar artificial diet no choice
LB	Lysogeny broth
LBAM	Light brown apple moth caterpillar
LCMS	Liquid-chromatography mass spectrometry
LOL	Loline locus/gene cluster
LTM	Lolitrem locus/gene cluster
Μ	Moles per litre
MIDAS	Modular Idempotent DNA Assembly System
m/s	Meters per second
Mcf	Makes caterpillars floppy
MEP	2-Methyl-D-erythritol-4-phosphate
mg	Milligram
mins	Minutes
MITEs	Miniature inverted transposable elements
mL	Millilitre
mM	millimoles per litre
mm	Millimetre
MQ	Milli-Q water
mRNA	Messenger RNA
	U U U U U U U U U U U U U U U U U U U

MS	Mass spectrometry
MT	Mass spectrometry Methyltransferase domains
	Nanograms
ng NMR	Nuclear magnetic resonance
	-
nptll	Geneticin resistance gene
NRPS	Non-ribosomal peptide synthetase
NZGL	New Zealand Genome Limited
OM	Osmoregulation buffer
OX	Oxidation domain
PADN	Porina caterpillar artificial diet no choice
Pax	Paxilline
PC	Porina caterpillar
PC1	Physical containment one
PC2	Physical containment two
PCR	Polymerase chain reaction
PD	Potato dextrose
PDTN	Porina caterpillar detached tiller no choice
PEG	Polyethylene glycol
PER	Peramine
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
PKS	Polyketide synthase
PM	Porina caterpillar
PM	Porina moth
RG	Regeneration
RNA	Ribonucleic acid
Rnase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT-qPCR	Real time quantitative PCR
RTX	Repeats-in-toxin
SADN	Stem boring fly artificial diet no choice
SBF	Stem boring fly
SD	Secretion domain
SDS	Sodium dodecyl sulfate
SDTN	Stem boring fly detached tiller no choice
sec	Seconds
SM	Secondary metabolite
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
ST	Sorbitol Tris
STC	Sorbitol Tris calcium chloride
SWPC	Stem boring fly whole plant choice
ТВ	Tris-boric
TBE	Tris-boric acid-EDTA
tBLASTn	BLAST search against a nucleotide database with a protein sequence
TE	Thioesterase domain

Tm	Melting temperature
TMD	Transmembrane domain
UV	Ultra violet
V	Volume
v/v	Volume/volume
w/v	Weight/volume
WA	Water agar
WPC	Whole plant choice
WT	Wild type
Δ	Gene deletion
μg	Microgram
μL	Microliter
μm	Micrometre

# **1.0 INTRODUCTION**

Chapter one: Introduction

#### 1.1 Epichloë endophyte as a commercialised bioprotectant

The fungal genus *Epichloë* contains both sexual and asexual species and is classified as belonging to the family Clavicipitaceae within the phylum Ascomycota. This endophyte forms interactions with cool season grasses from the sub-family Pooideae within the family Poaceae (Johnson *et al.*, 2013a), which contains agriculturally important pasture species such as *Lolium perenne* (ryegrass), *Festuca arundinacea* (tall fescue), and *Festuca pratensis* (meadow fescue). In this interaction, the endophyte provides both abiotic (*e.g.* drought tolerance) and biotic (*e.g.* mammalian and insect bioactive compounds) benefits to the host plant, while the host plant in turn provides nutrients and a mode of transmission to the fungus (Schardl & Leuchtmann, 2005). This symbiotic induced biotic activity has been commercialised in the agricultural industry as a bioprotectant agent.

#### 1.1.1 Insect pests of Lolium perenne

Ryegrass is an economically important pasture crop in New Zealand as it provides nutrition for cattle raised for the dairy and meat industries (Ferguson *et al.*, 2018). Controlling insects that target ryegrass is therefore important, as reduction in palatable pasture reduces the nutritional intake of grazing cattle and in turn affects milk and meat production. In addition, money and time spent managing the insect populations and supplementary feed adds to production costs (Ferguson *et al.*, 2018) Insect population control strategies that do not use synthetic insecticides or chemicals are advantageous as they have reduced environmental impact, can be safer to use, may have lower economic cost, and are more specific with few if any non-target effects (Ferguson *et al.*, 2018).

There is a range of different insect species that damage ryegrass, although only a few species are key economic pests. While most of these species are endemic or native, some are accidental introductions from other countries. The insects typically cause damage when the larvae feed on either the root or tiller systems of the plant, though for a few species the adults also inflict damage. Some species complete their life cycle in a year (univoltine) with the adult insect present in winter and the larvae present

# Chapter one: Introduction

during the summer breeding season, whereas others have 2 - 3 generations per year

(Table 1.1).

Table 1.1: Comparison of economically important insect pests of ryegrass in New Zealand

Common name	Scientific name	Туре	Distribution	Breading season	Tissue effected	Economic impact	Control	Reference
Grass grub	Costelytra zealandica	Moth	New Zealand (endemic)	One cycle per 1-2 years (Adults Oct- Dec and pupa Jan-Sep)	Tillers and roots (pupa). Yellowing patches.	\$275M-\$706M on dairy farms and \$75M-\$205 M on sheep and beef farms annually	None	(Ferguson et al., 2018)
Porina	Wiseana cervinate	Moth	New Zealand (endemic)	One cycle per year (Adults Oct-Mar and pupa Apr- Sep)	Tillers (pupa). Dead patches.	Up to \$84M on dairy farms and up to \$88M on sheep and beef farms annually	Epichloe festucae var lolii strain AR37 in ryegrass (bioactive= epoxy- janthitrems)	(Jense <i>et al.,</i> 2004)(Fergus on <i>et al.,</i> 2018)
Mealy bug	Balanococcus poae	Armored insect	Canterbury, Manawatu and Nelson NZ. (endemic)	All year round	Tillers (adult and pupa). Brown field.	N/A	None	(Ferguson et al., 2018)
Root aphid	Aploneura lentisci	Aphid	Mediterranea n, Middle East region (endemic)	All year round	Tillers (nymphs) and roots (adult). Reduced plant growth.	N/A	Epichloe festucae var lolii strain AR37 in ryegrass (unknown)	(Popay <i>et el.,</i> 2016) (Hume <i>et al.,</i> 2007)(Fergus on et al., 2018)
Black field cricket	Teleogryllus commodus	Cricket	New Zealand, and Australia (native)	One generation per year (Adult Feb- May, and	Tillers (adult and pupa). Dead patches.	N/A	Epichloe uncinata in Festuca pratensis or a festulolium	(Becker <i>et al.,</i> 2015)(Fergus on et al., 2018)
Argentine stem weevil	Listronotus bonariensis	Weevil	South America, New Zealand, and Australia (introduced)	Up to three generations between Sep- March	Tillers (pupa). Dead patches,	Up to \$200M on dairy farms and up to \$235M on sheep and beef farms annually	Epichloe festucae var lolii strain AR1 (bioactive= peramine). Epichloe festucae var lolii strain AR37 in ryegrass (unknown)	(Rowan, Dymock et al., 1990) (Popay et al, 1995)(Fergus on et al, 2018)
Black beetle	Heteronychus arator	Scarab	Africa, Upper North Island NZ, and Australia (introduced)	One generation per year (Adult Mar- Dec and pupa Jan-Mar)	Tillers and roots (adult and pupa). Yellowing patches	Up to \$223M on dairy farms and up to \$19M on sheep and beef farms annually	Epichloe festucae var lolii strain AR37 in ryegrass (unknown)	(Ball, Christensen <i>et al.</i> 1994)(Fergus on et al., 2018)

#### Chapter one: Introduction

There is currently no biological control for grass grub, and mealy bug. Porina, black beetle, Argentine stem weevil (ASW), and the root aphid are however, controlled by ryegrass infected with the *Epichloë festucae* var *lolii* strain AR37. The bioactive indolediterpene (IDT) *epoxy*-janthitrem is known to deter feeding of porina larvae (Hennessy *et al.*, 2016). ASW is also controlled by ryegrass infected with *Epichloë festucae* var *lolii* AR1 (Rowan *et al.*, 1990). Finally, *E. uncinata* has bioactivity towards black field cricket (Table 1.1).

#### 1.1.2 Commercialisation of Epichloë as an insect biocontrol agent

The discovery of the two *Epichloë* secondary metabolites (SMs), peramine (responsible for ASW deterrence) and lolitrem B (responsible for ryegrass staggers) prompted an investigation to identify *Epichloë* species which produced peramine but did not produce lolitrem B (Johnson *et al.*, 2013a). This investigation resulted in the commercialisation of Endosafe® by Department of Scientific and Industrial Research (DSIR) and later on AgResearch in 1991 (Johnson *et al.*, 2013a, Fletcher *et al.*, 2012, Milne, 2007). Unfortunately, summer slump and fescue foot were observed in cattle exposed to Endosafe, and further testing resulted in the identification of the mammalian toxin ergovaline, an endophyte produced ergot alkaloid in the grass (Table 1.2) (Fletcher *et al.*, 2012).

This prompted an investigation into identifying *Epichloë* species that produced peramine, but not lolitrem B or ergovaline (Popay *et al.*, 1999). This resulted in the identification and commercialisation of *E. festucae* var *lolii* AR1 strain in ryegrass in 2001 (Table 1.2) (Milne, 2007). Interestingly, cattle grazing on grass infected with AR1 produced 9% more milk then cattle grazing on grass infected with the common toxic strain (CT), defined as the lolB clade of *E. festucae* var *lolii* (*Christensen et al.*, 1993).

AR37, another *E. festucae* var *lolii* strain, which does not produce peramine, lolitrem B, or ergovaline, was also included in this investigation, and when *in planta* was shown to have bioactivity to a much wider range of insects than AR1 infected ryegrass. However, rare cases of staggers in grazing cattle were documented, although the symptoms were much milder than those caused by CT (Johnson *et al.*, 2013a). AR37 in ryegrass

was commercialised in 2007, and trials have shown that grass infected with AR37 has greater advantages compared to grass infected with AR1, CT or grass that is endophyte free (Milne, 2007). Interestingly, the only bioactive molecules that have so far been detected in AR37 infected material are the *epoxy*-janthitrems, a group of IDTs (Table 1.2) (Jensen & Popay, 2004, Popay & Gerard, 2007, Finch *et al.*, 2010).

A parallel trial investigating *Epichloë* that did not produce ergovaline but did produce lolines was carried out on tall fescue (Johnson *et al.*, 2013a) and resulted in the identification and commercialisation of AR542 in tall fescue, *E. coenophiala*, in the USA in 2000 (MaxQ<sup>®</sup>) and AR542 in tall fescue, *E. coenophiala*, in New Zealand and Australia in 2003 (MaxP<sup>®</sup>) (Table 1.2) (Bouton, 2009, Johnson *et al.*, 2013a).

Finally, the highest ergovaline and lolitrem B producing endophyte species from both trials were commercialised in grasses in 2010 (Avanex<sup>®</sup>), *E. festucae* var *lolii* AR94/95 in ryegrass and *E. coenophiala* AR601 in tall fescue, and used in areas such as airports, sports fields, and recreational parks to deter birds (Table 1.2) (Pennell & Rolston, 2011).

Table 1.2: AgResearch commercialised insect biopesticide <i>Epichloë</i> species (Johnson <i>et</i>
<i>al.</i> , 2013a)

Commercial or common name	Fungal species	Major alkaloids	Key traits	Hosts
Common-toxic	E. festucae var Iolii	Lolitrems Peramine Ergovaline	Ryegrass staggers and deterrence against ASW and BB	Ryegrass in New Zealand
Endosafe	E. festucae var Iolii	Peramine Ergovaline	Summer slump/fescue foot, and deterrence against ASW	Ryegrass in New Zealand
AR1	E. festucae var Iolii	Peramine	Deterrence against ASW	Ryegrass in New Zealand, Australia, and South America
AR37	E. festucae var Iolii	Epoxy- janthitrems	Broad insect deterrence	Ryegrass in New Zealand, and Australia
MaxP	<i>E. coenophiala</i> strain AR542 and AR584	Lolitrems Peramine	Broad insect deterrence	Tall fescue in New Zealand, and Australia
MaxQ	<i>E. coenophiala</i> strain AR542 and AR584	Lolitrems Peramine	Broad insect deterrence	Tall fescue in USA
Avanex	<i>E. festucae</i> var <i>Iolii</i> strain AR94/95	Peramine Ergovaline Lolitrem B	Bird and wild life deterrence	Ryegrass in sports fields and recreational parks

ASW: Argentine stem weevil (*Listronotus bonariensis*). BB: Black beetle (*Heteronychus arator*)

**1.1.3** Novel insect bioprotection of Epichloë festucae var lolii AR48 infected ryegrass The asexual *Epichloë festucae* var *lolii* strain AR48 was isolated from *Lolium perenne* in Tuscany and has two new bioactivities towards *Ceradontha australis* (stem boring fly-SBF) and *Agrotis ipsilon* (cutworm moth caterpillars-CC) (Figure 1.1, 1.2, and 1.3). Both insects are ryegrass pests in New Zealand agricultural (refer to section 1.5).

SBF bioactivity of AR48 was identified during an ASW plot trial at Ruakura AgResearch New Zealand. This trial was set up to screen for ASW bioactivity in a range of endophyte infected ryegrasses, however during the trial there was a clear lack of SBF damage to AR48 and AR47 infected plants. The SBF damage was therefore assessed alongside the ASW damage. AR48 and AR47 did indeed have bioactivity against the SBF (Figure 1.1). This bioactivity was then confirmed by subsequent SBF insect trials (Alison Popay, AgResearch; personal communication).

Two laboratory trials were conducted in Purdue University in the USA that assessed the CC bioactivity potential of a range of perennial ryegrass and endophyte combinations. For the first trial that was performed in 2008, the CC were individually assessed in petri dishes, which contained moist filter paper, and given detached tillers every 24 hours for 10 days. The settling response was measured after the first 24 hours and survival rate measured at 24-hour intervals. AR48 and AR47 had the lowest settling response (%) and lowest survival rate out of all treatments (Figure 1.2).

The second trial was performed in 2012, used the same method as the 2008 trial except that the CC were assessed for survival only on day 5 and day 10, and end CC biomass was measured. There was no difference in survival rates between the different treatments (data not presented) but AR48, AR47, and AR1 had the lowest end CC biomass (Figure 1.3).

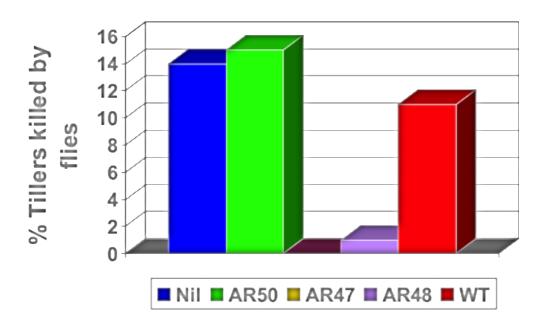


Figure 1.1: Stem boring fly (SBF-*Cerodontha australis*) bioactivity observed in an Argentine stem weevil (ASW-*Listronotus bonariensis*) plot trial, set up to screen for ASW bioactivity in a range of endophyte infected ryegrass, at Ruakura AgResearch New Zealand (unpublished data from David Hume, Grasslands AgResearch, New Zealand). Nil= no endophyte present. WT= wild type which is the common toxic strain found naturally occurring in ryegrass in New Zealand. AR= endophyte strain of interest.

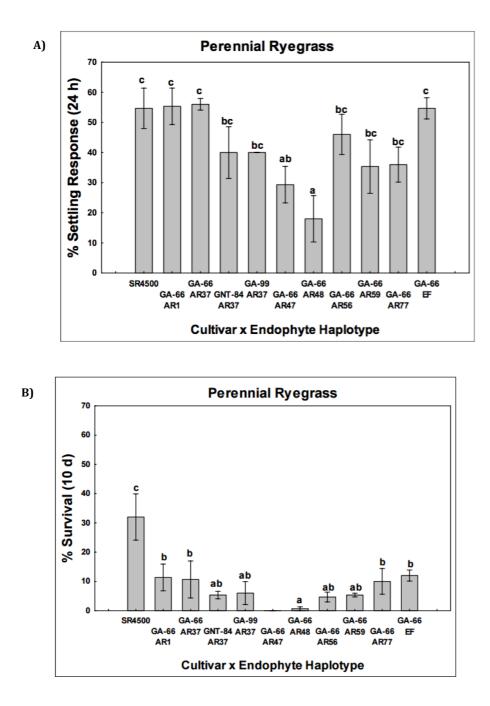


Figure 1.2: The detection of cutworm moth caterpillar (CC-*Agrotis ipsilon*) bioactivity in a range of perennial ryegrass (top label) and endophyte (bottom label) combinations under laboratory conditions 2008. CC contained in petri dishes with moist filter paper were given fresh detached tillers every 24 hours for 10 days. A) The settling response of CC after 24 hours exposure to different endophyte infected tiller combinations (data collected by Douglas S. Richmond, Purdue University, USA, and provided by David Humes, Grasslands AgResearch, New Zealand). B) Percentage of CC surviving on clippings of different endophyte infected tiller combinations after 10 days of laboratory feeding assay.

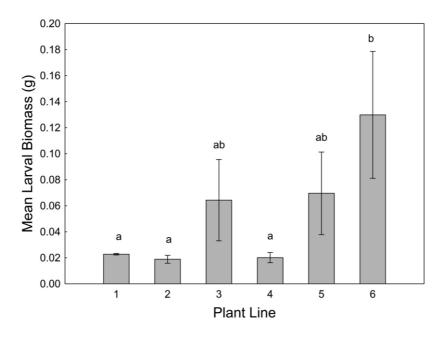


Figure 1.3: The detection of cutworm moth caterpillar (*Agrotis ipsilon*) bioactivity in a range of perennial ryegrass and endophyte combinations under laboratory conditions 2012. CC contained in Petri dishes with moist filter paper were given fresh detached tillers every 24 hours for 10 days, and end biomass measured (data collected by Stephanie Hathaway, Purdue University, USA, and provided by David Humes, Grasslands AgResearch, New Zealand). 1) AR1 in AGRLP-135. 2) AR1 in AGRLP-140. 3) AR47 in AGRLP-141. 4) AR48 in AGRLP-142. 5) HE toxic in FLp-322. 6) No endophyte in BG24T.

# 1.2 Epichloë endophyte molecular characterisation

# 1.2.1 Epichloë in culture and in planta morphologies

*In planta*, the endophyte is located in the aerial tissue within the intercellular space (apoplast) where in general single hyphae run parallel to the longitudinal axis of the leaf. These hyphae are physically attached to the plant cell walls, and while they are predominantly found at the base of the leaf, in some cases they form symbiotic relationships in the leaf blade as well (Figure 1.4) (Johnson *et al.*, 2013a, Clay, 1990).

Both endophyte and plant develop in parallel. In the cell division zone (CZ) of the plant leaf, endophyte hyphae are highly branched and grow from the hyphal tip. The plant cells, along with the attached hyphae, are then pushed out of the CZ by the dividing plant cells below into the expansion zone (EZ) of the plant leaf where the plant cells'

elongation and maturation occur. During this transition phase, the endophyte hyphae switch from tip to intercalary growth, allowing the hyphae to remain attached to the plant cell wall. Both plant and endophyte develop through intercalary growth in the EZ, but through different mechanisms *i.e.* the length of the plant cell compartments increase progressively during cell maturation, while endophyte hyphae compartments divide unevenly throughout the length of the hyphae (Figure 1.4) (Christensen *et al.*, 2008, Voisey, 2010, Schardl *et al.*, 2004).

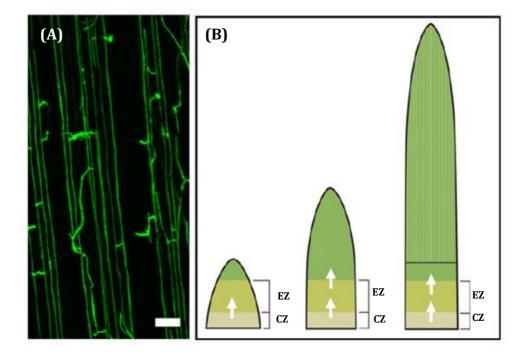


Figure 1.4: Fungal intercalary growth. (A) Morphology of hyphae in the grass leaf expansion zone using *E. festucae* strain Fl1 expressing green florescent protein (GFP) in ryegrass. Scale bar = 50  $\mu$ m. (B) Diagram of the different growth zones within a grass leaf, cell division zone (CZ), and leaf expansion zone (EZ). Arrows indicate direction of growth. Taken from (Voisey, 2010) with permission from Elsevier.

## 1.2.2 Epichloë asexual and sexual life cycles

The symbiotic interaction between the host plant and endophyte can be placed anywhere on the continuum of mutualistic to antagonistic depending on the endophyte strain. The placement of a specific strain is dictated by the ratio of the rate of vertical/asexual (through the seed) transmission to horizontal/sexual (through ascospore production) transmission, with the mode regulated at each tiller and ratio fixed for each strain. Given that asexual strains of *Epichloë* cannot undergo horizontal transmission, all strains therefore form a mutualistic interaction *e.g. E. festucae* var

*lolii* AR37. While sexual species of Epichloë can be placed anywhere on the continuum, e.g. *E. festucae* Fl1 rarely undergoes horizontal transmission and therefore forms a mutualistic interaction, *E. alumni* undergoes horizontal transmission frequently and therefore forms an intermediate mutualistic-antagonistic interaction (Schardl *et al.*, 2004), and *E. typhina* almost always undergoes horizontal transmission and therefore forms an antagonistic interaction (Chung & Schardl, 1997). Both the sexual and asexual reproductive processes coincide with the reproductive process of the host plant (Schardl *et al.*, 2004).

For asexual reproduction, the endophyte infects the ovary and ovules of each floret by growing from the vegetative apex into the inflorescence. Directly after fertilisation, the endophyte enters the embryonic sac, where during embryogenesis, it will infect both the embryo and surrounding structures. Finally, the endophyte colonises the shoot apex during germination, resulting in a systemic infection of the aerial tissue (Figure 1.5) (Philipson & Christey, 1986, Scott *et al.*, 2012).

For sexual reproduction to occur, a switch from restrictive growth to proliferative growth is required. This is dependent on the physiological state of both the grass and endophyte (Scott et al., 2012). Sexual reproduction of the endophyte takes place during the development of the inflorescence. The immature inflorescence is initially covered by the leaf sheath. This leaf sheath is infected by the endophyte which results in the inflorescence failing to emerge, and instead, a stroma develops (Schardl et al., 2004, Scott et al., 2012, Johnson et al., 2013a). For the development of ascospores from the stroma, cross-fertilisation between stroma of different mating types is required. This occurs via the female Botanophila fly that acts as a pollinator (Kohlmeyer & Kohlmeyer, 1974). Volatiles produced by the plant attract the fly to feed and oviposit. The fungal spores are then carried in the gut of the fly and fertilise the stroma of an opposite mating type upon excretion. This is a co-dependent relationship as the fly larva in turn requires the fertilised stroma as a food source. Once the stroma is fertilised, fruiting bodies (perithecia) develop with multiple sacs (asci) each containing 8 ascospores. These ascospores are then ejected into the air and infect neighbouring plants (Figure 1.5) (Schardl et al., 2004, Chung & Schardl, 1997).

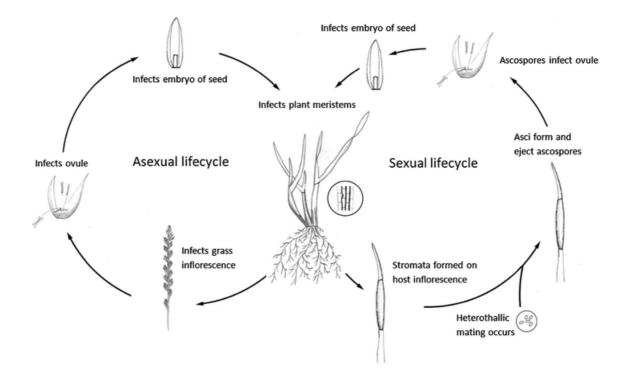


Figure 1.5: Diagram of both asexual and sexual life cycles of *Epichloë* species, taken from (*Johnson, 2013a*) with permission from Springer.

## 1.2.3 Establishment and maintenance of the Epichloë-host symbiosis

The evolutionary formation of the interaction that exists between endophytes and grasses is characterised by two key aspects: the ability of the endophyte to exist in harmony within the grass, and the evolutionary advantage that the endophyte provides to the grass. The use of both genetic and "omic" (transcriptomic and metabolomic) techniques, using *E. festucae* Fl1 and E2368 in ryegrass as a model system, has provided important insights into how this interaction is established and maintained (Scott *et al.*, 2012).

Through forward and reverse genetics, genes involved in the control of reactive oxygen species (ROS) (*noxA, noxR, bemA,* and *cdc24*)(Takemoto *et al.*, 2012, Scott *et al.*, 2012, Takemoto *et al.*, 2006, Tanaka *et al.*, 2006), iron up-take (*sidN*) (Johnson, 2008, Johnson *et al.*, 2007, Johnson *et al.*, 2013a, Johnson *et al.*, 2013b, Forester, 2014), hyphal anastomosis (*so*) (*Charlton et al.*, 2012), and response to environmental cues (*sakA* and *mpkA*) (Eaton *et al.*, 2010, Becker *et al.*, 2015, Eaton *et al.*, 2008, Qi & Elion, 2005, Scott & Eaton, 2008), have been identified as key components of this

interaction. Individual deletions of these genes resulted in *in planta* phenotypes associated with an antagonistic interaction *i.e.* the endophyte had increased hyphal branching, and hyphal proliferation, while the plant was stunted, had increased tillering, and underwent premature senescence.

The production of alkaloids by the endophyte provides a positive evolutionary selection pressure in favour of the interaction as these SMs provide bioprotection to the plant from both mammals and insects. These alkaloids were first identified by chromatography, and subsequently characterised by nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Ball *et al.*, 1997, Casabuono & Pomilio, 1997, Gallagher *et al.*, 1984). The biosynthetic pathway for each alkaloid was then characterised by knocking out or expressing the genes of interest and observing resultant end and intermediate products using MS (Tanaka *et al.*, 2005, Young *et al.*, 2005, Young *et al.*, 2006, Fleetwood *et al.*, 2007, Schardl *et al.*, 2006, Spiering *et al.*, 2002). The distribution of genes associated with alkaloid production has been characterised in a range of *Epichloë* species through PCR amplification and genomic sequencing. In addition, the corresponding alkaloid products of each gene has been confirmed through detection of the alkaloid in endophyte infected plant material by liquid chromatography combined with mass spectrometry (LCMS) (Takach & Young, 2014, Young *et al.*, 2005, Young *et al.*, 2009).

#### 1.2.4 Fungal secondary metabolite gene clusters

SMs are widely distributed throughout the kingdoms with diverse range of functionalities and can be defined in both the molecular and ecological perspective. SMs are low molecular weight compounds with restrictive taxonomic distribution that are dispensable to the host, and are produced at specific times during the organism's life cycle usually at low concentrations (Brakhage, 2013). However most importantly, SMs serve as survival functions for the producing organisms either by out-competing other organisms, scavenging for important nutrients, establishing and maintaining symbiotic associations, being sexual hormones or being differentiation effectors (Demain & Fang, 2000). This diverse range of functionalities of SMs makes these compounds of great interest to the scientific community especially in medicine and

agriculture. For example, antibiotics *e.g.* penicillin (Fleming, 1943), immunosuppression drugs *e.g.* cyclosporine (Freed *et al.*, 1987), cholesterol reducing drugs *e.g.* lovastatin (Downs *et al.*, 1998) and anti-insect *e.g.* bassianolide (Xu *et al.*, 2009). However, not all SMs are beneficial for humans, for example some are toxins *e.g.* aflatoxin from *Aspergillus* (Mishra & Das, 2003), and fumonisins from *Fusarium* (Scott, 2012), while others cause contact dermatitis *e.g.* urushiols from *Toxicodendron radicans* (Kalish & Johnson, 1990).

The focus of in this study is to identify SMs produced by the endophyte *in planta* that either have insect deterrent or toxic bioactivity. This is because only endophyte infected plants gain the ability to protect the host from the insects of interest therefore the SMs of interest are endophyte rather than plant derived. Identifying insect bioactive SMs will allow for exploitation of the activity in agriculture.

Genes that are involved in the same SM pathway in fungi *e.g.* synthesis, posttranslational modification, transportation, and regulation, are generally organised into gene clusters, with each gene under the control of its own promoter. The formation of these gene clusters makes synchronised regulation more effective due to the close proximity of the related genes. This arrangement is different to the arrangement of related genes in bacteria where the genes involved in the same pathway are controlled under one operon and are synthesised from a polysistronic mRNA (Keller *et al.*, 2005). These SM gene clusters are commonly located close to the telomeres *i.e.* subtelomeric, where DNA is frequently manipulated, promoting the gain, loss or modification of the SM producing genes. This can result in the production of new bioactive compounds and/or bioactive combinations that may have either a positive, neutral, or negative impact on the host species.

### 1.2.5 Regulation of fungal secondary metabolites

SM synthesis requires the synchronised regulation of genes in the correct tissue at a specific stage of the organism's life cycle under a specific set of environmental conditions. This requires both general regulation mechanisms *e.g.* global transcription

factors, and chromatin remodelling, as well as specific regulation mechanisms *e.g.* pathway specific transcription factors.

Global transcription factors are able to target genes either directly or indirectly upon activation by a specific stimulus. PacC is a fungal global transcription factor that targets a range of genes including SM genes. It is activated upon acidification *i.e.* pH stimuli causing multiple conformation changes which in turn result in activated PacC binding directly to target gene promoters (Luo et al., 2017). AreA is another example of a fungal global transcription factor that binds directly to the target genes but in response to nitrogen levels (Mihlan et al., 2003). CreA is a global transcription factor that targets a range of genes including those involved in the production of SMs but is specific to filamentous fungi. This global transcription factor is activated in response to carbon levels, and binds to regulatory elements at the promoters of target genes to repress transcription (Cepeda-García et al., 2014). LaeA is a global transcription factor specifically for fungal SMs. In Aspergillus nidulans, LaeA connects light, sexual development, and SMs through the formation of a protein complex with VelB (expressed during sexual development) and VeIA (up regulated in the dark) which in turn is proposed to block chromatin binding factors resulting in chromatin remodelling (Bayram *et al.*, 2008).

Some global regulators which also target SMs are species specific *e.g.* Tri6 is a global regulator of *Fusarium graminearum* (Nasmith *et al.*, 2011). For many SM gene clusters, the transcription factor which regulates the cluster is found within the cluster and is activated by a global transcription factor before binding to the promoters of each target gene *e.g.* aflR in the aflatoxin pathway (Yu *et al.*, 2004). However, in some cases, this transcription factor is not within the cluster *e.g.* PENR1 in the penicillin pathway (Litzka *et al.*, 1998).

Regulation of SM genes can also occur through chromatin remodelling either directly or indirectly. In the filamentous fungi *Fusarium fujikuroi* SM genes are directly regulated by the histone deacetylases, FfHda1 and FfHda2 (Studt *et al.*, 2013), while as mentioned earlier LaeA indirectly regulates histones through interaction with

chromatin binding factors (Bayram *et al.*, 2008). So far no global transcriptional factors or pathway specific transcription factors (within or outside the clusters) have been identified that target (positive or negative) the SM pathways currently linked to bioactivity in *Epichloë* (refer to section 1.2.6). A LaeA gene has been shown to be present in *Epichloë* but deletion of the gene has no effect on these pathways (Rahnama, 2016). However, histone modification has been directly linked to alkaloid regulation *in planta* (Chujo & Scott, 2014, Lukito, 2017).

## 1.2.6 Epichloë in planta induced bioactive alkaloids

The mammalian and insect bioactivities observed in *Epichloë* infected grasses has been linked to the presence of alkaloids produced by the endophyte such as lolitrem B (IDT alkaloid), ergovaline (ergot alkaloid), loline (1-aminopyrrolizidines), and peramine (pyrrolopyrazines) (Johnson *et al.*, 2013a). The biochemical pathways involved in the production of these key alkaloids have been proposed and annotated with the genes associated with each step (Figure 1.6).

Apart from lolines which have been detected at low levels in cultures of some *E. uncinata* strains (Blankenship *et al.*, 2001), these alkaloids are only produced *in planta* (Chujo & Scott, 2014), and are regulated by both the plant *e.g.* genotype (Faeth *et al.*, 2002) and hormones (Bastias *et al.*, 2017) as well as the environment *e.g.* light and temperature (Lane *et al.*, 2000, Hennessy *et al.*, 2016).

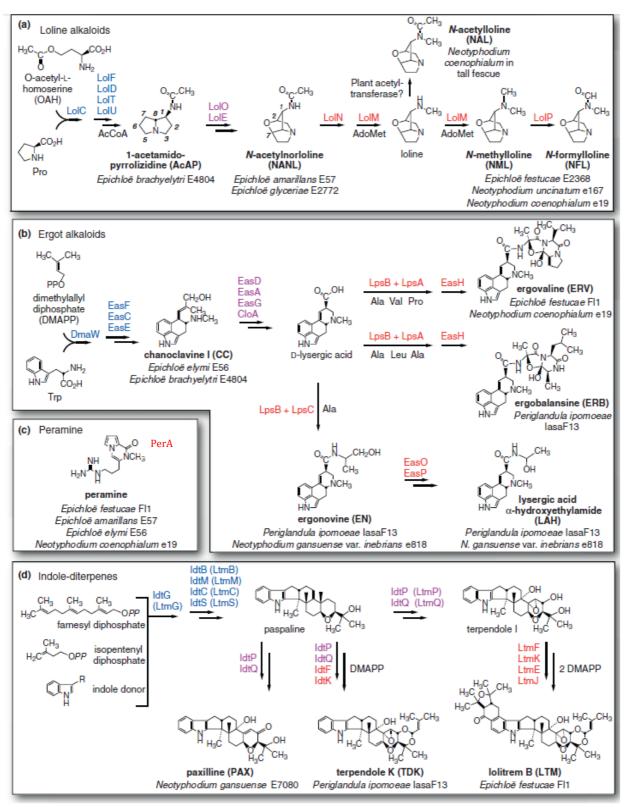


Figure 1.6: Simplified biochemical pathways that have been proposed for the production of secondary metabolites produced by *Epichloë* species. (A) Loline alkaloids. (B) Ergot alkaloids. (C) Peramine. (D) Indole-diterpenes. The corresponding gene(s) for each step in the pathway is highlighted in colour. Adapted from (Schardl *et al.*, 2013a) with permission from Elsevier.

## 1.2.6.1 Epichloë ergot alkaloids

Ergot alkaloids are produced by several fungi in the orders Hypocreales and Eurotiales within the phylum Ascomycota and are discontinuously distributed (Panaccione, 2005). They are classified into three groups of increasing complexity: clavines, ergoamides, and ergopeptines (Schardl *et al.*, 2009), with all groups containing a core ergoline ring. Ergot alkaloids are toxic to bacteria, nematodes, insects, and mammals. In mammals, these compounds are able to interact with receptors in either an agonistic or antagonistic manner (Schardl *et al.*, 2012, Gerhards *et al.*, 2014).

In *Epichloë*, the ergopeptine ergovaline has been identified as the metabolite responsible for summer slump and fescue foot in cattle grazing on endophyte infected pastures (Blythe *et al.*, 2007). The toxicities of compounds produced earlier in the ergovaline pathway are unknown, but other ergopeptines as well as ergoamides and clavines have been linked to insect bioprotection in endophyte infected plants without causing mammalian toxicity (Tor-Agbidye *et al.*, 2001, Schardl *et al.*, 2006). Ergovaline is synthesised by enzymes encoded in the sub-telomeric EAS gene cluster, which is comprised of 11 genes organised into three clusters separated by transposon relics and miniature inverted-repeat transposable elements (MITEs) (Figure 1.7) (Fleetwood *et al.*, 2007, Fleetwood *et al.*, 2011, Tanaka *et al.*, 2012, Schardl *et al.*, 2013b).

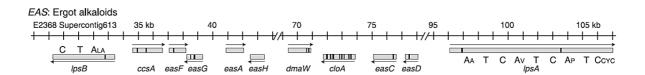


Figure 1.7: Ergot alkaloid (EAS) gene cluster in *Epichloë festucae* E2368. lpsA and lpsB (( $A_{LA}$ ) AMPylation domain binding lysergic acid, ( $A_A$ ) AMPylation domain binding alanine, ( $A_V$ ) AMPylation domain binding valine, ( $A_P$ ) AMPylation domain binding proline, (T) thiolation domain, (C) condensation domain, and ( $C_{cyc}$ ) cyclization condensation domain), adapted from (Schardl *et al.*, 2012) with permission from Elsevier.

## 1.2.6.2 Epichloë 1-Aminopyrrolizidines

1-Aminopyrrolizidines, commonly referred to as lolines, are a group of SM consisting of a saturated *exo*-1-amino pyrrolizidine with an unusual oxygen bridge between C(2) and

C(7), and variation of the R groups linked to N(1) (Schardl *et al.*, 2012). Lolines are produced by a small number of endophytes that infect some grasses in the Pooideae subfamily *i.e.* lolines are not generally produced by *Epichloë* species that infect ryegrass but there are a few exceptions *e.g. E. occultans*, but are produced by *Epichloë* species that infect fescue *e.g. E. coenophiala*, *E. festucae*, and *E. uncinata* (Popay *et al.*, 2009, Leuchtmann *et al.*, 2000). These compounds have a broad range of insect deterrence and toxic activities, but are not toxic or tremorgenic to mammals (Schardl *et al.*, 2009). Some of the insect species affected by lolines are: aphids (*Rhopalosiphum padi*), Japanese beetle larvae (*Popillia japonica*), large milkweed (*Oncopeltus faciatus*), army worm (*Spodoptera frugiperda*), and corn borer (*Ostrinia nubilalis*)(Schardl *et al.*, 2007). In *Epichloë*, lolines are produced by a series of enzymes encoded by genes in the *LOL* gene cluster, which contains 11 genes grouped into three clusters interspersed with MITEs (Figure 1.8) (Spiering *et al.*, 2005, Fleetwood *et al.*, 2011, Tanaka *et al.*, 2012).

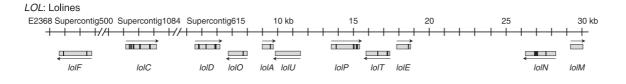


Figure 1.8: Loline (LOL) gene cluster in *Epichloë festucae* E2368, adapted from (Schardl *et al.*, 2012) with permission from Elsevier.

# 1.2.6.3 Epichloë pyrrolopyrazines

Peramine, the only known *Epichloë* produced pyrrolopyrazine, is responsible for deterring both larval and adult ASW from feeding on ryegrass. Peramine is synthesised by a single bimodular non-ribosomal peptide synthase (NRPS) encoded by the non subtelomeric gene *perA* (Tanaka *et al.*, 2005, Schardl *et al.*, 2012). The proposed amino acid substrates are 1-pyrroline-5-carboxylate, which binds to the first adenylation domain, and arginine, which binds to the second adenylation domain and is then methylated. There are no additional supporting genes associated with peramine synthesis *e.g.* transcription factors, post-transcriptional modifying enzymes, or transporters (Figure 1.9) (Berry *et al.*, 2015).

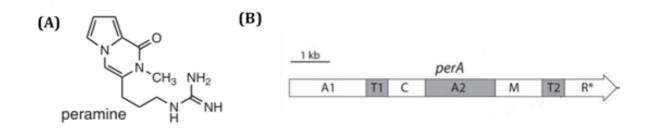


Figure 1.9: The structure of peramine (A) and the corresponding *perA* gene in *Epichloë festucae* Fl1 (B), adapted from (Schardl *et al.*, 2012) and (Berry *et al.*, 2015) with permission from Elsevier and American Society for Microbiology.

## 1.2.6.4 Epichloë indole-diterpenes

Lolitrem B is an IDT and the main metabolite responsible for ryegrass staggers (Gallagher *et al.*, 1981). It is synthesised through a complex metabolic network controlled by the *LTM/IDT* gene cluster which contains 11 genes split into three subtelomeric clusters by transposon relics (Figure 1.10) (Young *et al.*, 2005, Young *et al.*, 2006, Schardl *et al.*, 2012). IDT are reviewed comprehensively in section 1.3.

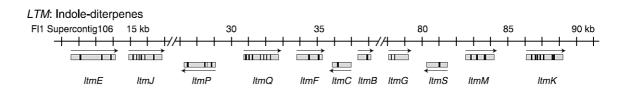


Figure 1.10: Indole diterpene (IDT/LTM) gene cluster in *Epichloë festucae* Fl1, adapted from (Schardl *et al.*, 2012) with permission from Elsevier.

# 1.3 Penicillium and Epichloë indole-diterpene characterisation

IDTs are produced by *Penicillium, Aspergillus, Claviceps,* and *Epichloë* fungi from the phylum Ascomycota (Saikia *et al.*, 2008). Structural diversity is brought about through different patterns of prenylation, hydroxylation, epoxidation, methylation, and oxidation, as well as differences in the stereochemistry of the IDT ring. IDTs can be grouped into paxilline-like compounds or non-paxilline-like compounds (Parker & Scott, 2004). Many of these compounds have either anti-mammalian or anti-insect activity and act by inhibiting calcium activated potassium channels (maxi-K) (Knaus *et al.*, 1994), or activating glutamate-gated chloride channels (GluCl) (Smith *et al.*, 2000) respectively. *Penicillium paxilli* is commonly used as a model system for studying IDT

synthesis due to its rapid growth rate, production of large quantities of IDT compounds in culture, and easy genetic manipulation (Young *et al.*, 2001)

### 1.3.1 Penicillium indole-diterpenes

Synthesis of the IDT compound paxilline by *P. paxilli* was first investigated through the analysis of the chemical components of the pathway using, chromatography, MS, synthetic chemical synthesis, crystallography, and NMR (Smith III & Mewshaw, 1985, Munday-Finch *et al.*, 1996, Mantle & Weedon, 1994, Weedon & Mantle, 1987, Miles *et al.*, 1992). The genetic component of the paxilline pathway was then investigated using plasmid insertional mutagenesis, chromosomal walking, and bioinformatics. Paxilline biosynthesis was linked to a 50 kb region on chromosome Va that contained 21 putative genes. Genes of interest were: a GGPP synthase (*paxG*), two FAD-dependent monooxygenases (*paxM* and *paxN*), a prenyltransfase (*paxC*), two cytochrome P450 monooxygenases (*paxR* and *paxQ*), a dimethylallyltryptophan synthase (*paxD*), two transcription factors (*paxR* and *paxS*), a dehydrogenase (*paxH*), a metabolite transporter (*paxT*), and an oxidoreductase (*paxO*). Deletion of *paxG* resulted in abolishment of paxilline production. The detection of *paxG* expression in *P. paxilli* wild type (WT) cultures along with this deletion provided evidence for the involvement of this cluster in paxilline synthesis (Young *et al.*, 2001).

The first stable intermediate in the proposed paxilline pathway is paspaline which was successfully synthesised using a construct expressing *paxG*, *paxC*, *paxM*, and *paxB* in a Pax<sup>-</sup> *P. paxilli* strain (Saikia *et al.*, 2006). The roles of *paxP* and *paxQ* in the conversion of paspaline to paxilline were confirmed through individual gene deletions that resulted in the accumulation of paspaline and 13-desoxypaxalline respectively as well as intermediate compound feeding assays (McMillan *et al.*, 2003, Saikia *et al.*, 2007). The paxilline pathway therefore consists of the early pathway genes *paxG*, *paxC*, *paxM*, and *paxP* and *paxQ* to 13-desoxypaxalline respectively. Paxilline and associated intermediate compounds from the proposed biosynthetic scheme were successfully synthesised in *Aspergillus oryzae* by introducing different combinations of the above 6 genes into the host genome (Tagami *et al.*, 2013).

The current paxilline pathway has PaxG synthesising geranylgeranyl pyrophosphate (GGPP) from isopentenyl diphosphate (IPP) and/or dimethylallyl pyrophosphate (DMAPP) and/or geranyl pyrophosphate (GPP) and/or farnesyl pyrophosphate (FPP). GGPP then undergoes a condensation reaction with indole-3-glycerol phosphate (IGP) by PaxC to produce 3-geranylgeranyl indole (3-GGI). This compound is then converted to paspaline through two consecutive rounds of epoxidation by PaxM and cyclisation by PaxB. Paspaline is then converted to 13-desoxypaxilline by PaxP, through multiple oxidation steps, and finally to paxilline through multiple oxidation steps by PaxQ (Figure 1.11).

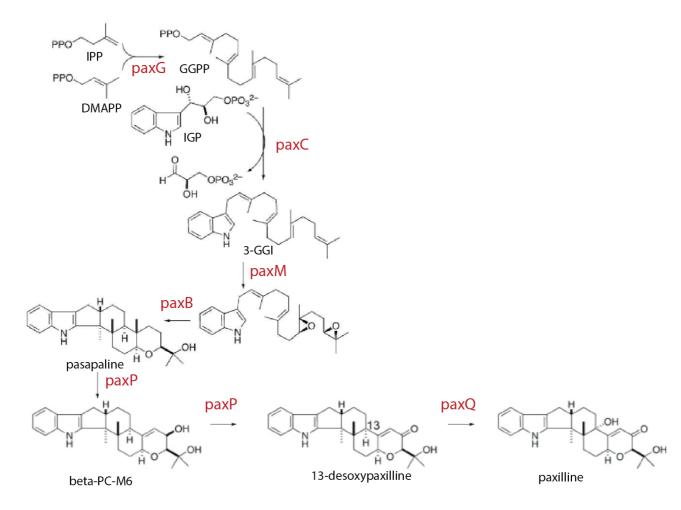


Figure 1.11: Simplified proposed paxilline biosynthetic pathway in *Penicillium paxilli* adapted from (Saikia *et al.*, 2006, Saikia *et al.*, 2007) with permission from John Wiley and Sons. The compounds are labelled below the corresponding chemical structure with genes responsible for the synthesis in red above the corresponding arrow. Isopentenyl diphosphate (IPP). Dimethylallyl pyrophosphate (DMAPP). Geranylgeranyl pyrophosphate (GGPP). Indole-3-glycerol phosphate (IGP). 3-geranylgeranyl indole (3-GGI).

#### 1.3.2 Epichloë indole-diterpenes

*Epichloë* can synthesise the paxilline-like compounds lolitrems, terpendoles, and janthitrems. Lolitrem B/A/C/F, lolitriol, janthitrems, paspaline, paxilline and terpendole C/M have been linked to mammalian tremorgenicity, through the inhibition of calcium activated potassium channels (Parker & Scott, 2004, Saikia *et al.*, 2008, Imlach *et al.*, 2011) while *epoxy*-janthitrems have been linked to insect deterrence (Jensen & Popay, 2004, Popay & Gerard, 2007). Initially the *Epichloë* IDT gene naming convention was *ltm* for lolitrem B synthesis genes given lolitrem B is the final product of the pathway. But the current naming convention is now *idt* for indole-diterpene given that a range of IDT compounds can be produced dependent on the functional gene combination.

One IDT of agricultural importance is lolitrem B, a transient mammalian neurotoxin, which has been linked to ryegrass staggers (Gallagher *et al.*, 1981). The biosynthetic pathway of lolitrem B was first modelled using the paxilline biosynthetic pathway from *P. paxilli*. PCR amplification of two conserved regions of the gene *paxG* followed by chromosomal walking identified *paxG* and *paxM* homologues in *Epichloë* (*idtG* and *idtM*), as well as an *Epichloë* specific P450 monooxygenase *idtK*. The genes were linked to lolitrem synthesis through deletion of *idtM* which abolished production and detection of both mRNA and proteins from *idtM*, *idtG*, and *idtK* genes in *Epichloë* WT infected ryegrass (Young *et al.*, 2005).

Due to the large AT-rich regions either side of this cluster suppression subtraction hybridization libraries followed by Southern blotting and sequencing rather than chromosome walking was used to identify other possible lolitrem genes. Bioinformatics analysis of this region identified homologous genes to *paxP*, *paxQ*, *paxB*, and *paxC* in *Epichloë* (*idtP*, *idtQ*, *idtB*, and *idtC*) along with the *Epichloë* specific prenyl transferase genes *idtF* and *idtE*, and another P450 monooxygenase *idtJ*. These genes are organized into 3 clusters with cluster 1 containing *idtG*, *idtM*, and *idtK*, cluster 2 containing *idtP*, *idtQ*, *idtF*, *idtC*, and *idtB*, and cluster 3 containing *idtE* and *idtJ*. RT-qPCR showed that all 10 genes are all up-regulated *in planta* but not expressed in culture suggesting that the genes are symbiotically regulated. No conserved consensus sequence was identified across all 10 genes that might explain universal up-

regulation through a transcription factor. Finally *idtC* and *idtM* were identified as *paxC* and *paxM* homologues through complementation into the respective *P. paxilli*  $\Delta paxC$  and  $\Delta paxM$  strains that restored paxilline synthesis (Figure 1.12) (Young *et al.*, 2006).

Genome sequencing of sexual, asexual, and hybrid strains identified that this SM gene cluster is sub-telomeric, and that the three clusters are separated by non-conserved AT-rich retro-transposable elements and MITE's (Young *et al.*, 2009). A novel gene, *idtS*, was identified through genome sequencing and is most similar to *paxA*. Both genes have unknown functions but are linked to IDT synthesis (Figure 1.12) (Schardl *et al.*, 2012, Schardl *et al.*, 2013c).

The lolitrem gene profile of 44 *Epichloë* isolates through PCR and Southern blotting identified three distinct groups; strains with all 10 genes, strains missing *idtE* and *idtJ*, and strains either missing at least one of the core genes (*idtG*, *idtM*, *idtB*, and *idtC*) or all of the genes. These profiles along with the corresponding chemical profiles were used to propose a biosynthetic pathway for lolitrem B production (Young *et al.*, 2009). This pathway was further investigated through individual gene deletions (*idtP*, *idtQ*, *idtF*, *idtK*, *idtE*, and *idtJ*) as well as recombinant gene expression (*idtP* and *idtQ*) followed by feeding assays and complementation of *paxP* and *paxQ* with *idtP* and *idtQ*, respectively (Saikia *et al.*, 2012).

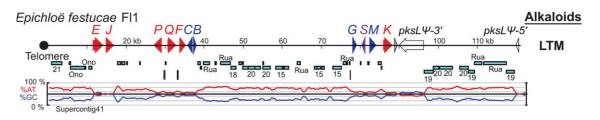


Figure 1.12: Indole diterpene (IDT/LTM) gene cluster in *Epichloë festucae* Fl1 strain adapted from (Schardl *et al.*, 2013c) with permission from PLOS. IDT genes as red/blue/purple arrows, transposable elements in blue boxes with corresponding name or size, and GC content below

The pathway is that IdtG, IdtC, IdtM, and IdtB catalyse the same steps as their corresponding Pax enzymes to produce paspaline (Introduction 1.3.1). Both IdtP and IdtQ are then able to bind paspaline creating a fork in the pathway. Typically, IdtQ will act first thereby directing the pathway down the main branch into lolitrem B synthesis,

but in some situations IdtP will act first and shunt the pathway down the minor branch into paxilline synthesis. Both pathways involve multiple oxidation steps catalysed by first IdtQ then IdtP for lolitrem B synthesis or first IdtP and then IdtQ for paxilline synthesis (the exact pathway is unknown). For lolitrem synthesis, terpendole I is then modified by IdtK and IdtF, which form the I ring structure, and/or IdtE and IdtJ, which form the A and B ring structures. These two pairs act independently. This results in a grid like biosynthetic pathway rather than a linear pathway resulting in a wide range of IDT produced through different terpendole I modifications. If any of these later pathway genes (*idtK*, *idtF*, *idtE*, and *idtJ*) are absent or non-functional then the minor branch towards paxilline synthesis is enhanced (Figure 1.13) (Saikia *et al.*, 2012).

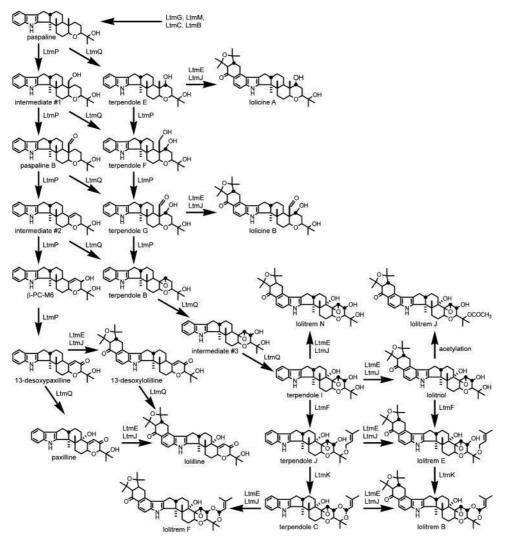


Figure 1.13: Proposed lolitrem B biosynthetic pathway in *Epichloë festucae* Fl1 strain from (Saikia *et al.*, 2012) with permission from John Wiley and Sons. The compounds are labelled below the corresponding chemical structure with genes responsible for the synthesis above the corresponding arrow. To note: *Itm* genes are the old nomenclature and are now referred to as *idt* genes *e.g. ItmP* is the same gene as *idtP*.

# 1.4 Makes caterpillars floppy (Mcf) as a possible *Epichloë* insect bioactive

The development of commercialised *Epichloë* strains has successfully arisen from understanding the biosynthesis, bioactivity, and distribution of the four SM pathways discussed above (Introduction 1.1 and 1.2). However, these pathways can only account for a proportion of the observed bioactivities. Research is therefore now focusing on the identification of new sources of bioactive molecules to explain new insect bioactivities. One newly identified gene of interest is a gene in the *Epichloë* genome called "makes caterpillars floppy" (*mcf*) identified by Ambrose in 2014. This gene is homologous to a bacterial gene that confers insecticidal activity and therefore has the potential to explain AR48 new bioactivity (Figure 1.14).

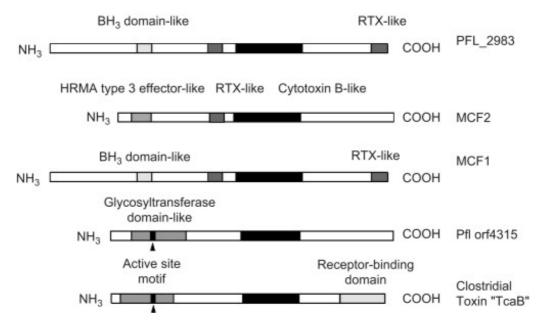


Figure 1.14: Comparison of makes caterpillars floppy (Mcf)-like proteins adapted from (Dowling & Waterfield, 2007), with permission from Elsevier. PFL\_2983 (*fitD* from *Pseudomonas fluorescens*). Mcf2 (*mcf2* from *Photorhabdus luminescens*). Mcf1 (*mcf1* from *Photorhabdus luminescens*). Pfl orf4315 (P. <u>f</u>luorescens insect <u>t</u>oxin (*fitD*) from *Pseudomonas fluorescens*). Toxin "TcdB" (*tcdB* from *Clostridium difficile*).

## 1.4.1 Photorhabdus luminescens Mcf

*Photorhabdus luminescens* is a Gram-negative Enterobacteriaceae bacterium that is both a symbiont of entomopathogenic nematodes from the Heterorhabditidae genus and a pathogen to the nematode's target insects. *P. luminescens* lives in the gut of the nematode, and upon infection of the target insect by the nematode, is released from the gut into the midgut of the insect. *P. luminescens* then secretes both

immunosuppressing compounds as well as toxins resulting in persistence of infection and death of the insect allowing both the nematode and bacteria to use the insect as a reservoir for breeding. The juvenile nematodes are reinfected with the bacterium before emerging from the insect corpse. This three-way interaction is used to study the switch between symbiotic and pathogenic life stages, and is regularly compared to *E. coli* which has a single symbiotic life stage (Forst *et al.*, 1997).

One of the many toxin genes identified from *P. luminescens* is one *mcf*. This gene was identified through screening a *P. luminescens* cosmid library using the tobacco hornworm caterpillar (*Manduca sexta*) as a model insect system. Normally, *E. coli* when injected into *M. sexta* is rapidly cleared from the insect, but when *E. coli* is expressing the correct toxin from the cosmid library, the bacteria persists in the insect and kills it. Using this screening method, a 33 kb cosmid was identified, and insertional mutagenesis showed that an 8.8 kb gene (*mcf*) within the cosmid was responsible for both the persistence and toxicity activities. The 324 kDa protein product from this gene is predicted to contain a N-terminal BH3 (Bcl2 homology domain 3) domain which is found in pro-apoptotic Blc-2 (B-cell lymphoma 2) proteins, a central transmembrane domain (TMD) from *Clostridium difficile* TcdA/TcdB, a RTX-like (Repeat-in-toxin) toxin repetitive region from *Actinobacillus pleuropneumoniae*, and a C-terminal RTX-like toxin repetitive region (Figure 1.14) (Daborn *et al.*, 2002).

*E. coli* expressing *mcf* has been shown to cause apoptosis in mammalian cell lines, haemocytes, and insect midgut through characteristic membrane blebbing, chromatin condensation, DNA fragmentation, and changes in biomarkers (Dowling *et al.*, 2004). Finally, the BH3 domain was shown to be important for this insect activity as mutations in this domain dramatically reduced bioactivity. Cells exposed to the Mcf BH3 domain have changes in mitochondrial membrane potential and release cytochrome c into the cytoplasm, showing that the toxin works by activating apoptosis *via* the mitochondrial pathway (Dowling *et al.*, 2007).

Further screening of the cosmid library identified a *mcf* gene allelic variant to *mcf*(1) called *mcf*2. This gene when expressed by *E. coli* and injected into *M. sexta* also results

in insect death. The 262 kDa protein product is predicted to contain the same central TMD and RTX-like toxin repetitive region. However, rather than a BH3 domain, it has an N-terminal HrmA domain, which is a type-III secreted effector from the plant pathogen *Pseudomonas syringae* and no C-terminal domain (Dowling *et al.*, 2004). All *Photorhabdus* strains studied, contain both *mcf* genes with *mcf1* in different genomic regions and sometimes next to a type IV exporter, and *mcf2* in the same genomic region and always next to a type I exporter (Figure 1.14) (Dowling & Waterfield, 2007).

#### 1.4.2 Pseudomonas fluorescens FitD

A mcf class protein was identified in Pseudomonas fluorescens, a Gram-negative bacterial species that forms a beneficial relationship with plants by colonizing the root system and producing bioactive compounds. Bioinformatics analysis of the genome sequence identified a mcf class gene, which is part of a cluster comprised of eight genes labelled *fitA* to *fitH*, with the *mcf* gene labelled *fitD*. The *fitD* gene is present as two allelic variants with a N-terminal glycosyltransferase-like domain or a BH3 domain, a central TMD, and no RTX-like domains. The genes that cluster around fitD are involved in the transport (fitA, fitB, and fitC) and regulation (fitE, fitF, and fitG) of the toxin. This cluster is not present in all *Pseudomonas* strains and there is syntony within but not outside the cluster. When *P. fluorescens* strains containing the Fit (Fluorescens Insect Toxin) cluster were injected into Galleria mellonella (wax moth) or M. sexta (tobacco hornworm moth) caterpillars the insects died in a similar manner and rate as P. luminescens mcf treated insects, while P. fluorescens strains without the cluster had minimal deaths. In addition, E. coli expressing fitD injected into the two above insect models resulted in death, and P. fluorescens infection of Drosophila melanogaster also results in death (Figure 1.14) (Dowling et al., 2007, Péchy - Tarr et al., 2008, Olcott et al., 2010).

## 1.4.3 Epichloë Mcf

In 2014, a paper published by Ambrose et al (2014) suggested that *mcf* had been horizontally transferred (HGT) from *P. luminescens* into the *Epichloë* genus. At that time, the gene had only been identified in bacterial species from the *Photorhabdus* and *Pseudomonus* genera, and in the fungal genus *Epichloë*. However, a subsequent

paper in 2017 (Dupont & Cox) identified *mcf* in other fungal species; *Aspergillus flavus* (*Eurotiomycetes*), *Metarhizium* species (*Sordariomycetes*), *Neonectria ditissima* (*Sordariomycetes*) and *Phlebiopsis gigantean* so the HGT event of *mcf* into *Epichloë* has been retracted (Tian *et al.*, 2017).

Annotation of all 13 *Epichloë* species analysed identified two conserved introns (a variably present 5' intron and a conserved 3' intron). The *mcf* mRNA from *E. typhina* subsp. *Poae Ps1* was extracted and sequenced. This confirmed the presence and location of both annotated introns. This cDNA sequence was then expressed in *E. coli*, and the transgenic *E. coli* injected into CC. This resulted in death of the caterpillar within 24 to 48 hours, while caterpillars injected with *E. coli* expressing empty vector or just water survived (Ambrose *et al.*, 2014).

# 1.5 AR48 infected Lolium perenne insect bioactivity

Ryegrass infected with *Epichloë festucae* var *lolii* AR48 confers new bioactivity towards the SBF (*Cerodontha australis*) and CC (*Agrotis ipsilon*) (Introduction 1.1.3). Currently, both these insects are minor pests to New Zealand agriculture however, identification of the bioactive/s responsible for these bioactivities will future proof the industry when or if these species become major pests. Also, the identified bioactive/s could possibly be applied on other agricultural insect pests depending on the bioactive/s insect target range.

## 1.5.1 Agromyzidae characterisation

The Agromyzidae family is within the true or two-winged fly order Diptera, and contains flies of 0.9 mm to 6.0 mm in length that are morphologically similar. These flies differ mainly in varying colour combinations of black and yellow, and occasionally green, grey, and blue as well. Worldwide approximately 2,500 species have been identified which are usually restricted to a single geographical location (Spencer, 2012). The Agromyzidae target a large range of dicots and monocot plants, in particular ornamentals, fruit, vegetables, and cereals, with most species restricted to a single family or genera, though true polyphagia has been identified in 13 species. The majority of the Agromyzidae target the leaf, which is why the flies are commonly

referred to as leaf mining flies despite approximately 25% of species feeding on alternative structures such as the root, stem, seed head, pod, twig, or flower (Spencer, 1973).

#### 1.5.1.1 Agromyzidae life cycle

The life cycle for each Agromyzidae species varies with the length being host as well as temperature dependent. In general, the complete life cycle takes 21-28 days under favourable conditions, although it can as much as double that under unfavourable conditions. There are multiple cycles per season, with the number dependent on the temperature *i.e.* the hotter the summer the shorter the cycle and the more cycles per season. The female ovipositor punctures the plant tissue creating wound sites for both feeding (80%) as well as for laying eggs (20%). A single female will lay approximately 500 eggs just under the tissue surface, one egg per wound, over its two-week long life span. The egg, which is initially clear, will become creamy white over time and hatch 3 days later. The emerging 1 mm long transparent larva will start to burrow into the plant tissue creating an enclosed transparent "mine" scattered with black excrement, avoiding any dead or potentially hazardous regions along the way. The larva will undergo three instars and grow to 1.9 mm over the next 4-6 days, finally forming a pupa containing the third instar. Pupation may occur either inside the plant tissue at the base of the mine or the larva will emerge from the tissue and develop just outside the plant or just below the soil surface. The pupa is initially yellow and will become brown over time with black pupa normally indicating parasitism. The adult fly will emerge approximately 9 days later and as SBF are considered "moderate" fliers the flies usually remain near the hatched location, although wind can aid in long distance dispersal. Outside the breeding season, there are few flies present and most Agromyzidae enter diapause over winter in the pupal stage (Jovicich, 2009, Georgia, 2018, Blancard, 2012).

## 1.5.1.2 Distribution of Agromyzidae New Zealand

Three extensive studies on Agromyzidae population composition and distribution have been conducted in New Zealand: Watt (1924) identified 12 new species, Harrison (1976) identified a further 4 species, and Spencer (1976) discovered a further 21 new

species. Currently 41 species of Agromyzidae have been identified in New Zealand and other sub-Antarctic Islands, of which 34 species are endemic and are thought to have arrived *via* 16 immigration events from Australia and 1 from South America. These species are from the following genera; *Melanagromyza* (1 spp.), *Hexomyza* (1 spp.), *Cerodontha* (sub-genera *Ictermyza* (1 spp.)), *Cerodontha* (3 spp.), *Liriomyza* (22 spp.), *Phytoliriomyza* (7 spp.), and *Phytomyza* (6 spp.) (Spencer, 1976).

The establishment of these particular species is most likely due to wind dispersal of flies from neighbouring countries or human introduction, alongside the establishment of the corresponding specific host plant. Australia has twice as many genera and three times as many species compared to New Zealand, and the two countries have only 7 Agromyzidae species in common. Distribution of this family within New Zealand is uneven but is wide spread throughout the North and South Islands. Plant hosts have been confirmed for 26/41 species as well as an additional 3 species with reasonable certainty. Other Agromyzidae hosts are present in New Zealand but the corresponding Agromyzidae are absent, supporting the randomness of the immigration events (Spencer, 1976).

#### 1.5.1.3 Cerodontha australis characterisation

The most common and widely distributed Agromyzidae species in New Zealand is *Cerodontha australis*, also known as the wheat sheath miner or SBF. This species is identified by a grey thorax, yellow legs, yellow frons, and the shape of the third antennal segment. New Zealand also has three other less common *Cerodontha* species, *C. sylvesterensis* (yellow notoplural, darker femur, and different aedeagus *i.e.* male sex organs), *C. angustipennis* (black thorax, and yellow notopleural), and *Icteromyza triplicate* (extended yellow lunule, and wide rounded antenna) (Figure 1.15 and 1.16). *C. australis* is present in meadows and the road side of both the North Island, South Island, and some sub-Antarctic Islands. Sydney Australia is the only location outside New Zealand that has been reported to have *C. australis. Cerodontha* species target cereal and pasture grasses such as *Hordeum vulgare* (Barley), *Lolium perenne* (Ryegrass), *Triticum aestivum* (Wheat), *Dactylus glomerata* (Cocksfoot), and the endemic New Zealand grass *Poa anceps* (Spencer, 1976). In New Zealand, the

breeding season peaks between December to January and slows down between February to March.

## 1.5.1.4 Economic impact and control management of Agromyzidae

Apart from the damage caused by the genus *Liriomyza*, which contains the top three Agromyzidae pests (globally invasive polyphagous leaf miners *i.e. L. huidobrensis*, *L. sativa*, and *L. trifolii*) all of which are not present in New Zealand (Spencer, 1973), damage to host plants from Agromyzidae insects most of the time remains just below the economic threshold. Damage from Agromyzidae depends on the host range, the host type, how the larvae feed, what part of the plant is affected, the stage of plant growth, and the size of the insect population. The presence of the mine can result in a loss of tissue function *i.e.* reduced photosynthetic capacity of the plant, and the puncture wounds are thought to increase secondary bacterial and fungal infections (Spencer, 1973).

However, these flies do have the potential to become pests under some conditions. In nature the Agromyzidae are controlled by very host specific parasitoid wasps from the Eulophidae and Braconidae families (Jovicich, 2009). This control can occasionally be lost due to an unsynchronised emergence of wasps and flies or a reduced number of wasps due to disease or climate change. This type of outbreak is, however, short lasting and minor. More serious and long term outbreaks occur due to direct or indirect long term extensive and misused insecticide application, which in some cases targets the Agromyzidae but more commonly the associated parasitoid (Spencer, 1973). This has led to varying degrees of resistant populations of Agromyzidae and/or the associated parasitoid compounding the detrimental effect of these species on the economy. Alternative control methods are other Agromyzidae targeting insects, border control, trapping, resistant crops cultivars, and dynamic environments have worked to varying degrees (Weintraub *et al.*, 2017, Hondo *et al.*, 2006, Huang *et al.*, 2009).

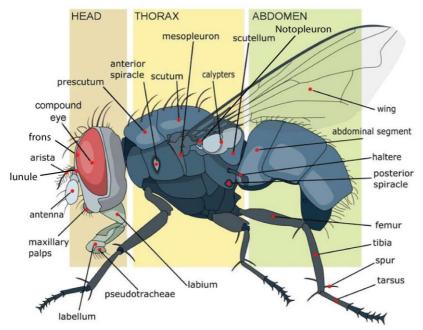


Figure 1.15: Schematic diagram of the morphology of Diptera, with key areas and structures labelled, adapted from (Wikipedia, 2018).

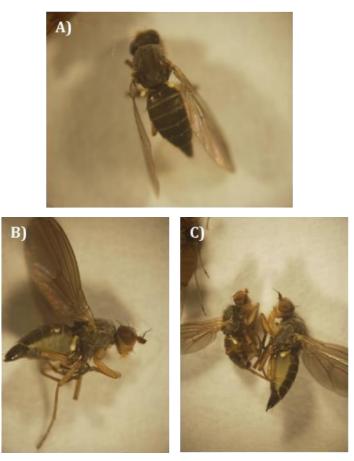


Figure 1.16: *Ceradontha australis* (stem boring fly-SBF). A) Top view. B). Side view C) Male (left) and female (right)

#### 1.5.2 Noctuidae characterisation

The Noctuidae family "owlet moths", within the Lepidoptera order (moths and butterflies), contains moths that have "owl-like eye" patterns on the top of the forewings that reflect light. Currently, Noctuidae is the largest Lepidoptera family containing over 20,000 species, however this family is commonly re-classified due to the diverse range of characteristics. In general, the moths are nocturnal ranging from 10 mm to 170 mm in length, are brown with lighter and/or darker patches, and have wings that are held upright to form a "roof" at rest. Many exceptions are found with some species being diurnal, and/or colourful, and/or having flat rested wings. Noctuidae are found worldwide with the exception of Antarctic regions. The moths feed on nectar and are important pollinators, while the larva feed on the leaves, flowers, fruits, and stems of the plant, inflicting enough damage to cause significant economic loss. The target plants and distribution of each species can range from broad to very specific (CSIRO, 2018, BAMONA, 2018). New Zealand has approximately 160 Noctuidae species, which is a relatively low number given the size of family, of which 139 are species endemic to New Zealand. Although the family has been identified as being important, it is not well studied in New Zealand, and many species have either not yet been discovered or correctly identified. Furthermore the corresponding host plants are commonly unknown (Landcare, 2018).

#### 1.5.2.1 Agrotis ipsilon characterisation

*Agrotis ipsilon* (previously known *Agrotis ypsilon*), commonly known as the black cutworm or greasy cutworm or dark sword moth or CC, is classified as belonging to the Noctuidae family. Although this moth is found worldwide, it is absent in some tropical and cold regions, and is more prevalent in the northern compared to the southern hemisphere. In America, *A. ipsilon* has been observed to migrate north in the spring and south in the fall to evade undesirable temperatures (Capinera, 2015).

Adult *A. ipsilon* are between 35 mm to 50 mm long with long thin forewings that are a mottled dark-brown colour, and at rest the wings sit flat and rectangular. The first two thirds of the forewing is dark-brown and the remaining third pale-brown to grey, with black 'dagger' shaped markings along the outer edge. The hind wing is white to light

grey but the veins stand out as dark markings. There is a distinct "Y" pattern on the forewings that gives the moth the name "ipsilon" (the Greek symbol for Y). The females are darker than the males, and have filiform rather than feathered antenna (Figure 1.17) (CABI, 2018, Taranaki Educational Resource: Research, 2018).

*A. ipsilon* larvae target approximately 50 plant species including but not limited to vegetables, maize, sweet corn, cereals, grasses, lucerne, white clover, plantain, and weeds such as dock (Khattab & Azazy, 2013). New Zealand currently has five *Agrotis* species; *A. admirationis, A. infusa, A. innominate, A. ipsilon,* and *A. munda,* with *A. ipsilon* being endemic and distributed throughout the country (Landcare, 2018).

#### 1.5.2.2 Agrotis ipsilon life cycle

The breeding season for *A. ipsilon* in New Zealand is October to April and peaks between February to March, with 2-3 life cycles occurring each summer depending on the temperature. The complete life cycle takes between 7 to 12 weeks. The female moth can lay between 600-800 eggs over its lifetime, with eggs ovideposited on to the surface of low lying leaves or leaf debris as a single egg or clusters of up to 3 eggs. The egg is 0.45 mm in diameter and changes from white to brown upon maturing, with the larva emerging 3-5 days later to reside on the soil surface. The emerging larva is lightgrey with stripes and dark spots along the sides and head. The larva will darken to light-brown/almost black over 5-8 instars over the next 4-6 weeks. Upon moulting to the fourth instar, the larva will build a burrow in the soil where it will hide during the day and emerge at night to feed on plant material brought into the burrow. At maturity the larva will be approximately 50 mm long and weigh about 150 mg, and will then form a 17 mm to 25 mm long red-brown pupa under the soil, which will darken over the 2-3 weeks before the adult moth emerges. Females are able to lay fertile eggs 3-5 days after emerging. A. ipsilon are found as either a late stage larva or pupa during the winter months, with only a few moths in existence (Allan, 1984, Agpest, 2018, CABI, 2018, Capinera, 2015, Harris et al., 1962).

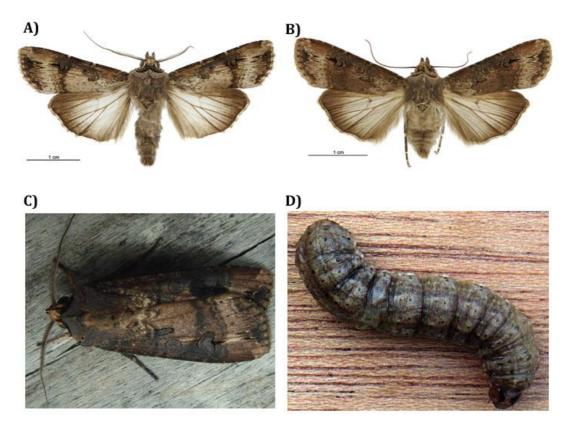


Figure 1.17: *Agrotis ipsilon* (cutworm moth (CM) and cutworm moth caterpillar (CC)). A) Male. B) Female. C) Top view resting male. D) Larva, adapted from (Landcare, 2018) and (Taranaki Educational Resource: Research, 2018).

## 1.5.2.3 Economic impact and control management of Agrotis ipsilon

Damage inflicted by *A. ipsilon* only occurs during the larval stage of the life cycle. Each larva on average consumes a total of 400 cm<sup>2</sup> of foliage during development; 10% during the second to last instar and 80% during the last instar. This is due to the quantity of plant material required to sustain the larvae as well as the technique of feeding, which involves severing the tiller at the base killing the whole leaf, even if the detached tiller is not completely consumed by the larva (Capinera, 2015). Alternatively, the larva may only damage the meristematic tissue rather than severing the stem, but this also results in leaf death. Damage tends to be observed in patches, as the larva will kill off one plant before moving to neighbouring plants. This means younger plants that have few leaves are more susceptible to infestation by this pest then mature plants (Jackson, 2013).

*A. ipsilon* outbreaks tend to occur after floods as moist conditions promote breeding. Outbreaks also occur if there is a large weed infestation in or near young crops as the

weeds act as reservoir for larvae that can then migrate to the crop. In general, yield loss ranges from 20%-80%, of plants before the two leaf stage with populations of 3 caterpillars/100 plants. When plants are at the two to four leaf stage an infestation of 6 caterpillars/100 plants results in a loss of 10,000 plants/ha (Agpest, 2018). The likelihood of an outbreak is hard to predict as it depends on the dynamic interaction between the crop (type, size, and stage of development), the *A. ipsilon* population (size and stage of development), and the environmental conditions (humidity, temperature, and wind).

Populations of *A. ipsilon* are controlled by crop management, insecticides and natural predators. Crop management techniques include not planting in or near fields previously infested with *A. ipsilon*, eliminating weeds or other host plants within and around the crop site, keeping the crop site well drained, monitoring for presence of larva, using light or pheromone trapping of moths, and burning/burying plant waste. Natural predators of *A. ipsilon* are viruses, bacteria, fungi, protozoa, nematodes, birds, mammals, and insects. Mixing *A. ipsilon* resistant Kentucky bluegrass with endophyte infected ryegrass was shown to reduce the insect population (*Richmond & Shetlar, 2001*)

# 1.6 Aims

The overall aim of this project is to identify the bioactive compound or compounds present in *E. festucae* var *lolii* strain AR48 infected ryegrass that have stem boring fly (*Cerodontha australis*) and cutworm moth caterpillar (*Agrotis ipsilon*) bioactivity. This is to be achieved by bioinformatics to identify the candidate genes followed by an analysis for bioactivity using reverse genetics in the model *E. festucae* strain Fg1 experimental system followed by insect bioactivity trials.

# Aim 1: Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *Iolii* AR48 and *Epichloë festucae* Fg1.

**Objective 1.1:** Sequence the genome of in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1.

**Objective 1.2:** Analyse the functionality and presence of the bioactive secondary metabolite genes in the genome of in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 through bioinformatics.

**Objective 1.3:** Detect the presence of the bioactive secondary metabolite compounds in in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass through mass spectrometry.

**Objective 1.4:** Analyse the structure of any newly identified compounds through mass spectrometry.

Aim 2: Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.

**Objective 2.1:** Analyse the distribution and functionality of the *mcf* gene in the *Epichloë* using bioinformatics.

**Objective 2.2:** Analyse the potential bioactivity of Mcf through reverse genetics.

# Aim 3: Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.

**Objective 3.1:** Design and perform a stem boring fly (*Cerodontha australis*) bioactivity trial on a range of wild type and a  $\Delta mcf$  mutant *Epichloë*-infected ryegrass.

**Objective 3.2:** Design and perform a cutworm moth caterpillar (*Agrotis ipsilon*) bioactivity trial on a range of wild type and a  $\Delta mcf$  mutant *Epichloë*-infected ryegrass.

**Objective 3.3:** Design and perform a porina caterpillar (*Wiseana* spp) bioactivity trial on a range of wild type and a  $\Delta mcf$  mutant *Epichloë* infected ryegrass.

**Objective 3.4:** Design and perform a light brown apple moth caterpillar (*Epiphyas postvittana*) bioactivity trial on a range of wild type and a  $\Delta mcf$  mutant *Epichloë*-infected ryegrass.

# **2.0 MATERIALS AND METHODS**

# 2.1 Molecular and biological materials

# 2.1.1 Details of strains used in this study

248 Wild type 21 Wild type 237 Wild type 2 Wild type 2 Wild type 2 Wild type 2 Mild type 3 I/pTM05 3 I/pLM12	AgResearchAgResearchAgResearchMassey UniversityMassey UniversityThis studyItoh (1994)Massey University	Wild typeWild typeWild typeWild typeWild typeWild type $\Delta mcf$ mutantComplete paxilline pathway
1 Wild type 37 Wild type 1 Wild type 1 Wild type 1/pTM05	AgResearch         AgResearch         Massey University         Massey University         This study         Itoh (1994)	Wild type         Wild type         Wild type         Wild type         Mild type         Amcf mutant         Complete paxilline         pathway
37 Wild type 1 Wild type 2 Wild type 1/pTM05	AgResearch         Massey University         Massey University         This study         Itoh (1994)	Wild type         Wild type         Wild type         ∆mcf mutant         Complete paxilline         pathway
1 Wild type 1 Wild type 1/pTM05 ild type	Massey University Massey University This study Itoh (1994)	Wild type         Wild type         Δmcf mutant         Complete paxilline         pathway
Wild type 1/pTM05	Massey University This study Itoh (1994)	Wild type         ∆mcf mutant         Complete paxilline         pathway
1/pTM05	This study Itoh (1994)	∆ <i>mcf</i> mutant Complete paxilline pathway
ild type	Itoh (1994)	Complete paxilline pathway
		pathway
		pathway
12031/pLM12	Massey University	AngyD
	McMillan (2003)	ΔραχΡ
12031/pRC3	Massey University McMillan (2003)	ΔραχQ
12258/TM06	This study	ΔpaxP AR48 idtP
		complementation
12258/TM06	This study	ΔpaxP AR48 idtP
		complementation
12258/TM06	This study	ΔpaxP AR48 idtP
		complementation
12258/TM06	This study	ΔpaxP AR48 idtP
		complementation
12258/TM06	This study	ΔpaxP AR48 idtP
10050 /TN 407	The state	complementation
12259/110107	This study	$\Delta paxQ$ AR48 <i>idtQ</i>
12250/TN 407		complementation
12259/110107	This study	$\Delta paxQ$ AR48 <i>idtQ</i>
	This study	complementation
12259/110107	This study	Δ <i>paxQ</i> AR48 <i>idtQ</i> complementation
	This study	$\Delta paxQ$ AR48 <i>idtQ</i>
12239/111107		complementation
12259/11/07	This study	$\Delta paxQ$ AR48 <i>idtQ</i>
12233/111107		complementation
17758/551	This study	$\Delta paxP P. paxilli paxP$
12230/331		complementation
12258/551	This study	ΔpaxP P. paxilli paxP
2230/331		complementation
	2031/pRC3 2258/TM06 2258/TM06	McMillan (2003)2031/pRC3Massey University McMillan (2003)2258/TM06This study2258/TM06This study2258/TM06This study2258/TM06This study2258/TM06This study2258/TM06This study2258/TM06This study2259/TM07This study

P.P-T8	PN2258/pSS1	This study	ΔpaxP P. paxilli paxP
			complementation
P.P-T9	PN2258/pSS1	This study	ΔpaxP P. paxilli paxP
			complementation
P.P-T10	PN2258/pSS1	This study	ΔpaxP P. paxilli paxP
			complementation
P.Q-T6	PN2259/pSS2	This study	ΔpaxQ P. paxilli paxQ
			complementation
P.Q-T7	PN2259/pSS2	This study	ΔpaxQ P. paxilli paxQ
			complementation
P.Q-T8	PN2259/pSS2	This study	ΔpaxQ P. paxilli paxQ
			complementation
P.Q-T9	PN2259/pSS2	This study	ΔpaxQ P. paxilli paxQ
			complementation
P.Q-T10	PN2259/pSS2	This study	ΔpaxQ P. paxilli paxQ
114 120	1112007 poor		complementation
P.P-T11	PN2258/pSS56	This study	$\Delta paxP$ FI1 <i>idtP</i>
	1112200, p0000		complementation
P.P-T12	PN2258/pSS56	This study	$\Delta paxP$ FI1 <i>idtP</i>
	1112200, p0000		complementation
P.P-T13	PN2258/pSS56	This study	$\Delta paxP$ FI1 <i>idtP</i>
1.1 115	1112230/03330	This study	complementation
P.P-T14	PN2258/pSS56	This study	$\Delta paxP$ Fl1 <i>idtP</i>
1.1 114	1112230/03330	This study	complementation
P.P-T15	PN2258/pSS56	This study	$\Delta paxP$ Fl1 <i>idtP</i>
1.1 115	1112230/03330	This study	complementation
P.Q-T11	PN2259/pSS58	This study	$\Delta paxQ$ Fl1 <i>idtQ</i>
1.0 111	1112233/03330	This study	complementation
P.Q-T12	PN2259/pSS58	This study	$\Delta paxQ$ Fl1 <i>idtQ</i>
1.0 112	1112233/03330	This study	complementation
P.Q-T13	PN2259/pSS58	This study	$\Delta paxQ$ Fl1 <i>idtQ</i>
1.0 115	11122337 03330	This study	complementation
P.Q-T14	PN2259/pSS58	This study	$\Delta paxQ$ Fl1 <i>idtQ</i>
1.0 114	1112233/03330		complementation
P.Q-T15	PN2259/pSS58	This study	$\Delta paxQ$ Fl1 <i>idtQ</i>
1.0 115	1112233/03330	This study	complementation
E.coli			complementation
2.001			
DH5a	F- Φ80 <i>lac</i> ZΔM15	ThermoFisher	Cell line
	$\Delta$ ( <i>lac</i> ZYA- <i>arg</i> F) U169	Scientific	_
	recA1 endA1 hsdR16	_	
	(rk-, mk+)		
	phoAsupE44λ-thi-1		
	gyrA96 relA1		
XL 1-	recA1 endA1 gyrA96	ThermoFisher	Cell line
	5,	Scientific	
Blue	thi-1 hsdR17 supE44	I SCIENTINC	

	<i>laclqlacZΔM15</i> Tn10		
	(Tet <sup>R</sup> )]		
TM03	DH5a/pTM03	This study	Fg1 <i>mcf</i> whole gene KO
TM04	DH5α/pTM04	This study	AR48 mcf
			complementation
TM05	DH5a/pTM05	This study	Fg1 <i>mcf</i> 5' gene KO
TM06	DH5a/pTM06	This study	AR48 <i>idtP</i> COMP
TM07	DH5α/pTM07	This study	AR48 idtQ COMP
PN1375	XL-1/pAN7-1	Massey University	Backbone
DB05	DH5a/pDB05	Massey University	P.paxilli heterologous
		Berry (2016)	expression Fl1 perA
PN4299	DH5α/pDB48	Massey University	Hyg resistant
		Berry (2016)	
DB49	DH5α/pDB49	Massey University	Gen resistant
		Berry (2016)	
PN1994	XL 1-Blue/pSS1	Massey University	paxP COMP
		Saikia (2007)	
PN1995	XL 1-Blue/pSS2	Massey University	paxQ COMP
		Saikia (2007)	
PN4078	DH5a/pSS56	Massey University	FI1 idtP COMP
		Saikia (2007)	
PN4079	DH5a/pSS58	Massey University	Fl1 idtQ COMP
		Saikia (2007)	
L. perenn	e		·
A13520	AR48 wild type/	Margo Forde	Seeds
,120020	Samson ryegrass	Germplasm centre	00000
A11751	AR47 wild type/	Margo Forde	Seeds
/(11/51	Samson ryegrass	Germplasm centre	50005
A16825	AR1 wild type/	Margo Forde	Seeds
	Samson ryegrass	Germplasm centre	
A17345	AR37 wild type/	Margo Forde	Seeds
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	Samson ryegrass	Germplasm centre	
A25872	Common toxic/	Margo Forde	Seeds
, ,23072	Samson ryegrass	Germplasm centre	
A11104	Endophyte negative/	Margo Forde	Seeds
A11104	Samson ryegrass	Germplasm centre	JEEUS
	Jambon yegiass		

# 2.1.2 Details of plasmids used in this study

Plasmids	Relevant characteristics	Source or reference	Comment
pTM03	mcf RB-PtrpC-hph-TtrpC-mcf LB;	This study	Fg1 <i>mcf</i> whole
	Hyg <sup>R</sup> /Amp <sup>R</sup>		gene knock-out
			(KO)
pTM04	<i>mcf</i> promoter- <i>mcf</i> ORF- <i>mcf</i>	This study	AR48 <i>mcf</i> whole
	terminater;Amp <sup>R</sup>		gene
			complementation
			(COMP)
pTM05	<i>mcf</i> RB-P <i>trpC-hph</i> -T <i>trpC-mcf</i> LB;	This study	Fg1 <i>mcf</i> 5' gene
	Hyg <sup>R</sup> /Amp <sup>R</sup>		КО
pTM06	nptII-PpaxM-AR48 idtP-TpaxM;	This study	AR48 idtP COMP
	Gen <sup>R</sup> /Amp <sup>R</sup>		
pTM07	nptll-P <i>paxM</i> -AR48 <i>idtQ</i> -T <i>paxM</i> ;	This study	AR48 idtQ COMP
	Gen <sup>R</sup> /Amp <sup>R</sup>		
pAN7-1	PtrpC-hph-TtrpC; Hyg <sup>R</sup> /Amp <sup>R</sup>	Massey University	Backbone
pDB05	P <i>pax</i> M- <i>per</i> A-T <i>pax</i> M; Amp <sup>R</sup>	Massey University	P. paxilli
		Berry (2016)	heterologous
			expression Fl1
			perA
pDB48	P <i>trp</i> C- <i>hph</i> -T <i>trp</i> C; Hyg <sup>R</sup> /Amp <sup>R</sup>	Massey University	Hyg resistant
		Berry (2016)	
pDB49	P <i>trp</i> C- <i>nptII</i> -T <i>trp</i> C; Gen <sup>R</sup> /Amp <sup>R</sup>	Massey University	Gen resistant
		Berry (2016)	
pSS1	<i>paxP</i> -P <i>trp</i> C- <i>nptII</i> -T <i>trp</i> C; Gen <sup>R</sup> /Amp <sup>R</sup>	Massey University	paxP COMP
		Saikia (2007)	
pSS2	paxQ-PtrpC-nptII-TtrpC;	Massey University	paxQ COMP
	Gen <sup>R</sup> /Amp <sup>R</sup>	Saikia (2007)	
pSS56	nptll-P <i>paxM</i> -Fl1 <i>idtP</i> -T <i>paxM</i> ;	Massey University	FI1 <i>idtP</i> COMP
	Gen <sup>R</sup> /Amp <sup>R</sup>	Saikia (2007)	
pSS58	nptll-P <i>paxM</i> -Fl1 <i>idtQ</i> -T <i>paxM</i> ;	Massey University	Fl1 idtQ COMP
	Gen <sup>R</sup> /Amp <sup>R</sup>	Saikia (2007)	

# 2.1.3 Details of primers used in this study

Primers	Sequence 5' to 3'	Comments	Product size bp
pTM03			
Hyg_F	AGCTTGGAACTGATATTGAA GG	Hygromycin F/ sequencing	- 2185
pDB33.7	CAGGTCGAGTGGAGATGTG G	Hygromycin R	2165
pRS425_F	GCTGTTTCCTGTGTGAAATTG	pAN7.1 F/ sequencing	2591
pRS425_R	GTCGTGACTGGGAAAACCC	pAN7.1 R/ sequencing	2591
TM49	GTAACGCCAGGGTTTTCCCA GTCACGACCCGGGCAAAGGT CTCTTCTCGTGGC	TM03 LB F/ TM04 F/ sequencing	1 1 2 2
TM50	CCACTCCACATCTCCACTCGA CCTGGCAACCCGAGAGAGA ACC	TM03 LB R	- 1428
TM51	ATGCTCCTTCAATATCAGTTC CAAGCTGGTTATGCTCGGCT ATTGAC	TM03 RB F	- 1394
TM52	GCGGATAACAATTTCACACA GGAAACAGCCCGGGCGGAG TTGGGGGGGCATTAT	TM03 RB R/ TM04 R/ sequencing	1594
TM55	CGTCTTTGGCACACAGTTGG	TM03 mcf screening F	1743
TM56	TATCGCCGCATCGATATCGG	TM03 mcf screening R	1/45
pTM04			
TM115	AGACGCATTGGGTCAACAGT	TM04 Screening LB F	
TM116	GTTGAAGACGAGGTGCGGT A	TM04 Screening LB R	1237
TM117	CGGCTTTGCGAAGTGATAGC	TM04 Screening RB F	1049
TM118	TTGGAGCGAACGACCTACAC	TM04 Screening RB R	1049
pTM05			
TM70	GTAACGCCAGGGTTTTCCCA GTCACGACCCGGGCACCATT TCGCAGTCGTAGC	TM05 LB F	1011
TM71	CCACTCCACATCTCCACTCGA CCTGAGCAAAGGACTAGGCC AACC	TM05 LB R	- 1911
TM72	ATGCTCCTTCAATATCAGTTC CAAGCTCTTCGTCGCATTGAC TTGGC	TM05 RB F	2005
TM73	GCGGATAACAATTTCACACA GGAAACAGCCCGGGATGTC GGCCTGTACAGAACG	TM05 RB R	2095

TM86	ATGTAGCGCTTCTCTGCAGG	TM05 hph screening F	2669	
TM87	ATGGGTCTTGAAGCCTCTGC	TM05 hph screening R	2009	
TM109	TACCGGGTTGATCGTGATGC	TM05 LB screen F	2528	
TM89	ACTGAGGAATCCGCTCTTGG	TM05 LB screen R	2528	
TM90	ATGTCCTCGTTCCTGTCTGC	TM05 RB screen F	2674	
TM110	CGTTGGGTTTGCAGATGAGC	TM05 RB screen R	2674	
TM111	TTTGGCTGCTCAGACTTGGG	TM05 Deleted region screen 1# F	1099	
TM112	CACGGTTCGCCTAACAAACC	TM05 Deleted region screen 1# R		
TM113	GGCCTAATCCTTTGCTTCCG	TM05 Deleted region screen 2# F	1442	
TM114	AGTGACACCAACTCCTGTGC	TM05 Deleted region screen 2# R		
pTM06				
TM74	GATCGCCCTTCCCAACAGTT GCGCAGTTGTTGGCATGGGA GTAGG	TM06 pDB49-P <i>paxM</i> (pDB05) F	1278	
TM75	TAAGACAGCATGCAACATAA ACATGGTTTCTGAATCTTAAA GATACATG	TM06 <i>idtP-PpaxM</i> (pDB05) R		
TM76	TGTATCTTTAAGATTCAGAAA CCATGTTTATGTTGCATGCTG TCTTAGC	TM06 P <i>paxM</i> (pDB05)- <i>idtP</i> F/Screening	1907	
TM77	GAAAACCAAAAATTGCTCCA ATGGTTCATGTCCTATCACTC CTGTCG	TM06 T <i>paxM</i> (pDB05)- <i>idtP</i> R/Screening		
TM78	AAACGACAGGAGTGATAGG ACATGAACCATTGGAGCAAT TTTTGG	TM06 <i>idtP-TpaxM</i> (pDB05) F	- 1322	
ТМ79	GCGTCGCGCCATTCGCCATT CAGGCCGAATTGAGAAGCTG ATTTGC	TM06 pDB49-T <i>paxM</i> (pDB05) R		
ТМ80	GACAGCAAATCAGCTTCTCA ATTCGGCCTGAATGGCGAAT GGCGC	TM06 T <i>paxM</i> (pDB05)-pDB49 F	75.00	
TM81	TCCATCCTACTCCCATGCCAA CAACTGCGCAACTGTTGGGA AGGG	TM06 P <i>paxM</i> (pDB05)-pDB49 R	7508	
pTM07				
TM74	GATCGCCCTTCCCAACAGTT GCGCAGTTGTTGGCATGGGA GTAGG	TM06 pDB49-P <i>paxM</i> F	1278	

TM82	AAAGTCAAAACGCTCTGTTA	TM07 <i>idtQ</i> -P <i>paxM</i> R	
	ACATGGTTTCTGAATCTTAAA		
	GATACATG		
TM83	ATGTATCTTTAAGATTCAGAA	TM07 PpaxM-idtQ F/Screening	
	ACCATGTTAACAGAGCGTTT		
	TGACTTTC		2000
TM84	GAAAACCAAAAATTGCTCCA	TM07 TpaxM-idtQ R/Screening	2060
	ATGGTTCACAGATCCTTCATC		
	CTAGCTC		
TM85	AAGAGCTAGGATGAAGGAT	TM07 <i>idtQ</i> -T <i>paxM</i> F/Screening	
	CTGTGAACCATTGGAGCAAT		
	TTTTGGT		4222
TM79	GCGTCGCGCCATTCGCCATT	TM06 pDB49-T <i>paxM</i> R	1322
	CAGGCCGAATTGAGAAGCTG		
	ATTTGC		
TM80	GACAGCAAATCAGCTTCTCA	TM06 T <i>paxM</i> -pDB49 F	
	ATTCGGCCTGAATGGCGAAT		
	GGCGC		7500
TM81	TCCATCCTACTCCCATGCCAA	TM06 P <i>paxM</i> -pDB49 R	7508
	CAACTGCGCAACTGTTGGGA		
	AGGG		
P. paxilli	transformation screening		
TM92	TTGATGAGGATGGGCGTTGC	<i>P. paxilli paxP</i> screen F (pSS1)	
TM93	CCGATTTCCACATCTCAACAC	P. paxilli paxP screen R (pSS1)	1871
	с		
TM94	AACCACAGCTTTCATGTCAG	<i>P. paxilli paxQ</i> screen F (pSS2)	
	G		
TM95	AGCCTTACAGAGAGATTCGT	<i>P. paxilli paxQ</i> screen R (pSS2)	2037
	GG		
TM125	TAATGTTGCACGCTGTCCCA	Fl1 <i>idtP</i> screen F (pSS56)	
TM126	TTCAACATACGGTAACTGTCT	Fl1 <i>idtP</i> screen R (pSS56)	1826
	тстсс		
TM127	GGAGATGTTAACAGAGCATT	Fl1 <i>idtQ</i> screen F (pSS58)	
	TTGACT	, , , , , , , , , , , , , , , , , , ,	2012
TM128	TCACAGATTCTTCTCCCCAGC	Fl1 <i>idtQ</i> screen R (pSS58)	
		· · · · · · · · · · · · · · · · · · ·	

# 2.2 Sterile conditions

Unless stated otherwise, all media and reagents were prepared using Mill-Q (MQ) water (Barnstead NANOpure water purification system-Thermo Scientific<sup>TM</sup> Barnstead<sup>TM</sup>) and sterilised by autoclaving (20 mins at 121°C). All equipment involved with *E. coli* and *Epichloë* were sterilised by autoclaving, and all experiments were carried out under sterile conditions within a UV sterilised laminar flow hood. All equipment involved with *P. paxilli* were sterilised by autoclaving, and all experiments were carried out under sterile conditions within a UV sterilised by autoclaving.

# 2.3 Analyse the bioactive secondary metabolite pathways in Epichloë

# festucae var lolii AR48 and Epichloë festucae Fg1

# 2.3.1 Epichloë festucae growth conditions

Colonies were maintained on Potato dextrose (PD) (2.4% (w/v) PD at pH 6.5) as a liquid medium or on media solidified using 1.5% (w/v) agar, with or without selection, at 22°C for 5-7 days for *E. festucae* strains, and for 14-30 days for *E. festucae* var *lolii* strains.

# 2.3.2 Penicillium paxilli growth conditions

Colonies were maintained on Czapek Dox Yeast extract (CDYE) (3.34% (w/v) CD, 0.5% (w/v) YE, and 5% (v/v) of trace element mix) as a liquid medium or with 1.5 % (w/v) agar, with or without selection, at 22°C for 7 days. The trace element mix contained 1.7 mM FeSO<sub>4</sub>.7H<sub>2</sub>O, 1.73 mM ZnSO<sub>4</sub>. 7H<sub>2</sub>O, 0.59 mM MnSO<sub>4</sub>.H<sub>2</sub>O, 0.2 mM CuSO<sub>4</sub>. 5H<sub>2</sub>O, and 0.17 mM CoCl<sub>2</sub>. 6H<sub>2</sub>O.

# 2.3.3 Epichloë festucae genomic DNA extraction

Mycelia from 50 mL of liquid culture (Method 2.3.1) grown for 4 days was filtered through a nappy liner and washed with 250 mL of MQ water. The mycelia were frozen at -80°C for at least 2 hours, lyophilised overnight, and then ground to a fine powder under liquid nitrogen using a mortar and pestle. The macerated mycelia were then transferred into 2 mL Eppendorf tubes in 15-20 mg aliquots, with excess mycelia stored in Nunc tubes at -20°C. 800  $\mu$ L of extraction buffer (150 mM EDTA, 50 mM Tris-

HCl, at pH 8.0) was added to the tubes and mixed before addition of DNase-free RNase (final concentration of 10 mg/mL). The samples were incubated for 30 mins at 37°C after which proteinase K (final concentration of 2 mg/mL) was added and samples incubated for a further 30 mins at 37°C. Insoluble material was removed by centrifugation at 17,000 g for 15 mins and the top transparent aqueous solution transferred into a fresh Eppendorf tube containing a ½ volume of phenol and a ½ volume of chloroform, the tube was then mixed, and centrifuged at 17,000 g for 15 mins. This phenol and chloroform step was repeated 2 more times. The final transparent aqueous solution was then transferred into a fresh Eppendorf containing 1 volume of chloroform and centrifuged at 17,000 g for 15 mins. The transparent aqueous layer was then transferred into a fresh Eppendorf containing 1 volume of isopropanol and precipitated overnight at -20°C. DNA was then pelleted by centrifugation at 17,000 g for 15 mins after which the aqueous solution discarded. If a polysaccharide pellet was visible, 800  $\mu$ L of 1M NaCl was added to the tube, and the mixture incubated at room temperature for 10 mins before centrifuging at 17,000 g for 10 mins. The aqueous solution was then transferred into a fresh Eppendorf containing 1 volume of isopropanol and precipitated for 10 mins at room temperature before the DNA was pelleted by centrifugation at 17,000 g for 10 mins. After the supernatant was removed, the pellet was washed with 70% (v/v) ethanol, before being left to air-dry. The DNA was then resuspended in MQ and stored at 4°C (Byrd *et al.*, 1990).

#### 2.3.4 Epichloë festucae crude DNA extraction

Approximately 1 cm<sup>2</sup> of mycelia from a 5-7 day old solid culture (Method 2.3.1) was macerated in a 1.5 mL Eppendorf tube using a pestle. 150  $\mu$ L of lysis buffer (100 mM Tris-HCL, 100 mM EDTA, 1% SDS at pH 8.0) was added to the Eppendorf tube, mixed, and the sample incubated at 70°C for 30 mins. Next, 150  $\mu$ L of 5 M CH<sub>3</sub>CO<sub>2</sub>K was added to the tube, which was then incubated on ice for 10 mins and then centrifuged for 20 mins at 17,000 g. The supernatant was transferred to a new Eppendorf tube containing 140  $\mu$ L of isopropanol and incubated at -20°C overnight to precipitate the DNA. The sample was then centrifuged for 20 mins at 17,000 g, and the isopropanol removed, and the pellet washed with 70% (v/v) ethanol. The DNA was repelleted by centrifugation 17,000 g for a further 5 mins, the ethanol removed, and the pellet airdried at 37°C. The pellet was then resuspended in 50  $\mu$ L of MQ, heated at 70°C for 10 mins, and centrifuged for 10 mins at 17,000 g to remove any insoluble material. The supernatant was carefully removed, then aliquoted in to a 96 well plate, and stored at -20°C until required.

#### 2.3.5 Penicillium paxilli crude DNA extraction

In a 2 mL screw cap tube 200  $\mu$ L of spore stock (Method 2.3.10) was added to approximately 50  $\mu$ L of 0.5 mm glass beads. A Fastprep machine (ThermoSavant FP120) set at 20 secs at 4 m/s was then used to disrupt the spores, which were then placed on ice. Next, the samples were centrifuged for 20 mins at 30,000 g at 4°C and placed back on ice. The top 50  $\mu$ L of the supernatant was removed, and transferred to a 96 well plate, and used immediately or kept at -20°C.

# 2.3.6 Epichloë festucae glycerol stocks

Into a Nunc tube, 2 mL of PD liquid culture containing 5-day-old mycelia (with or without selection) (Method 2.3.1) was mixed with 2.5 mL 30% (v/v) glycerol and stored at  $-80^{\circ}$ C.

# 2.3.7 Penicillium paxilli glycerol stocks

Into a Nunc tube, 800  $\mu$ L of spore stock (Method 2.3.10) was mixed with 200  $\mu$ L of 50% (v/v) glycerol and stored at -80°C.

# 2.3.8 Penicillium paxilli protoplast preparation

For each strain, 25 mL 30 hour old liquid culture (Method 2.3.2) was filtered through a sterile funnel containing a nappy liner. The collected mycelia were initially washed with 200-300 mL of MQ water, then equilibrated using 50-100 mL of OM buffer (1.2 M MgSO<sub>4</sub> and 10 mM NaHPO<sub>4</sub> at pH 5.8 using NaH<sub>2</sub>PO<sub>4</sub>) and dried with paper towels. Into pre-weight flasks, 4 g lots of wet mycelia were added along with 40 mL of twice syringe filtered (0.2 µm Ahlstrom Relia Perp) lysing enzyme (10 g/ml, *Trichoderma harzianum*, Sigma L1412), and incubated at 30°C overnight with shaking at 80 rpm. The next day, the presence of the protoplasts were checked under the microscope using a haemocytometer. The protoplasts from identical flasks were then collectively filtered

through a funnel containing a nappy liner and collected in a 200 mL Schott bottle. Next, 5 mL aliquots of the protoplast solution were transferred into 15 mL Falcon tubes, and carefully overlayed with 2 mL of ST buffer (0.6 M sorbitol, and 100 mM Tris-HCL, at pH 8.0). The tubes were then centrifuged at 20,000 g for 5 mins at 4°C. The protoplasts (white layer) were removed from the interface along with the top colourless layer and transferred to 15 mL Falcon tubes containing 5 mL STC buffer (1 M sorbitol, 50 mM Tris-HCL, and 50 mM CaCl<sub>2</sub>, at pH 8.0). The two solutions were mixed, and centrifuged for 5 mins at 20,000 g at 4 °C. The STC buffer rinse step was repeated 3 more times with tubes pooled until the protoplasts were in a single tube. The protoplasts were then resuspended in 500 µL of STC buffer, and the concentration calculated using a haemocytometer. To make stocks, the protoplasts were diluted to 1.25 x 10<sup>8</sup> protoplasts/mL in STC buffer, and 80 µL of this protoplast solution was added to 20 µL 40% (v/v) PEG aliquots, which were stored at -80°C.

#### 2.3.9 Penicillium paxilli protoplast transformation

For each transformation, 50 µL of the appropriate protoplast (Method 2.3.8) was defrosted on ice. To the protoplasts, 2  $\mu$ L of spermidine (7.11 mg/mL) and 5  $\mu$ L of heparin (5 mg/mL) were added alongside 1.0–2.5  $\mu$ g of DNA (in no more than 30  $\mu$ L), mixed by pipetting, and incubated on ice for 30 mins. To the mixture, 900 µL of 40% (v/v) PEG was added, mixed by pipetting, and incubated back on ice for 20 mins. Then, 300  $\mu$ L of this mixture was added to 3 mL of 0.8% (w/v) RG (2.4% PD (w/v), 0.8 M sucrose, and 0.8% (w/v) agar, at pH 6.5) previously heated to 50°C, mixed by pipetting, and poured onto plates containing 1.5% (w/v) RG (2.4% PD (w/v), 0.8 M sucrose, and 1.5% (w/v) agar, at pH 6.5). The plates were incubated overnight at 22°C, and then overlayed with 5 mL of 0.8% (w/v) RG containing the antibiotic of choice. For this project, hygromycin at 150  $\mu$ g/mL and geneticin at 100  $\mu$ g/mL. The plates were then incubated for a further 4-6 days at 22°C, and 20 tranformants picked and streaked out onto individual CDYE plates containing the antibiotic of choice. A single spore colony was then picked from each transformant and re-plated out onto individual CDYE plates containing the antibiotic of choice, with four identical colonies per plate. These were then incubated at 22°C for 7 days.

#### 2.3.10 Penicillium paxilli spore stocks

*P. paxilli* was grown on CDYE plates (with or without selection) as 4 separate colonies for 5-7 days (Method 2.3.2). Using a scalpel, 1 cm<sup>2</sup> sections of each centre of the 4 colonies were cut out of the plate and placed into a universal glass bottle containing 3 mL of 0.01% (v/v) triton X-100. The spores were then washed off the colonies by gently shaking the bottle. This solution was then transferred into 2 mL Eppendorf tubes, and stored at 4°C for up to 6 months.

#### 2.3.11 Bioinformatics analysis of Epichloë festucae insect bioactive genes

FASTA formatted genome sequences (AR48, Fg1, AR1, AR37, AR5, and E2368) were imported into Geneious as individual BLAST databases. Fl1 gene models for ergot alkaloid, peramine, and indole-diterpene (IDTs) biosynthetic genes together with E2368 gene models for loline biosynthetic genes were used as queries to interrogate each of the genome databases using BLASTn. Gene homologues were manually annotated, spliced, and virtually translated to generate encoded protein products. Pairwise and multiple sequence alignments of gene or protein sequences were generated using the global alignment tool within Geneious allowing for free end gaps and 65% similarity. IdtP and IdtQ substrate binding site and catalytic amino acids were predicted using Phyre2 advanced analysis.

# 2.3.12 Lolium perenne seed accession

The plants required for the insect assay trials were grown from seeds sourced from the Margot Forde Germplasm Centre (Palmerston North, New Zealand) where each seed harvest is associated with a seed accession number. AR48 in Samson ryegrass (A13520), AR47 in Samson ryegrass (A11751), AR1 in Samson ryegrass (A16825), AR37 in Samson ryegrass (A17345), CT in Samson ryegrass (A25872), and nil in Samson ryegrass (A11104).

# 2.3.13 Lolium perenne seed DNA extraction

For each extraction, 0.1 g of seed (Method 2.3.12) was distributed into 6 seeds (maximum) per 2 mL micro tube (Sestet) containing one ceramic bead. The seeds were disrupted using FastPrep-24 tissue homogenizer for 20 secs at speed 6.5. The tubes

were then centrifuged for 30 secs at 20,000 g, and the beads removed. Next, 160  $\mu$ L of NaOH (0.133 mM) was added to each tube, and the un-capped tubes microwaved at low power (10% of 1100W) for 1 min, alongside a small beaker of water. Then, 600  $\mu$ L of 1x TE (1M Tris-HCL, 0.1M EDTA, pH 8.0) solution was added, and mixed by vortexing. The tubes were incubated at 4°C overnight, then centrifuge at 20,000 g for 5 mins, and supernatant transferred to a 96 well plate.

# 2.3.14 Simple sequence repeat (SSR) polymerase chain reaction (PCR)

Each SSR PCR reaction contained 10  $\mu$ L PCR reaction (1× TE buffer (1M Tris-HCL, 0.1M EDTA, pH 8.0), 1.5 mM MgCl<sub>2</sub>, 50  $\mu$ M dNTPS, 1 U Taq polymerase, 0.2  $\mu$ M of each primer (B11 or ans014), 4  $\mu$ L of 30 fold diluted seed extract (Method 2.3.13), and 4  $\mu$ L MQ), with the following conditions used: 1 cycle at 94°C for 4 min, 35 cycles at 94°C for 30 secs, 60°C for 30 secs, 72°C for 30 secs, and a final extension of 72°C for 7 min.

# 2.3.15 SSR genotyping

Into each well of a PCR 96 well plate, 2  $\mu$ L of SSR PCR product (Method 2.3.14) was added to 9  $\mu$ L of Hi-Di containing 1.5% (v/v) Genes (500LIZ) as a size marker, and denatured under the following conditions: 1 cycle of 94°C for 5 min, and 1 cycle of 4°C for 5 min. The PCR products were then analysed on an ABI 3100 Genetic Analyser (Applied Biosystems), with the resultant electropherograms analysed using ABI Prism GeneScan (v 3.7, Applied Biosystems).

# 2.3.16 DNA sequencing

PCR (Method 2.4.21), plasmid (Method 2.4.3), and genomic DNA (Method 2.3.3) were sequenced by New Zealand Genomics Limited (NZGL) as instructed by the company. Genomic DNA was sequenced in a single lane of 250 bp paired-end generated on an Illumina MiSeq run by NZGL to give 14.8 million high quality reads (200× genome coverage). The reads were dynamically trimmed using the SolexaQA package to their longest fragment such that the base call error rates did not exceed a P value of 0.05, and paired end reads of less than 100 bp discarded. *De novo* assembly was performed using ABySS version 2.0.0 with default parameters and a *k*-mer length of 95 bp.

#### 2.3.17 Indole-diterpene extraction from Epichloë festucae herbage and seed

50 mg of milled freeze-dried pseudostem tissue (Method 2.3.1) or milled seed (Method 2.3.12) was mixed with 1 mL of extraction buffer 1 (80% (v/v) acetonitrile, 1% (v/v) acetic acid, and 0.99  $\mu$ g/mL of N-benzyl-1, 8-naphthaleneimide as internal standard) and mixed by end-over-end rotation in the dark for 1 hour. Samples were centrifuged at 21,000 g for 5 mins, and the supernatant collected. 600  $\mu$ L of this supernatant was then diluted with 4.6 mL of 1% (v/v) acetic acid before loading onto an Isolute C18 spin column, which was previously prepared by washing with 2 mL of 50% (v/v) methanol, and then 2 mL of 1% (v/v) acetic acid. The column was then washed with 2 mL of 1% (v/v) acetic acid, and then 1 mL of 50% (v/v) methanol. The IDT fraction was eluted from the column using by washing with 1 ml of methanol containing 1% (v/v) ammonia and syringe filtered.

#### 2.3.18 Indole-diterpene extraction from Penicillium paxilli cultures

Approximately 50 mg of milled freeze-dried culture (Method 2.3.2) was mixed by endover-end rotation in the dark for 1 hour with 1 mL of extraction buffer 1 (80% (v/v) acetonitrile, 1% (v/v) acetic acid, and 0.99  $\mu$ g/mL of N-benzyl-1, 8-naphthaleneimide as internal standard). Samples were centrifuged at 21,000 g for 5 mins, the supernatant collected and syringe filtered.

# 2.3.19 Chromatography of indole-diterpene extracts for triple quadrupole (TSQ) analyses

IDT compounds in the extracts (Method 2.3.17 and 2.3.19) were separated by high performance liquid chromatography (HPLC) prior to mass spectrometry (MS) analysis using an Accela 1250 pump and a Kinetix C18 2.6 $\mu$ ; 150x2.1 mm column set to a flow rate of 0.3 mL/min. For each sample the liner gradient was as follows using elutent A (100% water with 0.1% foric acid) and eluent B (100% acetonitrile, 0.1% formic acid): T<sub>0</sub> 90% A, T<sub>6</sub> 40% A, T<sub>17</sub> 0% A, T<sub>19</sub> 0% A, T<sub>20</sub> 50% A, T<sub>25</sub> 90% A.

# 2.3.20 Chromatography of indole-diterpene extracts for triple quadrupole (TSQ) analyses

IDT compounds extracts (Method 2.3.17 and 2.3.18) were separated by HPLC prior to MS analysis using an Accela 1250 pump and a Gemini-NX 3u C18 110A; 150x2.0 mm column set to a flow rate of 0.2 mL/min. For each sample the liner gradient was as follows using elutent A (100% water with 0.1% foric acid) and eluent B (100% acetonitrile, 0.1% formic acid):  $T_0$  50% A,  $T_{20}$  30% A,  $T_{40}$  0% A,  $T_{43}$  0% A,  $T_{44}$  50% A,  $T_{50}$  50% A.

# 2.3.21 Mass spectrometry analysis TSQ of indole-diterpenes from Epichloë festucae

HPLC separated IDT compounds (Method 2.3.19) were then analysed using a Thermo TSQ Quantum mass spectrometer. The mass spectrometer was set to scan selected MRMs with Q1 and Q3 isolation widths of 0.7 amu (Table 2.1). The data was processed using LCQuan software for quantification.

# 2.3.22 Mass spectrometry analysis LTQxl of indole-diterpenes from Epichloë festucae

HPLC separated IDT compounds (Method 2.3.20) were then analysed using a Thermo LTQ XL mass spectrometer. The mass spectrometer was set to scan  $MS^1$  (180 – 800 m/z) and collect  $MS^2$  of selected parent ions in each of the four method segments, with the isolation width for  $MS^2$  precursor ions set to ±1 amu (Table 2.2). The data was processed using LCQuan software for quantification

Parent ion	Fragment ion	Collision energy	Tube lens
420.3	130.1	20	80
	402.2	5	80
422.3	130.1	20	80
	404.2	5	80
436.3	130.1	20	83
	418.2	5	83
438.3	130.1	20	85
	420.2	5	85
454.3	130.1	20	80
	436.2	5	80

Table 2.1: Indole-diterpenes analysis by triple quadrupole (TSQ) selected reaction monitoring the following chromatogram segments

# Table 2.2: Indole-diterpenes analysis by linear ion trap (LTQxl) selected reaction monitoring the following chromatogram segments.

Segment 1 (Time 0–13.5 n	nin)		
Analyte Retention	MS1 precursor ion $(m/z)$	MS2 filter ions ( $m/z$ )	time (min)
lolitrem N	620.4	562.4	9.0
lolitriol	620.4	562.4	10.8
Segment 2 (Time 13.5–22	.0 min)		
Analyte Retention	MS1 precursor ion ( $m/z$ )	MS2 filter ions ( $m/z$ )	time (min)
Paxitriol	438.3	420.3	13.0
Paspaline B	436.3	420.3	15.4
Paxilline	436.3	420.2	16.2
Terpendole E	438.3	422.2	16.7
Lolitrem K	602.3	544.4	16.8
Lolitrem M	604.3	546.4	15.5
Lollicine	604.3	546.4	18.6
Lolitrem J	662.4	604.4	15.0
Segment 3 (Time 22.0–28.	6 min)		
Analyte Retention	MS1 precursor ion $(m/z)$	MS2 filter ions ( $m/z$ )	time (min)
13-desoxypaxilline	420.3	402.2, 405.2	22.8
Lolitrem A	702.4	644.3	25.7
Segment 4 (Time 28.6–40.	0 min)		
Analyte Retention	MS1 precursor ion( $m/z$ )	MS2 filter ions ( $m/z$ )	time (min)
Paspalline	422.3	130.2, 406.3	33.9
Terpendole C	520.3	504.3	28.4
Lolitrem B	686.4	628.3	29.8
Lolitrem E	688.4	630.3	31.3

#### 2.3.23 Mass spectrometry analysis LTQxl of indole-diterpenes from Penicillium paxilli

HPLC separated IDT (Method 2.3.20) were then analysed using a Thermo LTQ XL mass spectrometer with an insolation width of 2.00 *m/z*, normalised collision energy of 35.00%, and an isolation window of 400 *m/z* to 600 *m/z*. The MS<sup>2</sup> was collected in a data dependent manner by selecting the most intense ion from the parent list of 406.20 *m/z*. 418.20 *m/z*, 420.20 *m/z*, 422.20 *m/z*, 438.20 *m/z*, 452.20 *m/z*, 454.20 *m/z*, 474.30 *m/z*, 488.30 *m/z*, 490.30 *m/z*, 504.30 *m/z*, 506.30 *m/z*, 522.30 *m/z*, 542.30 *m/z*, 554.30 *m/z*, 556.30 *m/z*, 572.30 *m/z*, 574.30 *m/z*, 588.30 *m/z*, and 590.30 *m/z*, using dynamic exclusion of repeat count 3, repeat duration of 10 secs, exclusion list size of 50 masses, and exclusion duration of 10 secs. Where a parent ion was not detected, the most dominant peak in the MS<sup>1</sup> spectrum was fragmented.

#### 2.3.24 Fragmentation in tree generation for Epichloë festucae indole-diterpenes

HPLC separated IDT compounds (Method 2.3.20) were then analysed using a Thermo LTQ XL mass spectrometer with an insolation width of 2.00 *m/z*, normalised collision energy of 35.00%, and an isolation window of 400 *m/z* to 600 *m/z*. Spectra collected were (MS<sup>1</sup> 420.30 *m/z* -> MS<sup>2</sup> 404.30 *m/z*), (MS<sup>1</sup> 422.30 *m/z* -> MS<sup>2</sup> 406.30 *m/z*), (MS<sup>1</sup> 436.30 *m/z* -> MS<sup>2</sup> 418.30 *m/z* -> MS<sup>3</sup> 346.30 *m/z*), (MS<sup>1</sup> 438.30 *m/z* -> MS<sup>2</sup> 420.30 *m/z*), (MS<sup>1</sup> 436.30 *m/z* -> MS<sup>2</sup> 418.30 *m/z* -> MS<sup>3</sup> 346.30 *m/z*), (MS<sup>1</sup> 438.30 *m/z* -> MS<sup>2</sup> 420.30 *m/z*), (MS<sup>1</sup> 436.30 *m/z* -> MS<sup>2</sup> 418.30 *m/z* -> MS<sup>3</sup> 346.30 *m/z*), (MS<sup>1</sup> 438.30 *m/z* -> MS<sup>2</sup> 420.30 *m/z*), (MS<sup>1</sup> 436.30 *m/z* -> MS<sup>2</sup> 418.30 *m/z* -> MS<sup>3</sup> 400.30 *m/z*) and (MS<sup>1</sup> 436.30 *m/z* -> MS<sup>2</sup> 360.30 *m/z*).

# 2.4 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

#### 2.4.1 Bioinformatics analysis of makes caterpillars floppy (mcf)

FASTA formatted genome sequences (AR48, Fl1, Fg1, AR1, AR37, AR5, and E2368) were imported to Geneious as individual BLAST databases. The E. typhina subsp Poae Ps1 mcf gene (Ambrose et al., 2014) was used as a query sequence to interrogate each of the genome databases using BLASTn. Pairwise and multiple sequence alignments were generated using the global alignment tool within Geneious allowing for free end gaps and 65% similarity. Additional genomes from the Kentucky Endophyte Genome database (Schardl et al., 2013c) were searched, using the BLASTn search engine provided, with the same E. typhina subsp Poae Ps1mcf gene. All gene homologues were manually annotated, spliced, and virtually translated to generate encoded protein products. Domains were annotated from a selection of these manually annotated Mcf protein sequences as well as literature Mcf protein sequences (Dowling & Waterfield, 2007) using a range of online tools: BLASTp, InterPro Scan, Phyre2, and Hhpred. The *Epichloë mcf* gene location was annotated using the completely assembled E. festucae FI1 genome sequence (Winter et al., 2018) annotated with E2368 gene models (EfM3.0) and RNA sequencing data (Schardl et al., 2013c). The selected genes were then either searched in other Epichloë genomes using the BLASTn search engine provided by the Kentucky Endophyte Genome database or the FASTA

formatted genome sequence (AR48, Fg1, AR1, and AR37) databases on Geneious using the BLASTn function.

#### 2.4.2 Escherichia coli growth conditions

Colonies were maintained on Luria Broth (LB) (1% (w/v) tryptone, 0.5% (w/v) yeast extract, and 86 mM NaCl) as a liquid medium or with 1.5% (w/v) agar, with or without selection, at 22°C overnight.

#### 2.4.3 Escherichia coli plasmid extraction

Plasmids from 1 day old liquid cultures (Method 2.4.2) were extracted using the High Pure Plasmid Isolation Kit (Roch) as instructed by the manufacturer.

#### 2.4.4 Chemically competent Escherichia coli DH5α cells

A 24-hour-old streaked plate (Method 2.4.2) was used to inoculate a single colony into 50 mL of SOB medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, and 2.5 mM KCl) in a 500 mL flask. The culture was grown at 18°C at 150-250 rpm for 19-50 hours until the A<sub>600</sub> was between 0.4-0.8. The flask was immediately put on ice and incubated for 10 mins. The culture was then transferred into GSA bottles, centrifuged at 10,000 g for 15 mins at 4°C, and supernatant discarded. The pellet was resuspended in 17 mL (1/3 volume of the SOB medium) of ice-chilled transformation buffer (TB) (10 mM PIPES, 15 mM CaCl<sub>2</sub>.2H<sub>2</sub>O, ad 250 mM KCl) and incubated on ice for 10 mins. Next, the solution was centrifuged at 10,000 g for 15 mins at 4°C, and the supernatant discarded. The pellet was then resuspended in 4 mL (1/12.5 volume of the SOB medium) of ice-chilled TB, 300 µL of DMSO (final concentration 7% (v/v)) added and incubated on ice for 10 mins. The chemically competent *E. coli* cells were then transferred into 1.5 mL Eppendorf tubes in 0.1 mL aliquots, flash frozen in liquid nitrogen, and stored at -80°C.

# 2.4.5 Escherichia coli DH5 $\alpha$ cell transformation

For each transformation, 50  $\mu$ L of chemically competent *E. coli* cells (Method 2.4.4) were thawed on ice. To the cells, 5  $\mu$ L of Gibson assembly mixture (Method 2.4.23) or 1 ng DNA (Method 2.4.3) was added and incubated back on ice for 30 mins. The cells were then heat shocked for 30 secs at 42°C and placed back on ice for 2 mins. To the

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cells, 900  $\mu$ L of SOC stock (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>.7H<sub>2</sub>O, and 20 mM Glucose) was added, and incubated at 37°C with shaking at 225 rpm for 1 hour. Typically, the cells were plated out as 30  $\mu$ L and 270  $\mu$ L aliquots onto LB with 150  $\mu$ g/mL ampicillin or kanamycin (Method 2.4.2).

#### 2.4.6 Escherichia coli glycerol stocks

A single colony was spread out across a LB plate (with or without antibiotic selection) so that a lawn of bacterial growth can establish (Method 2.4.2). To the plate, 5 mL of 50% (v/v) glycerol was added, and colonies scrapped off the plate surface using a glass rod. The glycerol solution containing the suspended cells was then collected using a pipette, transferred into Nunc tubes, and stored at -80°C.

#### 2.4.7 Escherichia coli plasmid design

Plasmids were designed in silico using the MacVector software system, with primers designed using the in-software application (Length: 18-25 bp, GC%: 45-55 %, and Tm: 55-80°C, 2 bp GC clamp, and maximum Tm difference between primers of 4°C)

#### 2.4.8 Epichloë festucae protoplast preparation

For each strain, 50 mL of a 4-6 day old mycelia in liquid culture (Method 2.3.1) was filtered through a funnel containing a nappy liner. The collected mycelia was initially washed with 200-300 mL of MQ water, then equilibrated using 50-100 mL of OM buffer (1.2 M MgSO<sub>4</sub> and 10 mM NaHPO<sub>4</sub>, at pH 5.8 using NaH<sub>2</sub>PO<sub>4</sub>), and dried with paper towels. Into pre-weighed flasks, 4 g lots of wet mycelia was added along with 40 mL of twice syringe filtered (0.2 µm Ahlstrom Relia Perp) lysing enzyme (10 mg/ml, *Trichoderma harzianum*, Sigma L1412), and incubated at 22°C overnight with shaking at 80 rpm. The next day, the presence of the protoplasts were checked under the microscope using a haemocytometer. The protoplasts from identical flasks were then collectively filtered through a funnel containing a nappy liner and collected in a 200 mL Schott bottle. Next, 5 mL aliquots of the protoplast solution were transferred into 15 mL Falcon tubes, and carefully overlayed with 2 mL of ST buffer (0.6 M sorbitol, and 100 mM Tris-HCL, at pH 8.0). The tubes were then centrifuged at 20,000 g for 5 mins at 4°C. The protoplasts (white layer) were removed from the interface along with the top

transparent layer, and transferred to 15 mL Falcon tubes containing 5 mL STC buffer (1 M sorbitol, 50 mM Tris-HCL, and 50 mM CaCl<sub>2</sub>, at pH 8.0). The two solutions were mixed, and centrifuged for 5 mins at 20,000 g at 4°C. The STC buffer rinse step was repeated 3 more times with tubes pooled until the protoplasts were in a single tube. The protoplasts were then resuspended in 500  $\mu$ L of STC buffer, and the concentration calculated using a haemocytometer. To make stocks, the protoplasts were diluted to 1.25 x 10<sup>8</sup> protoplast/mL in STC buffer, and 80  $\mu$ L of this protoplast solution was added to 20  $\mu$ L 40% (v/v) PEG aliquots, which were kept at -80°C.

#### 2.4.9 Epichloë festucae protoplast transformation

For each transformation, 50  $\mu$ L of the appropriate protoplasts (Method 2.4.8) were defrosted on ice. To the protoplasts, 2  $\mu$ L of spermidine (7.11 mg/mL) and 5  $\mu$ L of heparin (5 mg/mL) were added along with 1.0-2.5  $\mu$ g of DNA (in no more than 30  $\mu$ L), mixed by pipetting, and incubated on ice for 30 mins. To the mixture, 900  $\mu$ L of 40% (v/v) PEG was added, mixed by pipetting, and incubated back on ice for 20 mins. Then, 50  $\mu$ L of this mixture was added to 3 mL of 0.8% (w/v) regeneration medium (RG) (2.4% PD (w/v), 0.8 M sucrose, and 0.8% (w/v) agar, at pH 6.5) previously heated to 50°C, mixed by pipetting, and poured on to plates containing 1.5% (w/v) RG (2.4% PD (w/v), 0.8 M sucrose, and 1.5% (w/v) agar, at pH 6.5). The plates were incubated overnight at 22°C, and then overlayed with 5 mL of 0.8% (w/v) RG containing the antibiotic of choice. Transformants were selected on final concentrations of hygromycin at 150  $\mu$ g/mL and geneticin at 100  $\mu$ g/mL. The plates were then incubated for a further 7-14 days at 22°C, with 100-200 transformants picked onto PD agar plates containing the antibiotic over this time period. Each transformant was nuclear purified three times.

#### 2.4.10 Epichloë festucae spore isolation

*E. festucae* was subcultured onto PD plates, with each plate containing 5 colonies, and grown until the colonies were 1.5-2 cm in diameter *i.e.* approximately 10 days (Method 2.3.1). Next, 2 mL of PD broth was added to the plate and scrubbed with a glass rod. This PD spore suspension was washed over the colonies, and scrubbed several times to increase the spore concentration, before being removed, and filtered through a 1 mL

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tip containing glass wool into a 1.5 mL Eppendorf tube. The spore stock was then plated out on to PD plates to obtain single spore purified colonies (Method 2.3.1).

#### 2.4.11 Lolium perenne growth conditions

Wild type (WT) endophyte infected ryegrass seeds (Method 2.3.12) were germinated in seedling trays containing non-fungicidal soil (provided by AgResearch) in a PC1 glasshouse. Ryegrass seedlings infected with mutant endophyte strains (Method 2.4.13) were planted to root trainers containing non-fungicidal soil (provided by AgResearch) in a PC2 glasshouse. All seedlings were top watered until a root system was established and then bottom watered. The presence of the endophyte was detected by immunoblotting (Method 2.4.14).

#### 2.4.12 Lolium perenne seed sterilisation

Uninfected Samson *L. perenne* seeds (Method 2.3.12) were soaked in 50% (v/v)  $H_2SO_4$  for 30 mins and rinsed 3 times with MQ water. The seeds were then soaked in 50% (v/v) chlorine bleach for 30 mins and rinsed 3 times with MQ water. The seeds were then spread onto filter paper lined petri dishes and air-dried in a laminar flow cabinet. The seeds were then kept in these containers at 4°C until require for plant inoculations (Method 2.4.13).

# 2.4.13 Lolium perenne seedling inoculation

Sterilised uninfected Samson *L. perenne* seeds (Method 2.4.12) were placed right side up and with the shoot end of the seed pointing upwards onto 3% (w/v) WA plates. The plates were placed in a light tight container and positioned so that the seed shoot end was upwards and incubated in the dark at 22°C for 7 days. The meristems of the seedlings were then inoculated with 7-day old endophyte mycelia from PD agar plates (without selection) (Method 2.4.11), by making an incision into the meristem with a staple blade under a dissection microscope and inserting mycelia into the cut. The seedlings were then placed back into the light tight container and incubated for a further 7 days, again with the seeds positioned with shoot upright. The seedlings were then exposed to light for a further 7 days before being planted into root trainers (Method 2.4.11).

#### 2.4.14 Epichloë festucae infected Lolium perenne plant immunoblotting

Immunoblotting (Simpson et al., 2012) was performed on plants from inoculated seedlings (Method 2.4.13) or plants from endophyte infected seeds (Method 2.3.12 and 2.4.11). Each tiller was cut with a scalpel horizontally at the base, as close to the soil as possible without contaminating the newly exposed surface. The freshly cut detached tiller end was then blotted onto a nitrocellulose membrane (0.45  $\mu$ m). The membrane was then incubated in milk protein blocking solution (BS) (20 mM Tris (hydroxymethyl) methylamine, 50 mM NaCl, 0.5% (w/v) non-fat milk powder, pH 7.5) to coat any non-protein bound areas, for at least 2 hours on an orbital shaker at room temperature. The BS was then removed, and the membrane was rinsed twice with fresh BS. Primary antibody (polyclonal rabbit anti-endophyte produced at AgResearch in conjunction with Massey University's Small Animal Production Unit) (25 ml in 25 ml BS) was then incubated with the membrane on an orbital shaker overnight at 4°C. The antibody solution was removed, and the membrane washed twice with BS. Secondary antibody (goat anti-rabbit IgG-AP, sc-2034, Santa Cruz Biotechnology, USA) (6.25 mL in 25 ml BS) was then incubated with the membrane on an orbital shaker for at least 2 hours at room temperature. The antibody solution was removed, and the membrane washed twice with BS. Chromogens were prepared as manufacturer's instructions (SIGMAFAST<sup>™</sup> Fast Red TR/Naphthol AS-MX). The chromogen solution was then incubated with the membrane on an orbital shaker for 15 mins or until red colour developed on control positive blot, at room temperature. Development was then stopped by rinsing the membrane three times in MQ water.

#### 2.4.15 Epichloë festucae infected Lolium perenne plant macroscopy

Mycelia plugs were gown on PD plates next to each other at 22°C for 7 days (Method 2.3.1), with colony morphology compared in size, shape, colour, and texture.

#### 2.4.16 Epichloë festucae infected Lolium perenne plant microscopy

Microscopy slides were placed onto 1.5% (w/v) water agar (WA) plates and then overlayed with 1.5% (w/v) WA. Mycelia plugs of *E. festucae* grown on 3% (w/v) WA plates for 5-7 days at 22°C were placed on these pre-prepared plates so that the mycelia were on the surface of the agar directly above the edge of the embedded

slide, and grown for 5-7 days at 22°C. A block of the newly formed mycelia was cut and place on a new slide, stained with 4-5 μL of Calcofluor white (3 mg/mL) (fluorescent Brighter 28; Sigma), and covered with a cover slip. The slide was imaged using an Epifluorescence microscopy (Olympus IX83) under 40× oil immersion objective lens, NA=0.075, outfitted with U-MWUS filter tubes for Calcofluor white. Images were captured with Retiga 600M (QImaging) camera using a Bin2X2. Images were produced with ImageJ software (NIH).

# 2.4.17 DNA gel extraction

DNA was extracted from agarose gel electrophoresis (Method 2.4.22) using the Wizard SV Gel and PCR Clean-up System (Promega) as instructed by the manufacturer.

# 2.4.18 DNA column purification

DNA was purified from PCR solutions (Method 2.4.21) using the QIAquick PCR Purification Kit (Qiagen) as instructed by the manufacturer

# 2.4.19 DNA Qubit

DNA was quantified using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific). For each sample, 199  $\mu$ L or 190  $\mu$ L of working solution (1  $\mu$ L broad range double stranded DNA reagent and 199  $\mu$ L of corresponding buffer) was added to 1  $\mu$ L of DNA solution or 10  $\mu$ L of either standard 1 or 2 (used for calibration) respectively, and incubated at room temperature for 2 minutes, before being measured on the Qubit using the correct settings for the chosen reagent.

# 2.4.20 DNA Nanophotometer

For each DNA sample, 3.5  $\mu$ L of DNA solution was analysed using lid factor 10 (for concentrations between 15-800 ng/ $\mu$ L) and factor 50 (for double stranded DNA). Readings of 260/280 and 260/230 were taken along with the concentration (ng/ $\mu$ L).

#### 2.4.21 PCR

Taq DNA polymerase (Roch) or Q5  $^{\circ}$  DNA polymerase (New England Biolabs, inc.) reaction mixture were used as instructed by the manufacturer, with the addition of 2% (v/v) DMSO when required.

#### 2.4.22 DNA agarose gel electrophoresis

Gels were prepared using 0.8% or 1.5% (w/v) agarose in 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na<sub>2</sub>EDTA) were loaded with DNA samples pre-mixed with 5:1 of DNA loading dye (20% (w/v) sucrose, 5 mM EDTA, 1% (w/v) SDS, and 2% (w/v) bromophenol blue) alongside 1 Kb plus ladder (Thermo Fisher Scientific). The samples were then run using a Molecular Imager<sup>®</sup> Gel Doc<sup>TM</sup> XR + System, stained in ethidium bromide (1 µg/mL) solution for 15-30 mins, and de-stained in MQ for 10-15 mins before imaging using a UV transilluminator Gel Documentation System (Bio-Rad).

#### 2.4.23 Gibson assembly

For each assembly, 10  $\mu$ L 2x Gibson assembly master mix (4  $\mu$ L of 5x Isothermal buffer, 0.08  $\mu$ L of T5 exonuclease, 0.25  $\mu$ L of Phusion polymerase, 2  $\mu$ L Taq ligase, and 3.67  $\mu$ L MQ water) was thawed on ice. To this, 50-100 ng of linear vector backbone along with 3-fold excess of each insert fragment, using the relative molar concentrations, was added, and made up to 20  $\mu$ L with MQ water. The fragments were designed to have a 15 bp overlap between neighbouring fragments with 100% bp matching and were generated using PCR (Method 2.4.21) followed by column purification (Method 2.4.18). This mixture was incubated at 50°C for 60 mins in a thermo cycler. The mixture can be used straight away or stored at 4°C. The Gibson assembly mixture is then transformed into *E. coli* (Method 2.4.5)

#### 2.4.24 Clone checker

Single colonies from the Gibson assembly mixture transformed *E. coli* plates (Method 2.4.5) were picked, and resuspended in individual PCR tubes containing 6  $\mu$ L of LB. Next, 3  $\mu$ L of this suspension was transferred to PCR tubes containing 8  $\mu$ L of green clone checker solution and heated to 100°C for 30 secs using a thermo cycler. Once the tube had cooled, 1  $\mu$ L of the restriction enzyme, chosen based on the plasmid *in silico* 

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restriction map (Method 2.4.7), and 1  $\mu$ L of the corresponding restriction enzyme buffer was added to the tube, and incubated at the appropriate restriction enzyme temperature for 30 mins. The samples were then analysed using gel electrophoresis (Method 2.4.22). The remaining 3  $\mu$ L of LB suspension can be used to start cultures (Method 2.4.2) for plasmid extractions (Method 2.4.3).

# 2.4.25 Restriction analysis

The amount of enzyme used depended on the amount of DNA digested and was calculated based on the assumption that 1 enzyme unit can digest 1  $\mu$ g of DNA in 1 hour in 50  $\mu$ L therefore. The enzyme was chosen based on the restriction map (Method 2.4.7) with the buffer type and temperature recommended by manufacturer.

# 2.4.26 Epichloë festucae Fg1 mcf whole gene replacement construct

For the generation (Method 2.4.7) of *mcf* whole gene replacement construct (pTM03, appendix 23), *mcf* left-boarder (LB) (TM40/TM50) and *mcf* right-boarder (RB) (TM51/TM52) were amplified (Method 2.4.21) from Fg1 WT genomic DNA (Method 2.3.3), and the hph cassette (hph\_F/DB33.7) and backbone (pRS426\_F/pRS426-R) amplified from pAN7-1 (Appendix 25 and Method 2.4.3). All the products (RB 1428 bp, LB 1394 bp, hph 2185 bp, and backbone 2591 bp) were purified (Method 2.4.17), assembled using Gibson assembly (Method 2.4.23), and sequenced (Method 2.3.16).

# 2.4.27 Epichloë festucae Fg1 mcf 5' gene replacement construct

For the generation (Method 2.4.7) of *mcf* 5' gene replacement construct (pTM05, appendix 24), *mcf* LB (TM70/TM71) and *mcf* RB (TM72/TM73) were amplified (Method 2.4.21) from Fg1 WT genomic DNA (Method 2.3.3), and the hph cassette (hph\_F/DB33.7) and backbone (pRS426\_F/pRS426-R) amplified from pAN7-1 (Appendix 25 and Method 2.4.3). All the products (RB 1911 bp, LB 2095 bp, hph 2185 bp, and backbone 2591 bp) were purified (Method 2.4.17), assembled using Gibson assembly (Method 2.4.23), and sequenced (Method 2.3.16).

#### 2.4.28 Epichloë festucae var Iolii AR48 mcf gene complementation construct

For the generation (Method 2.4.7) of *mcf* gene complementation construct (pTM04, appendix 27), AR48 *mcf* open reading frame (ORF) (TM49/TM52) was amplified (Method 2.4.21) from AR48 WT genomic DNA (Method 2.3.3), and backbone (pRS426\_F/pRS426-R) amplified from pAN7-1 (Appendix 25 and Method 2.4.3). All the products (AR49 mcf ORF 11786 bp and backbone 2591 bp) were purified (Method 2.4.17), assembled using Gibson assembly (Method 2.4.23), and sequenced (Method 2.3.16).

#### 2.4.29 Epichloë festucae var Iolii AR48 idtP gene complementation construct

For the generation (Method 2.4.7) of *idtP* gene complementation construct (pTM06, appendix 10); *idtP* (TM76/TM77) was amplified (Method 2.4.21) from AR48 WT genomic DNA (Method 2.3.3), PpaxM (TM74/TM75) and TpaxM (TM78/TM79) from pDB05 (Appendix 16 and Method 2.4.3) and backbone (TM80/TM81) from pDB49 (Appendix 12 and Method 2.4.3). All the products (*idtP* 1907 bp, PpaxM 1278 bp, TpaxM 1322 bp, and backbone 7508 bp) were purified (Method 2.4.17), assembled using Gibson assembly (Method 2.4.23), and sequenced (Method 2.3.16).

#### 2.4.30 Epichloë festucae var Iolii AR48 idtQ gene complementation construct

For the generation (Method 2.4.7) of *idtQ* gene complementation construct (pTM07, appendix 14); *idtQ* (TM83/TM84) was amplified (Method 2.4.21) from AR48 WT genomic DNA (Method 2.3.3), PpaxM (TM74/TM82) and TpaxM (TM85/TM79) from pDB05 (Appendix 16 and Method 2.4.3) and backbone (TM80/TM81) from pDB49 (Appendix 12 and Method 2.4.3). All the products (*idtQ* 2060 bp, PpaxM 1278 bp, TpaxM 1322 bp, and backbone 7508 bp) were purified (Method 2.4.17), assembled using Gibson assembly (Method 2.4.23), and sequenced (Method 2.3.16).

# 2.4.31 DIG probe preparation for Southern blotting

DNA was prepared by purifying 1  $\mu$ g of linearized DNA produced either by PCR (Method 2.4.21 and 2.4.18) or restriction digest of a plasmid (Method 2.4.25. 2.4.22, and 2.4.17). The volume was adjusted to 16  $\mu$ L with MQ water and the DNA denatured by heating at 100°C for 5 mins and cooled on ice. Then, 4  $\mu$ L of DIG-High Prime (DIG

High Prime DNA Labelling and Detection Starter Kit-Roch) was added, and the mixture incubated overnight at 37°C. The reaction was stopped by adding EDTA (final concentration 20 mM pH 8.0) and heated at 65°C for 10 mins. This resulted in approximately 100 ng/ $\mu$ L of DIG labelled DNA probe.

#### 2.4.32 Genomic DNA preparation for Southern blotting

Genomic DNA was extracted from *E. festucae* mycelia using the small scale Byrd method (Method 2.3.3), and the concentration quantified using the Qubit (Method 2.4.19). For each restriction digest of each sample,  $1 \mu g$  of this genomic DNA was added to a 1.5 mL Eppendorf tube along with 20 U restriction enzyme (chosen based on the plasmid and gene in silico restriction map (Method 2.4.7)), 1x restriction buffer (recommended by the manufacturer), 10  $\mu$ g of Rnase A, and MQ water up to 100  $\mu$ L. The tube was then incubated at the appropriate temperature for the chosen restriction enzyme (recommended by the manufacturer) overnight. Before stopping the reaction, 5 µL of the reaction was analysed using gel electrophoresis (Method 2.4.22) to check the extent of the DNA digestion. Once satisfied the reaction was complete, the restriction reaction was stopped by adding 0.3 mM NaOAc (pH 5.2) and 2.5% (v/v) of 100%(v/v) ethanol and incubated at 25°C for at least 3 hours or overnight. The DNA was pelleted by centrifuging at 17,000 g for 15 mins at 4°C, and supernatant discarded. The DNA was then washed with 300  $\mu$ L 70% (v/v) ethanol, centrifuging at 17,000 g for 5 mins, air dried, and re-suspended in a mixture of 16  $\mu$ L of MQ water and 4  $\mu$ L SDS loading dye.

#### 2.4.33 Gel electrophoresis for Southern blotting

A 0.8% (w/v) agarose gel was loaded with prepared DNA (Method 2.4.32) alongside 20  $\mu$ L of 1 Kb plus ladder (Thermo Fisher Scientific). The gel was then run at 80 V for 3-5 mins or until the samples had entered the gel, and then 30 V overnight. With the gel kept in the cast, the gel was stained with ethidium bromide, washed in MQ water, and image taken with and without a ruler as a sizing reference (Method 2.4.22).

#### 2.4.34 DNA blotting for Southern blotting

The gel (Method 2.4.33) was washed by agitation in solution 1 (0.25 M HCl) for 15 mins, and the solution removed. Then the gel was washed in solution 2 (0.5 NaOH and 0.5 M NaCl) by agitation for 45 mins, and the solution removed. Next, the gel was washed in solution 3 (0.5 M Tris pH 7.4, and 2 M NaCl) with agitation for 60 mins, and solution removed. Finally, the gel was washed in 2x SSC (0.3 M NaCl, and 30 mM Na citrate) with agitation for 2 mins. The blot was then set up as follows: into the gel stand 20x SSC (3 M NaCl, and 0.3 mM Na citrate) buffer was placed into the two side wells, a 3MM Whatman (MM) sheets placed on top (cut slightly larger than the blotting stand so that the MM sides dipped into each well), and gladwrap added over the wells but not the gel blotting stand. The gel was then placed onto the blotting stand, 2x SSC wetted nylon membrane (cut to gel size and marked for orientation) placed on top, then 2 sheets of 2x SSC wetted MM sheets (cut to gel size) on top of the membrane, then 2 dry MM sheets (cut to gel size) on top of the two wetted MM sheets, and lastly a stack of paper towels weighted down with 1 L bottle filled with water. This was left at room temperature overnight to transfer that DNA from the gel to membrane through capillary action. The membrane was then dried between MM sheets, and DNA linked to the nylon membrane by UV irradiation for 2 min using CEX-800 UV cross-linker.

#### 2.4.35 Hybridization of the probe for Southern blotting

The DIG Easy Hyb machine was pre-heated to the appropriate temperature, calculated by Tm = 49.82 + 0.41 x (% G + C) – (600 / I) with I = length of probe in bp. Topt = Tm – 25. The probe (Method 2.4.31) was denatured by heating at 100°C for 5 mins and cooled on ice. The membrane (Method 2.4.34), washed in 10 mL prehybridisation solution (3 fold dilution of DIG Easy Hyb Granules at 37°C-DIG High Prime DNA Labelling and Detection Starter Kit-Roch) for 1 hour with gentle agitation in a PYRX<sup>TM</sup> hybridization tube in the DIG Easy Hyb machine, and solution removed. To the PYRX<sup>TM</sup> hybirdisation tube containing the membrane, 0.06% (v/v) denatured probe (prehybridisation solution DIG High Prime DNA Labelling and Detection Starter Kit-Roch) was added and incubated overnight with gentle agitation in the DIG Easy Hyb machine.

#### 2.4.36 Stringency washes for Southern blotting

The membrane (Method 2.4.35) was incubated in 250 mL of 2x SSC (50 mL 20x SCC (3 M NaCl, and 0.3 mM Na citrate), 5 mL 10% (w/v) SDS, and 445 mL MQ water) for 5 mins with gentle agitation. This step was repeated. The membrane was then incubated in 250 mL of 0.5x SSC (12.5 mL 20x SCC (3 M NaCl, and 0.3 mM Na citrate), 5 mL 10% (w/v) SDS, and 482.5 mL MQ water pre-heated to 70°C) for 15 mins with gentle agitation. This step was repeated.

# 2.4.37 Immunological detection for Southern blotting

The membrane (Method 24.36) was incubated in 100 mL washing buffer (100 mL maleic acid buffer (0.1 M maleic acid, and 0.15 M NaCl, pH 7.5), and 1.5 mL Tween 20) with agitation for 5 mins, and solution removed. Then the membrane was incubated in 50 mL blocking solution (5 mL blocking solution, and 45 mL maleic acid buffer) with agitation for 30 mins, and solution removed. Next, the membrane was incubated in 10 mL antibody solution (2  $\mu$ L Anti-Digoxigenin-AP (DIG High Prime DNA Labelling and Detection Starter Kit-Roch), and 10 mL blocking solution) with agitation for 30 mins, and solution removed. Solution) with agitation for 30 mins, and solution removed. Finally, the membrane was incubated in 200 mL washing buffer solution with agitation for 15 mins two times, followed by 100 mL of detection buffer (0.1 M Tris, and 0.1 NaCl, pH 9.5) with agitation for 5 mins. The membrane is then developed without agitation using 10 mL colour substrate solution (100  $\mu$ L NBT/BCIP stock solution (DIG High Prime DNA Labelling and Detection Starter Kit-Roch), and 5 mL detection buffer) in the dark. Typically, bands can be seen within 6 hours, and stopped using 100 mL MQ water. The membrane was air dried and stored in a dry dark place.

# 2.5 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass

#### 2.5.1 Stem boring fly whole plant choice trial plant preparation

Seeds from the Margot Forde Germplasm Centre (Method 2.3.12) infected with a range of endophyte strains were germinated (Method 2.4.11) and then at 6 weeks immunoblotted to check for the presence of endophytes (Method 2.4.14). In preparation for the insect trial, 20 replications worth of plants were selected, with

each replication containing one ryegrass plant infected with AR48, AR47, AR1, AR37, CT, or nil endophytes, and tillers trimmed to a maximum height of 15 cm. Both the position of each plant in each replication and the position of each replication within the glasshouse were randomised.

#### 2.5.2 Stem boring fly preparation

Stem boring fly preparation (SBF-*Cerodontha australis*) were collected from ryegrass fields at Grasslands AgResearch by sweeping the grass surface with a net and transferring the insects into containers. At Massey University Palmerston North, CO<sub>2</sub> gas was used to sedate the insects and, using a dissection microscope, the SBF were sorted out. SBF were identified by their grey thorax, yellow legs, yellow frons, black notopleura, and 3rd antennae segment (Introduction 1.5.1). The SBF were re-sedated and sorted into groups of 20 females (x 20) and 20 males (x 20) SBF and transferred into Eppendorf tubes. Females SBF were identified by the lower abdomen being larger, pointier, and black (Figure 1.15 and 1.16).

# 2.5.3 Stem boring fly whole plant choice trial

Two Eppendorf tubes (one containing female SBF and one containing male SBF) (Method 2.5.2) were added to the plants (now covered by a fine nylon mesh bag) (Method 2.5.1), tubes open, and bags tied closed. The adult SBF are therefore not naïve, though most likely exposed to ryegrass as a major food source, but the eggs, larva, and, pupa are naïve and only exposed to ryegrass. The plants were then bottom watered for 6 weeks. At the end of the trial the number of mines, presence of a pupa, and resultant fly emergence was recorded for each plant. One-way analysis of variance (ANOVA) was carried out on average mines per tiller (%), average pupa per mine (%), and average fly per pupa (%) to examine the significance of endophyte differences. The analyses were conducted using GenStat 17th edition (VSN International).

# 2.5.4 Cutworm moth caterpillar whole plant choice trial plant preparation

Seeds from the Margot Forde Germplasm Centre (Method 2.3.12) infected with a range of endophyte strains were germinated (Method 2.4.11) and then at 6 weeks immunoblotted to check for the presence of endophytes (Method 2.4.14).

Alternatively, endophytes were inoculated in to ryegrass seedlings (Method 2.4.13), which were then planted (Method 2.4.11), and at 6 weeks plant tissue was analysed by immunoblot to check for the presence of endophytes (Method 2.4.14). In preparation for the insect trial, 20 replications worth of plants were selected, with each replication containing one ryegrass plant infected with AR48, AR47, AR1, AR37, Fl1, Fg1, CT, or nil endophytes, and tillers trimmed to a maximum height of 15 cm and tillers counted. Both the position of each plant in each replication and the position of each replication within the glasshouse were randomised

#### 2.5.5 Cutworm moth caterpillar preparation

Cutworm moth (CM-Agrotis ipsilon) were caught using a light trap, placed in a container containing dock leaves and moist paper towels, and left to lay eggs over 1-2 weeks. Once hatched, the cutworm moth caterpillar (CC-Agrotis ipsilon) were kept in these containers, and the detached dock leaves were replaced every 2-3 days or when needed. After 1 week, the caterpillars were moved to new containers containing detached dock leaves and fed on detached dock leaves for 2-3 weeks or until the star of the CWPC trial. The containers were kept in a shaded area in the same glasshouse as the ryegrass plants at AgResearch.

#### 2.5.6 Cutworm moth caterpillar whole plant choice trial

In to each replication, 40 caterpillars (Method 2.5.5) were added to the plants (Method 2.5.4) and covered by a fine nylon bag. The CC are therefore not naïve as the initial food source was dock leaves (Method 3.5.5) rather than the tested plant source which was ryegrass. The plants were then bottom watered for 2 weeks. At the end period the number of tillers, and score (5= No damage, 4= 25% damage, 3= 50% damage, 2= 75% damage, and 1= Dead) was recorded for each plant. One-way analysis of variance (ANOVA) was carried out on average difference in tiller number and average plant visual score using GenStat 17th edition (VSN International) to examine the significance of endophyte differences.

# 2.5.7 Cutworm moth or porina caterpillar detached tiller no choice trial plant preparation

Seeds from the Margot Forde Germplasm Centre (Method 2.3.12) infected with a range of endophyte strains were germinated (Method 2.4.11) and then at 6 weeks immunoblotted to check for the presence of endophytes (Method 2.4.14). Alternatively, endophytes were inoculated in to ryegrass seedlings (Method 2.4.13), which were then planted (Method 2.4.11), and at 6 weeks plant tissue was analysed by immunoblot to check for the presence of endophytes (Method 2.4.14).

#### 2.5.8 Porina caterpillar preparation

Porina moths (PM-Wiseana spp) were caught using a light trap, placed in a container containing artificial diet (2.5% (w/v) agar, and 1% (w/v) freeze-dried endophyte free ryegrass up to 1 L carrot juice) and moist paper towels, and left to lay eggs over 1-2 weeks. Once hatched, the porina caterpillars (PC-Wiseana spp) were kept in these containers, and the artificial diet was replaced every 2-3 days or when needed. After 1 week, the caterpillars were moved to new containers containing artificial diet and bark, where the caterpillars remained for 2-3 weeks or until the start of the PDTN trial. The artificial diet was made fresh every week and stored at 4 °C during the week. The containers were kept in a temperature controlled cabinet at 18°C. The CC (Method 2.5.5) or PC were weighed before being placed into separate yellow screw top containers that were filled 34 of the way with bark. After being ranked by weight, the caterpillars to be used for the trial were selected so that there was the smallest weight difference between the lightest and heaviest. Selected caterpillars were then assigned an endophyte strain in a repeating order so that each endophyte was assigned caterpillars from each weight range. These were then randomly distributed based on endophyte label in an air-conditioned laboratory (with light) for the CDTN or an 18°C incubator (without light) for the PDTN and starved overnight.

#### 2.5.9 Cutworm moth or porina caterpillar detached tiller no choice trial

The CC are not naïve as the initial food source was dock leaves (Method 3.5.5) rather than the tested plant source which was ryegrass. The PC are also not naïve as the initial food source though contained ryegrass also contained agar and carrot juice

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(Method 3.5.8) rather than the tested plant source which was only ryegrass. Each day, the caterpillars were weighed, and fed the pre-weighed ryegrass tiller. Any uneaten tillers were removed and weighed. This was done until 8 tillers (CC) or 10 tillers (PC) had been added and removed from the containers. One-way analysis of variance (ANOVA) and repeated measurements linear mixed model was carried out on CC/PC survival and CC/PC weight using GenStat 17th edition (VSN International) to examine the significance of endophyte differences.

# 2.5.10 Light brown apple moth caterpillar artificial diet no choice trial plant preparation

Seeds from the Margot Forde Germplasm Centre (Method 2.3.12) infected with a range of endophyte strains were germinated (Method 2.4.11) and then at 6 weeks immunoblotted to check for the presence of endophytes (Method 2.4.14). Alternatively, endophytes were inoculated in to ryegrass seedlings (Method 2.4.13), which were then planted (Method 2.4.11), and at 6 weeks plant tissue was analysed by immunoblot to check for the presence of endophytes (Method 2.4.14). The grass was then harvested, freeze-dried, and milled before being added to the artificial diet (2.5% (w/v) agar, and 1% (w/v) freeze-dried endophyte infected or free ryegrass up to 1 L carrot juice).

# 2.5.11 Light brown apple moth caterpillar preparation

The light brown apple moth caterpillars (LBAM- *Epiphyas postvittana*) were ordered from Plant and Food New Zealand a week before the start of the trial. Day old LBAM were added to separate yellow screw top containers and assigned an endophyte strain. The caterpillars were then randomly distributed based on endophyte label in an 18°C incubator (without light).

# 2.5.12 Light brown apple moth caterpillar artificial diet no choice trial

On day one of each week, artificial diet (2.5% (w/v) agar, and 1% (w/v) freeze-dried endophyte infected or free ryegrass up to 1 L carrot juice) was added to the containers (Method 2.5.11) and on day 4 of each week the diet was replaced. The LBAM are therefore naïve as they are only exposed to the tested food source. At the start of each

week the artificial diet was made fresh and stored at 4°C during the week. LBMA establishment (production of silk cocoon), and moulting times (head colour and size change) were recorded over this time period. One-way analysis of variance (ANOVA) was carried out on caterpillar establishment times and moulting times using GenStat 17th edition (VSN International) to examine the significance of endophyte differences

# **3.0 RESULTS**

## 3.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1

Secondary metabolites (SMs) are the main source of bioprotective compounds produced by fungi. In *Epichloë,* four SM pathways have been identified as producing bioactive compounds that target insects; lolitrem B, ergovaline, loline, and peramine (Introduction 1.2.6). These pathways were analysed in the *E. festucae* var *lolii* asexual strain AR48 that has two new insect bioactivities against the stem boring fly (SBF-*Cerodontha australis*) and cutworm moth caterpillar (CC-*Agrotis ipsilon*) (Introduction 1.1.3). During this analysis the *E. festucae* strain Fg1 was identified as being AR48-like, and important as a model system for genetic manipulation, therefore this strain was also analysed

## 3.1.1 Analysing the functionality and presence of the bioactive secondary metabolite genes in the genome of AR48 and Fg1 through bioinformatics.

The genome of AR48 was sequenced (Method 2.3.16) to gain an insight into which known bioactive SM pathways were present. The final assembly consisted of 1580 scaffolds containing 36 million bp with a GC content of 43%. The average scaffold length was 22,900 bp, with the largest scaffold 238,100 bp, and a scaffold  $N_{50}$  of 46,100 bp (Table 3.1).

Statistics	AR48	Fg1
Number of reads	21 million	14 million
Genome assembly size	36 Mbp	35 Mbp
Fold coverage of	127 fold	250 fold
sequence		
Scaffold number	1580	3769
Scaffold $N_{50}^*$	46,100 bp	70,535 bp
Contig number	4,618	11,141
Contig N <sub>50</sub> *	15,710 bp	19,839 bp
GC%	43%	44%

Table 3.1: Genome assemble statistics of AR48 and Fg1

\* The contig/scaffold length at which 50% of the contig lengths are longer

An analysis of the genome sequence of AR48 identified all eleven indole-diterpene (IDT) genes involved in lolitrem B synthesis (Figure 3.1 and Method 2.3.11). However, both *idtF* and *idtK* each had single base deletions that would result in premature translational termination of the transcripts thereby rendering the genes non-functional (Figure 3.2). For the remaining known alkaloid pathways, no genes were identified from either the ergovaline or loline pathways, though the single gene responsible for peramine production *perA* was identified. Analysis of this gene showed that it contained a 230 bp deletion identical to the *perA* allele in the *E. festucae* sexual strain Fg1 (Figure 3.3). The presence of the same null allele suggests that AR48 and Fg1 have a close ancestry.

To further investigate this ancestry the genome of Fg1 was sequenced (Method 2.3.16). The final assembly consisted of 3769 scaffolds containing 35 million bp with a GC content of 44%. The average scaffold length was 9458 bp, with the largest scaffold 400,034 bp, and a scaffold  $N_{50}$  of 70,535 bp (Table 3.1).

The genome of Fg1 was also analysed for the presence of known bioactive SM genes (Method 2.3.11). As expected, no genes involved in ergovaline synthesis or loline synthesis were identified, but all eleven IDT genes involved in lolitrem B synthesis were present (Figure 3.1). Again *idtF* and *idtK* each had single base deletions, with *idtF* sharing an identical change to AR48, and *idtK* having an alternative deletion closer to the 5' end of the gene (Figure 3.2). The phylogenetic relationship between AR48 and Fg1 was investigated through simple sequence repeat (SSR) analysis using 23 loci (Method 2.3.15). Fg1 and AR48 share a common ancestor that is unique to the AR48 clade, which supports the alkaloid bioinformatics analysis that these strains are closely related (Figure 3.4).

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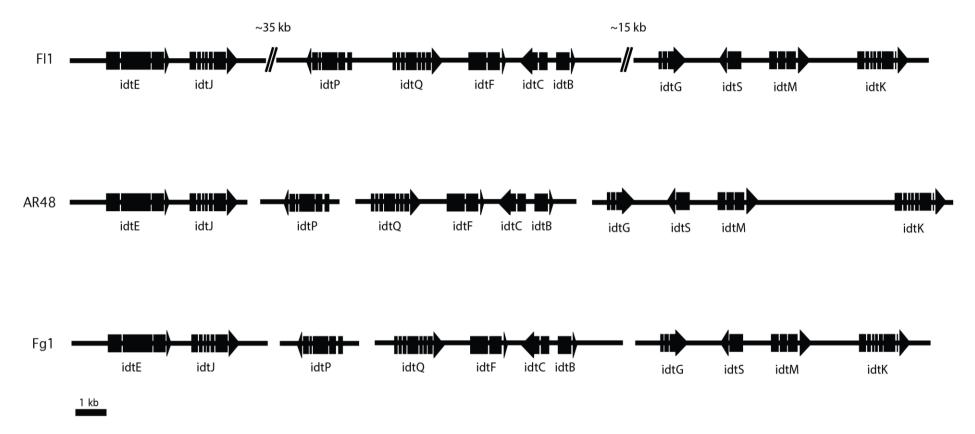


Figure 3.1: Organisation of indole-diterpene (IDT) genes in different *Epichloë festucae* strains. Contigs from strains Fl1, AR48, and Fg1 were annotated in Geneious for genes involved in indole-diterpenes synthesis: cluster 1 contains *idtG*, *idtS*, *idtM*, and *idtK*, cluster 2 contains *idtQ*, *idtF*, *idtC*, and *idtB*, and cluster 3 contains *idtE*, and *idtJ*. While *idtP* was on a separate contig it is likely to be linked to cluster 2 based on the known organization of these genes in strain Fl1. Contigs are represented by horizontal black lines. Genes are represented by black arrows, introns as white boxes and the gene name directly below. Double slashed lines represent large genomic regions of repetitive DNA sequence, with the approximate size of the region shown above.

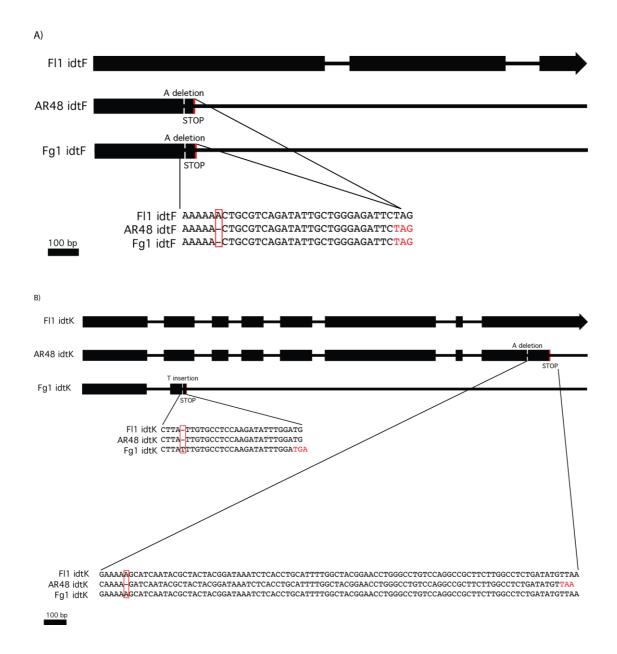


Figure 3.2: Indole-diterpene (IDT) *IdtF* and *idtK* gene alignment in different *Epichloë festucae* strains. Gene sequences of *idtF* (A) or *idtK* (B) from Fl1, AR48, and Fg1 were aligned using Geneious global alignment tool with free end gaps and 65% similarity. Genes are represented by thin black horizontal lines. The transcripts are represented by black arrows, with introns as gaps. The region around the deletion is enlarged, with the missing base represented by a dash, and the deletion highlighted by a red box and the premature stop codon highlighted in red text.

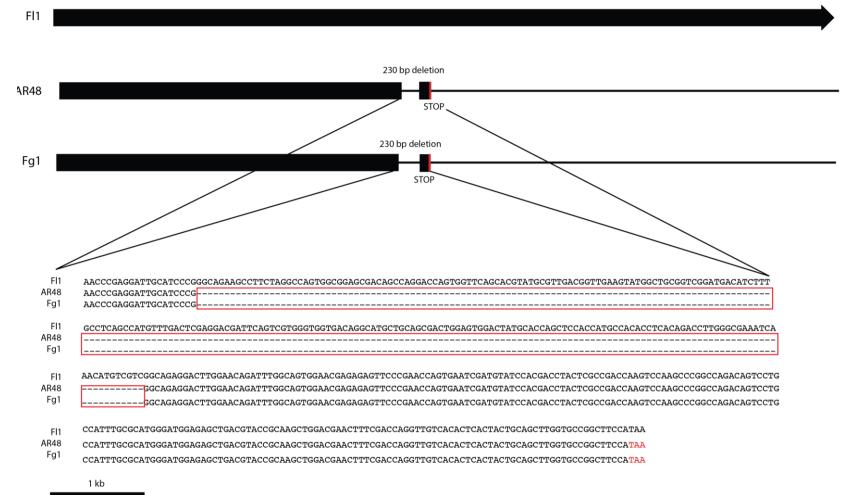


Figure 3.3: *PerA* gene alignment for different *Epichloë festucae* strains. Gene sequences of *perA* from Fl1, AR48, and Fg1 where aligned and translated using Geneious global alignment tool with free end gaps and 65% similarity. Genes are represented by thin black horizontal lines. Transcripts are represented by black arrows, with introns as gaps. The deleted region is enlarged, with missing bases represented by a dash, the deleted region highlighted by a red box and the premature stop codon highlighted in red text

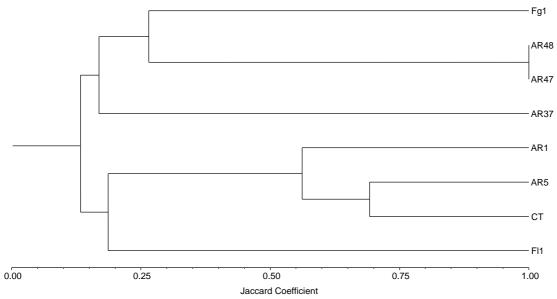


Figure 3.4: Simple sequence repeat (SSR) dendrogram of *Epichloë festucae* strains using 23 loci. Only the key strains used in this study are shown.

The genomes from the *E. festucae* strains E2368, AR1, AR37, and AR5 also contain *idt* genes but are missing *idtE* and *idtJ*, and in addition E2368 does not contain *idtG*, *idtS*, *idtM*, and *idtK* (Method 2.3.11). The encoded Idt proteins from these genomes when aligned to the corresponding proteins from AR48 and Fg1 all had high sequence identity ranging from 99.1%-100%, with the exception of IdtP and IdtQ which dropped to 92.6% and 90.3% respectively. However, the protein sequences of IdtP and IdtQ from AR48 and Fg1 share sequence identity of 99.8% and 99.1% respectively (Figures 3.5, 3.6, and Appendix 1).

The amino acid changes for IdtP and IdtQ for both AR48 and Fg1 sequences were compared to the remaining *E. festucae* corresponding genes (Method 2.3.11). For IdtP there were 34 changes in total, with one non- conservative change specific to Fg1 *i.e.* S226P. Of these shared changes 14/34 (41%) were identified as being non-conservative, and of these non-conservative changes 10/14 (71%) were from polar or charged amino acids to hydrophobic or uncharged amino acids. One of the changes removes a cysteine (C12W), which could affect the tertiary structure of the protein.

E2368 was the only other strain that shared changes with AR48 and Fg1, with 3 of the changes conservative and 3 other changes non-conservative. Many of the changes are located in or near P450 predicted pockets *e.g.* I191H, S208F, and K395I, with most of these changes to more hydrophobic amino acids (Figure 3.5, Appendix 2 and 3, and Method 2.3.11).

For IdtQ, there were 53 changes in total, with two specific to Fg1 *i.e.* S162F (nonconservative) and R532M (conservative) and two specific to AR48 *i.e.* 1177S (nonconservative) and T217I (non-conservative). Of these shared changes 27/49 (55%) were identified as being non-conservative, and of these non-conservative changes 8/27 (29%) were from polar or charged amino acids to hydrophobic or uncharged amino acids, however 8/27 (22%) were from hydrophobic or uncharged to polar or charged amino acids. In contrast to IdtP, one of the changes adds a cysteine *i.e.* C306R, which could affect the tertiary structure of the protein. There was some variability between the remaining *E. festucae* strains but all are conservative amino acid substitutions. E2368 again shared some changes with AR48 and Fg1, with three of the changes conservative and one change non-conservative. Again, many of the changes are located in or near P450 predicted pockets *e.g.* H62Y, T470I, and E484A, with approximately equal balance of polar/charged and hydrophobic/aromatic changes (Figure 3.6, Appendix 2 and 4, and Method 2.3.11).

For both AR48 and Fg1 IdtP and IdtQ the haem binding site that is required for catalytic activity has no amino acid changes in the key positions. Also, the key amino acids in Helices I, J, and K are conserved (Figure 3.5 and 3.6) (McMillan *et al.*, 2003).

[		
AR48	1 MFMLHAV LAS IWLLLWYVVYGTRRKECIPTIRRW	
Fg1	1 MFMLHAVLAS IWLLLWYVVYGTRRKECIPTIRRW	
FI 1	1 MLMLHAVPVGICLLLWYVVYGTKRKECIPTIRRW	PRLLPQFLDRLSYNDHAARLVKHGYE 60
E2368	1 MLMLHAVAVGICLLLWYVVYGTKRKECIPTIRRW	
AR1	1 MLMLHAVPVGICLLLWYVVYGTKRKECIPTIRRW	
AR37		PRLLPQFLDRLSYNDHAARLVKHGYE 60 PRLLPOFLDRLSYNDHAARLVKHGYE 60
AR5	1 MLMLHAVPVGICLLLWYVVYGTKRKECIPTIRRW	PRELPOPEDRESTNDHAARLVNHGTE 60
AR48	61 KHKNQP FRILK I DMDLIVIPLQYALELRAVTSDK	LDPLTASEDDNAGKVTRILLGSELHT 120
Fg1	61 KHKNQPFRLLKIDMDLIVIPLQYALELRAVTSDK	LDPLTASEDDNAGKVTRILLGSELHT 120
FI1	61 KHKNQPFRLLKMDMDLIVIPLQYALELRAVTSDK	
E2368	61 KHKNQPFRLLKMDMDLIVIPLQYALELRAVTSDK	
AR1	61 KHKNQPFRLLKMDMDLIVIPLQYALELRAVTSDK	
AR37	61 KHKNQP FRLLKMDMDLIVIPLQYALELRAVTSDK	
AR5	61 KHKNQPFRLLKMDMDLIVIPLQYALELRAVTSDK	LDPLTASFDDNAGKVTRILLGSELHT 120
AR48	121 RAIQQRLTPRLPQALPVLLDELNHAFGQVLPAS	DGSNAWVSVNPYELVLNLATRAAARL 180
Fg1	121 RAIQQRLTPRLPQALPVLLDELNHAFGQVLPAS	DGSNAWVSVNPYELVLNLATRAAARL 180
FI1	121 RAIQQR LTPK LPQT LPV LLDELNHAF GQV LPAGN	DGSNAWISVNPYELVLNLATRATARL 180
E2368	121 RAIQORLTPKLPQTLPVLLDELNHAFGQVLPAGN	DGSNAWVSVNPYELVLNLATRAAARL 180
ARI	121 RAIQQRLTPKLPQTLPVLLDELNHAFGQVLPAGN	DGSNAWISVNPYELVLNLATRATARL 180
AR37	121 RAIQQR LTPK LPQTLPVLLDELNHAF GQVLPAGN	DGSNAWISVNPYELVLNLATRATARL 180
ARS	121 RAIQORLTPKLPQTLPVLLDELNHAFGQVLPAGN	DGSNAWISVNPYELVLNLATRATARL 180
AR48	181 FVGDPICRNEHFLETTASFSRNTFDTIFTSRTFC	NLFTYYLARWISTAKKAHGQLQYIQN 240
Fg1		NLFTYYLARWIPTAKKAHGQLQYIQN 240
FI1	181 FVGDLICRNEIFLETTASFSRNTFDTISTSRSFC	NLFTHY FARWISTAK EAHGQLQYIQN 240
E2368	181 FVGDLICRNEIFLETTASFSRNTFDTISTSRSFC	NLFTYYFARWISTAKKAHGQLQYIQN 240
AR1	181 FVGDLICRNEIFLETTASFSRNTFDTISTSRSFC	NLFTHY FARWISTAK EAHCQLQYIQN 240
AR37	181 FVGDLICRNEIFLETTASFSRNTFDTISTSRSFC	NLFTHY FARWISTAK EAHGQLQYIQN 240
ARS	181 FVGDLICRNEIFLETTASFSRNTFDTISTSRSFO	NLFTHY FARWISTAK EAHGQLQYIQN 240
AR48	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	RPEALAHRTLGILSMAVVHTTAMAST 300
Fg1	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
FI1	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
E2368	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
AR1	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
AR37	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
ARS	241 LLGSEVQRRKLNSEEKHDDFLQWCTELAVTEDEA	
111111111111		
AR48	301 HILFDMISDNILKESLQKEQQDVLKHGWTEITQQ	TMLDMKQLDSLMRESQRVNPVGEFTF 360
Fg1	301 HILFDMISDNILKESLQKEQQDVLKHGWTEITQO	
FI1	301 HILFDMISDDSLKESLRREQQNVLKHGWTEITQQ	
E2368	301 HILFDMISDDSLKESLRREQQDVLKHGWTEITQQ	
AR1	301 HILFDMISDDSLKESLRREQQNVLKHGWTEITQQ	
AR37 ARS	301 HILFDMISDDSLKESLRREQQNVLKHGWTEITQQ 301 HILFDMISDDSLKESLRREQQNVLKHGWTEITQQ	
rins .	SUT HILF DWISDDSLKESLKKEQQ NVERHOW ETTQC	IMEDINKQ EDSENKESQKINTVEETIE 500
AR48	361 RRIVRERITLSDC FQLQPGQQIAIPAKCINTDST	ILPDAHLFQPFRWLKQSSTAPTSFSN 420
Fg1	361 RRIVRERITLSDG FQLQPGQQIAIPAKCINTDST	I LP DAHLFOP FRWLKOSSTAPTSFSN 420
FI1	361 RRIVRERITLSDGYQLQPGQQIAIPAKCINTDST	
E2368	361 RRIVRERITLSDGYQLQPGQQIAIPAKCINTDST	
AR1	361 RRIVRERITLSDGYQLQPGQQIAIPAKCINTDST	KLSDAHLFQPFRWLKQSGTATTSFSN 420
AR37	361 RRIVRERITLSDGYQLQPGQQIAIPAKCINTDST	
ARS	361 RRIVRERITLSDGYQLQPGQQIAIPAKCINTDST	KLSDAHLFQPFRWLKQSGTSTTSFSN 420
4.0.40	AN FEATNING CEEDVACACACACACACACACACACACACACACACACACACAC	EV DEVIDEEED CODODALLUUCDVILLE
AR48	421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL	
Fg1	421 SSALNLHEGEGRYACPGREIASYMIKAIMSRILL	
FI1 E2368	421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL 421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL	
E2368 AR1	421 SSALNLHFGFGRYACPGRFIASYMIKAIMSKILL 421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL	
ARI AR37	421 SSALNLHFGFGRYACPGRFIASYMIKAIMSKILL 421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL	
ARS	421 SSALNLHFGFGRYACPGRFIASYMIKAIMSRILL	
AR48	481 NRNAVVL FRRLEKTVPVC SGNDRSDRT	507
Fg1	481 NR NAVVL FRR LEKTVPVC SGNDR SDR T	503
		507
FI1	481 NR NAVVLLRRLEKTVTVC	498
E2368	481 NR NA V V L L R R L E K T V T V C	498 498
AR1	481 NR NAVVLLRR LEKTVTVC 481 NR NAVVLLRR LEKTVTVC 481 NR NAVVLLRR LEKTVTVC	498 498 498
E2368	481 NR NA V V L L R R L E K T V T V C	498 498

Figure 3.5: Amino acid sequence alignment of indole-diterpene (IDT) IdtP in *Epichloë festucae* strains AR48, Fg1, Fl1, E2368, AR1, AR37, and AR5 using Geneious. The amino acids are colour coded by similarity to the amino acid in the corresponding position in the remaining sequences, with dark purple as most similar and white as least similar. The amino acid number is labelled either side of the sequence. The sequences within the yellow box are a conserved motif found in Helix I (E/H(T/S)(T/S)(S/T/A)). The sequences within the orange box are conserved amino acids found in Helix J (PE). The cyan box contains conserved sequence motifs found in Helix K (ExxR). The red box contains sequences comprising the haem-binding site (F(G/S)xGx(H/Y)xCxGxx(I/L/F)A) where x represents any amino acid.

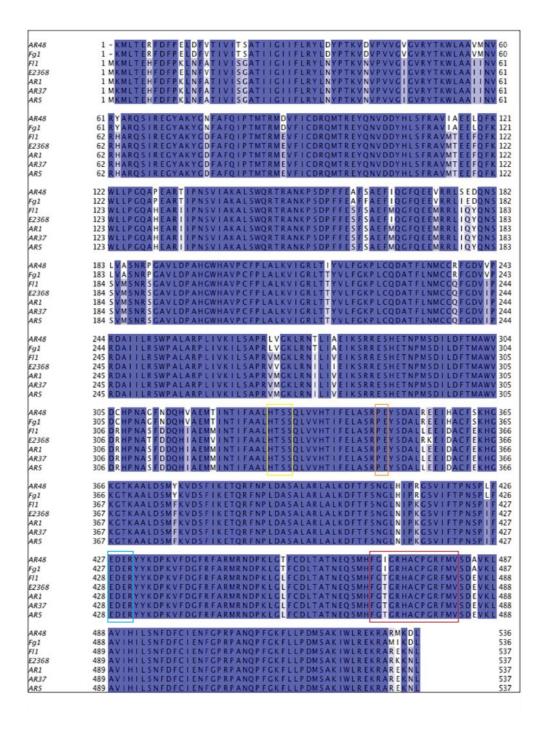


Figure 3.6: Amino acid sequence alignment of indole-diterpene (IDT) IdtQ in *Epichloë festucae* strains AR48, Fg1, Fl1, E2368, AR1, AR37, and AR5 using Geneious. The amino acids are colour coded by similarity to the amino acid in the corresponding position in the remaining sequences, with dark purple as most similar and white as least similar. The amino acid number is labelled either side of the sequence. The sequences within the yellow box are a conserved motif found in Helix I (E/H(T/S)(T/S)(S/T/A)). The sequences within the orange box are conserved amino acids found in Helix J (PE). The cyan box contains conserved sequence motifs found in Helix K (ExxR). The red box contains sequences comprising the haem-binding site (F(G/S)xGx(H/Y)xCxGxx(I/L/F)A) where x represents any amino acid.

## 3.1.2 Detection of the bioactive secondary metabolite compounds in AR48 and Fg1 infected ryegrass through mass spectrometry

IDT compounds were extracted from pseudostem of ryegrass infected with AR48, Fg1, AR1, AR37, and Fl1, and analysed by mass spectrometry (MS) (Method 2.3.17, 2.3.20 and 2.3.22). The control strain FI1 in ryegrass contained all IDT compounds normally observed in the lolitrem B synthetic pathway when using this protocol. This IDT profile was mirrored in the common toxic (CT) infected plant material as expected from previous analysis (unpublished data. Plant material infected with AR1 contained all early- and mid- pathway compounds, e.g. paspaline B, terpendole E, terpendole F, 13desoxypaxilline, and paxitriol, but did not contain any IdtE and IdtJ derived latepathway IDT compounds e.g. lolitriol, lolitrem K, lolitrem J, and lolitrem B. This is due to the absence of *idtE* and *idtJ* genes in the genome of AR1. However, IdtK, and IdtF derived late-pathway IDT compounds were identified as expected due to the presence of *idtK* and *idtJ* genes which are predicted to be functional *e.g.* terpendole M and terpendole C. AR37 and AR1 infected plant materials have a very similar IDT profile due to the absence of *idtE* and *idtJ*, and presence of functional *idtK* and *idtF* genes. However, the presence of the additional functional janD gene enables AR37 to produce epoxy-janthitrem IDT compounds (data not shown) (Table 3.2, Figure 3.11, and Appendix 1). The gene janD (given the epoxy-janthitrem product) is orthologous to *paxD* (prenyl transferase) and is located close to the known IDT cluster in AR37. Additional genes hypothesized to be involved in *epoxy*-janthitrem synthesis have been identified (Richard Johnson, AgResearch; personal communication).

Both AR48 and Fg1 infected material contained all early- and mid- pathway compounds confirming that the early- to mid- pathway enzymes IdtG, IdtM, IdtC, IdtB, IdtP, and IdtQ are functional supporting the bioinformatics analyses. Fg1 infected plant material contains terpendole K and lolitriol indicating that the late-pathway proteins IdtE, and IdtJ, are functional as predicted by bioinformatics. However, AR48 infected plant material, which also has *idtE* and *idtJ* genes predicted to be functional, does not contain either these compounds or any other IdtE/IdtJ derived IDT. Both Fg1 and AR48 infected plant materials do not contain IdtK and IdtF derived IDT as predicted by the bioinformatics analysis, which indicated that the genes responsible for the production

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of terpendole C, lolitrem E, and lolitrem B were non-functional. In addition, no *epoxy*janthitrem IDT compounds were identified as expected due to the absence of *janD* from both genomes (data not shown) (Table 3.2, Figure 3.11, and Appendix 1.1).

## 3.1.3 Analysing the structure of any newly identified compounds through mass spectrometry

During the routine IDT analysis (Method 2.3.20 and 2.3.22) four new IDT compound peaks were identified only in extracts from the AR48 and Fg1 infected plant material; new IDT compound one which is terpendole E-like (438 m/z and 10.3 mins), new IDT compound two which is 13-desoxypaxilline-like (420 m/z and 11.8 mins), new IDT compound three which is paxitriol-like (438 m/z and 19.4 min), and new IDT compound four which is terpendole I-like (454 m/z and 8.7 min). The new IDT compound peaks one, two, and four have marginally different retention times then the corresponding known IDT with the same m/z ratio *i.e.* no more than 0.3 min, while the new IDT compound peak three has a large retention time difference than the corresponding known IDT with the same m/z ratio *i.e.* 1 minute. Each peak showed the characteristic MS<sup>2</sup> fragments of early- and mid-pathway IDTs (Table 3.5, and Appendix 5).

The new IDT compound three was further investigated (Method 2.3.19 and 2.3.21). This analysis produced characteristic 130 *m/z*, 420 *m/z*, and 402 *m/z* daughter ions confirming this peak was an IDT compound. By comparing the fragmentation patterns of paxitriol and terpendole E whose structures are known and have the same multiple reaction monitoring (MRMs) a tentative structure for this new IDT compound three peak was proposed. The new IDT compound three is not a stereoisomer of either paxitriol and terpendole E as though the MRMs is the same and retention times differ (a characteristic of some stereoisomers) the fragmentation pattern differs (a characteristic of a constitutional isomer) (Figure 3.8, 3.9, 3.10, and Appendix 6, 7, and 8)

## 3.1.4 New indole-diterpene compound three synthesis through Penicillium paxilli complementation

Bioinformatics analysis suggested that the genes *idtP* and *idtQ* from AR48 and Fg1 encode the proteins that are most likely to be responsible for the synthesis of the new IDT compound three (Results 3.1.1). To understand this synthetic pathway, *Penicillium paxilli* single gene knock-out strains  $\Delta paxP$  and  $\Delta paxQ$  (Saikia *et al.*, 2007) were transformed with either *idtP* from *P. paxilli* (pSS1) (Saikia *et al.*, 2007), AR48 (pTM06), and Fl1 (pSS56/pDB49) (Saikia *et al.*, 2012) or *P. paxilli* (pSS2) (Saikia *et al.*, 2007), idtQ from AR48 (pTM07), and Fl1 (pSS58/pDB49) (Saikia *et al.*, 2012) (Method 2.3.9, and Appendix 9 to 15). Spore stocks of a representative number of these hygromycin or geneticin transformants were prepared, and PCR screened for the presence of the transformed gene for IDT analysis using MS (Method 2.3.5, 2.3.10, 2.3.18, 2.3.20, 2.3.22, 4.2.21, and 2.4.22, and Appendix 17).

The IDT profiles of all the controls were as expected. For the *P. paxilli* WT control paspaline, post paspaline products (paspaline B, paxitriol, PC-M6, and 13-desoxypaxilline) and paxilline were detected. This IDT profile was mirrored for both the *paxP* and *paxQ* complementation controls. For the AR48 *idtP* or Fl1 *idtP* complementations into *P. paxilli*  $\Delta paxP$ , paspaline accumulated therefore the paxilline biosynthesis pathway was not restored. For the AR48 *idtQ* or Fl1 *idtQ* complementations into *P. paxilli*  $\Delta paxQ$ , 13-desoxypaxilline accumulated therefore the paxilline biosynthesis pathway as not restored. In addition, no new IDT compound three or other new IDT compounds were detected in extracts of only the AR48 *idtP* and *idtQ* complementations (Table 3.3 and 3.4, Figure 3.11, and Appendix 17).

AR48 and Fg1 new indole- diterpene compond	Sample Name	Retention Time (min)	FI1	СТ	AR1	AR37	AR48	Fg1
	Paspaline		108	63	157	39	1179	192
	Terpendole F		7	3	8	2	1	0
One	Terpendole E-like	10.3	0	0	0	0	5	1
	Terpendole E	10.5	11	9	20	12	0	0
	Paspaline B		3	2	3	2	99	9
Two	13-Desoxypaxilline-like	11.8	0	0	0	0	23	23
	13-Desoxypaxilline	12.1	9	5	15	2	130	30
	Paxilline		2	2	9	2	91	31
Three	Paxitriol-like	11.9	NA	NA	NA	NA	NA	NA
	Paxitriol	10.0	7	7	12	11	2	1
	Terpendole I	8.6	32	20	79	41	2	1
Four	Terpendole I-like	8.7	0	1	2	1	27	2
	Terpendole J		4	4	4	2	0	0
	Terpendole C		13	36	39	2	0	0
	Terpendole K		31	85	90	1	0	0
	Terpendole M (?)		74	85	106	9	0	0
	Terpendole A (?)		23	25	31	9	0	0
	Terpendole N		186	276	521	5	0	0
	Lolilline		312	17	0	0	0	0
	Lolicine A	11.1	117	36	0	0	0	0
	Lolitrem M	10.25	348	89	0	0	0	0
	Lolitriol		3856	365	0	0	0	18
	Lolitrem K		160	50	0	0	0	410
	Lolitrem E		211	75	0	0	0	0
	Lolitrem B		901	1000	0	0	0	0
	Lolitrem A		194	83	0	0	0	0
	Lolitrem N		0	0	0	0	0	0
	Lolitrem F		43	50	0	0	0	0
	Epoxy-Janthitriol		0	0	0	1704	0	0
	Epoxy-Janthitrem I		0	0	0	3	0	0
	Epoxy-Janthitrem II		0	0	0	429	0	0
	Epoxy-Janthitrem III		0	0	0	170	0	0
	Epoxy-Janthitrem IV		0	0	0	169	0	0

Table 3.2: Mass spectrometry indole-diterpene (IDT) compound profiles of extracts from ryegrass pseudostem infected with different *Epichloë festucae* strains (ppm).

<u>Underlined name</u>: unknown peak with different retention time but same *m*/*s* as known IDT. (?): compound without standard. NA: not assessed. The stereochemistry of the compounds and standards are unknown.

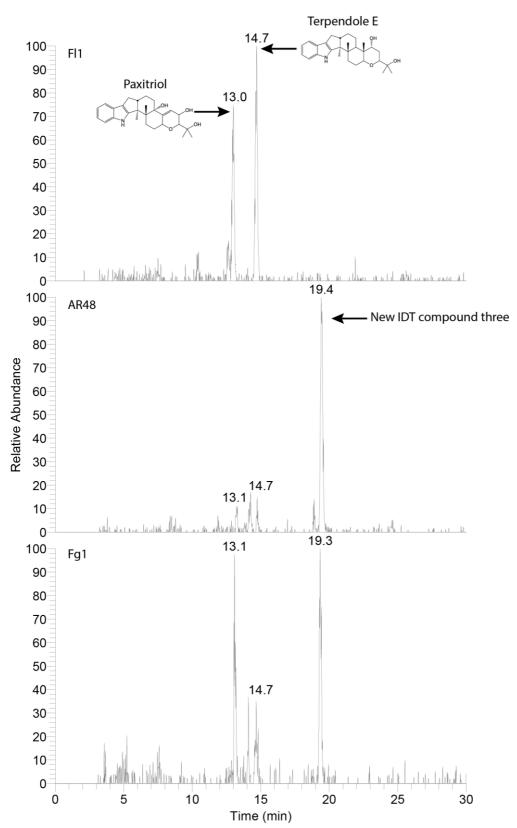


Figure 3.7: Liquid chromatography mass spectrometry (LC-MS) analysis of indolediterpenes (IDTs) from *Epichloë festucae* infected ryegrass. Single ion extracted (438 m/z) chromatograms of Fl1, AR48, and Fg1. Key ions are paxitriol (13.1 mins), terpendole E (14.7 mins), and new IDT compound three (19.3 mins), with the compound structure and name where known placed beside the corresponding peak. The stereochemistry of the compounds and standards are unknown.

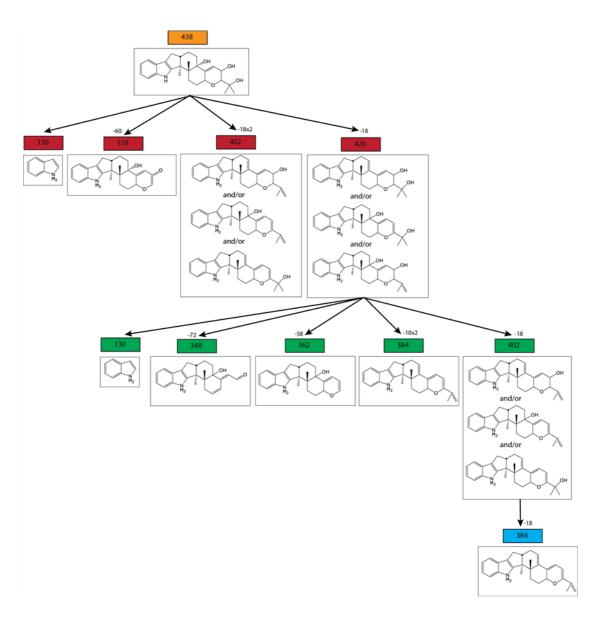


Figure 3.8: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of paxitriol from *Epichloë festucae* Fl1 infected ryegrass. Collision-inducted fragmentation of the 438 m/z parent ion results in key ions 420 m/z (loss of H<sub>2</sub>O), 402 m/z (loss of two H<sub>2</sub>O), 378 m/z (loss of C<sub>3</sub>H<sub>8</sub>O) and 130 m/z (core fragment for early- and midpathway IDTs). Collision-inducted fragmentation of the 420 m/z MS<sup>2</sup> daughter ion results in key ions 402 m/z (loss of second H<sub>2</sub>O), 384 m/z (loss of second and third H<sub>2</sub>O), 362 m/z (loss of C<sub>3</sub>H<sub>6</sub>O), 348 m/z (loss of C<sub>4</sub>H<sub>8</sub>O) and 130 m/z (core fragment). Collision-inducted fragmentation of the 402 m/z MS<sup>3</sup> daughter ion results in a third H<sub>2</sub>O loss to afford the 384 m/z ion. The stereochemistry of the compounds and standards are unknown.

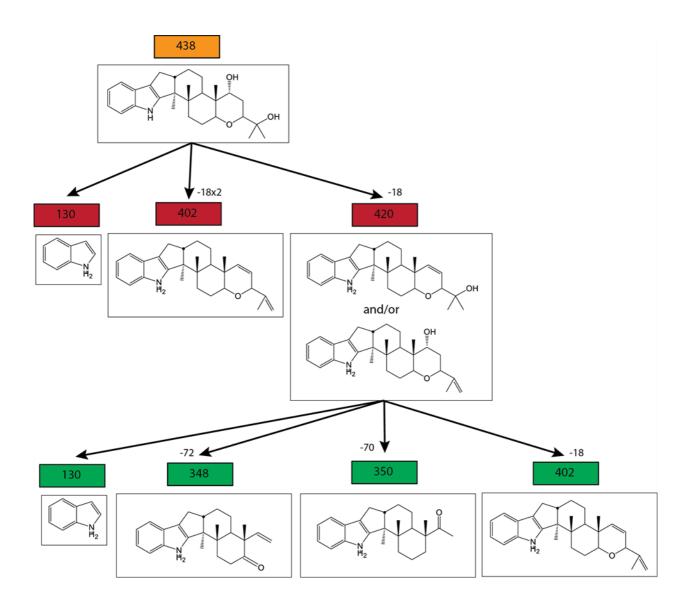


Figure 3.9: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of terpendole E from *Epichloë festucae* Fl1 infected ryegrass. Collision-inducted fragmentation of the 438 m/z parent ion results in key ions 420 m/z (loss of H<sub>2</sub>O), 402 m/z (loss of two H<sub>2</sub>O) and 130 m/z (core fragment for early- and mid-pathway IDTs). Collision-inducted fragmentation of the 420 m/z MS<sup>2</sup> daughter ion results in key ions 402 m/z (loss of second H<sub>2</sub>O), 350 m/z (loss of C<sub>4</sub>H<sub>6</sub>O), 348 m/z (loss of C<sub>4</sub>H<sub>8</sub>O) and 130 m/z (core fragment). The stereochemistry of the compounds and standards are unknown.

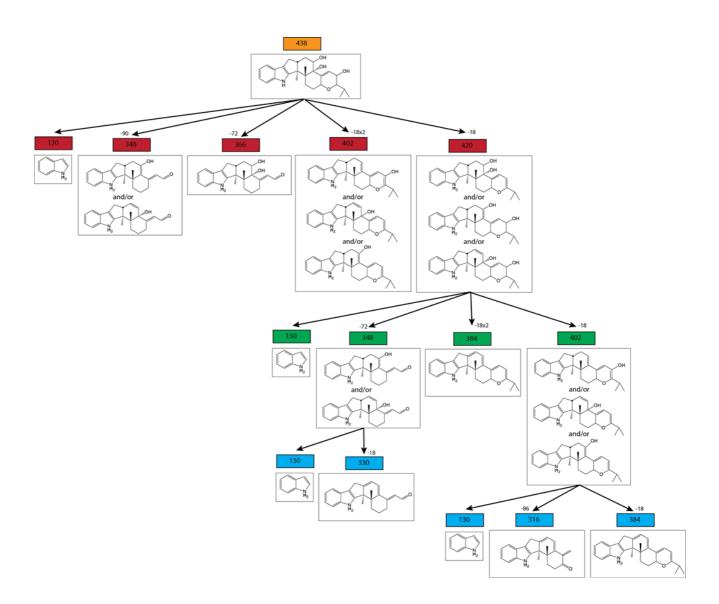


Figure 3.10: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of new indole-diterpene compound three from *Epichloë festucae* AR48 and Fg1 infected ryegrass. Collision-inducted fragmentation of the 438 *m/z* parent ion results in key ions 420 *m/z* (loss of H<sub>2</sub>O), 402 *m/z* (loss of two H<sub>2</sub>O), 366 *m/z* (loss of C<sub>4</sub>H<sub>8</sub>O), 348 *m/z* (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>8</sub>O) and 130 m/z (core fragment for early- and mid-pathway IDTs). Collision-inducted fragmentation of the 420 *m/z* MS<sup>2</sup> daughter results in key ions 402 *m/z* (loss of second H<sub>2</sub>O), 384 *m/z* (loss of second and third H<sub>2</sub>O), 348 *m/z* (loss of C<sub>4</sub>H<sub>8</sub>O) and 130 m/z (core fragment). Collision-inducted fragmentation spectrum of the 348 *m/z* MS<sup>3</sup> daughter ion results in key ions of 330 *m/z* (loss of second H<sub>2</sub>O) and 130 m/z (core fragment). Collision-inducted fragmentation spectrum of the 402 *m/z* MS<sup>3</sup> daughter ion results in key ions of 330 *m/z* (loss of second H<sub>2</sub>O) and 130 m/z (core fragment). Collision-inducted fragmentation spectrum of the 402 *m/z* MS<sup>3</sup> daughter ion results in key ions of 330 *m/z* (loss of second H<sub>2</sub>O) and 130 m/z (core fragment). The stereochemistry of the compounds and standards are unknown.

Table 3.3: Average intensity of indole-diterpene (IDT) compounds identified in *Penicillium paxilli* wild type, Δ*paxP* (KO-knock-out), and Δ*paxP* complemented with *paxP* from *Penicillium paxilli* or *idtP* from *Epichloë festucae* strains AR48 or Fl1 (ppm). The stereochemistry of the compounds and standards are unknown.

IDT		Emindole SB	Paspaline	Paspaline B	Paxitriol	PC-M6	13-Desoxypaxilline	Paxilline	Prenyl-Paxilline
WT		18	38	8	3	33	10	26	1
paxP K	ο	3	91	0	0	0	0	0	0
	1	7	105	1	1	64	1	19	2
David	2	1	59	1	0	19	0	19	1
Pax	3	17	135	1	1	55	2	20	1
paxP	4	14	123	0	1	68	2	18	0
	5	13	103	1	1	46	6	24	1
	1	2	108	0	1	1	3	0	0
4040	2	2	127	0	0	4	0	1	0
AR48	3	1	93	0	0	1	0	0	0
idtP	4	1	78	0	0	1	0	0	0
	5	0	102	0	1	4	0	1	0
	1	0	0	0	0	0	0	1	0
	2	9	3	2	0	2	17	0	0
FI1	3	0	2	0	0	0	0	0	0
idtP	4	2	38	0	0	1	0	0	0
	5	0	59	0	0	2	0	0	0

Table 3.4: Average intensity of indole-diterpene (IDT) compounds identified in *Penicillium paxilli* wild type,  $\Delta paxQ$  (KO-knock-out), and  $\Delta paxQ$  complemented with *paxQ* from *P. paxilli* or *idtQ* from *Epichloë festucae* strains AR48 or Fl1 (ppm). The stereochemistry of the compounds and standards are unknown.

IDT		Emindole SB	Paspaline	Paspaline B	Paxitriol	PC-M6	13-Desoxypaxilline	Paxilline	Prenyl-Paxilline
WT		18	38	8	3	33	10	26	1
paxQ I	Ó	13	18	2	1	10	22	0	0
	1	2	4	0	0	3	0	19	0
Pax	2	1	2	0	0	3	0	16	0
	3	1	3	0	0	3	0	15	0
paxQ	4	5	17	1	1	11	1	23	1
	5	1	2	0	0	0	0	4	0
	1	24	28	0	1	19	18	1	0
AD40	2	17	20	2	1	15	20	1	0
AR48 idtQ	3	13	11	2	0	9	20	1	0
aty	4	8	6	2	0	7	19	1	0
	5	0	1	2	0	1	14	1	0
	1	1	3	1	0	4	12	1	0
FI1	2	0	0	0	0	0	0	0	0
	3	3	14	1	1	7	18	1	0
idtQ	4	5	8	0	1	14	16	0	0
	5	23	35	2	0	20	26	1	0

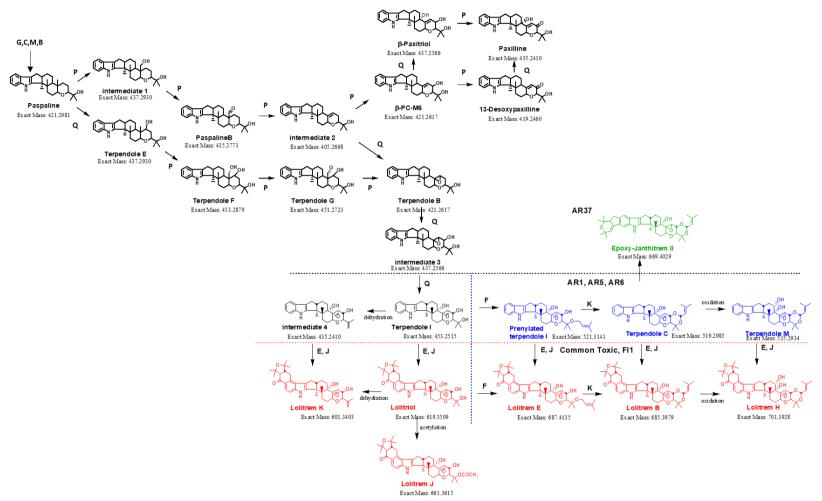


Figure 3.11: Simplified lolitrem B pathway in *Epichloë*. Genes (single letter *e.g.* indole-diterpene (IDT) = *idtP* = P) next to associated reaction. The section containing the end products of a strain is indicated. P/Q derived IDTs (black). E/J derived IDTs (red). F/K derived IDTs (blue/red). *Epoxy*-janthitrem IDT (green).

# 3.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

Only a set number of compounds from the known bioactive second metabolite (SM) pathways currently identified in *Epichloë* (indole-diterpenes (IDTs), lolines, ergot alkaloids and peramine) can explain the wide range of insect bioactivities observed in infected plant material (Introduction 1.1 and 1.2). Understanding the genetic basis of all these bioactivities *i.e.* new gene and/or compound identification is crucial for development of the *Epichloë* commercialisation programme.

Ambrose *et al.* (2014) linked the presence of the makes caterpillars floppy (*mcf*) gene to cutworm moth caterpillars (CC-*Agrotis ipsilon*) bioactivity by injecting *E. coli* expressing the *mcf* gene from the sexual *E. typhina* subsp *Poae Ps1* strain into CC which resulted in death, most likely through activation of apoptosis via the mitochondrial pathway (Introduction 1.4). The product of this gene is therefore a good candidate for CC bioactivity observed in AR48 Infected ryegrass, which of the known bioactive SM pathways only produces intermediate IDT from the lolitrem B pathway (Results 3.1). The bioactivity of *mcf* might extend to stem boring fly (SBF-*Cerodontha australis*) bioactivity depending on the host bioactivity range of the protein (Introduction 1.1.3).

## 3.2.1 Analysing the distribution and functionality of the mcf gene within the Epichloë festucae species through bioinformatics

A comparative analysis of the *mcf* gene across *Epichloë* was performed using bioinformatics to understand both the distribution and functionality of the gene, and how this might relate to CC bioactivity (Method 2.4.1). If the presence of a functional *mcf* gene is found only in strains with CC bioactivity, and conversely the presence of a non-functional gene or absence of the gene is found only in strains with no CC bioactivity, this will provide further supporting evidence that Mcf could be responsible for this insect bioactivity. The *E. typhina* subsp *Poae Ps1 mcf*, referred to as "*E. typhina mcf* model", from Ambrose et al. (2014) was used as the gene model and

corresponding protein model as there is experimental evidence supporting both the gene's structure *e.g.* DNA and cDNA sequencing, as well as resultant protein bioactivity, *e.g.* expression levels and artificial CC insect assay (Ambrose *et al.*, 2014).

The initial analysis focused on strains from the species *E. festucae* as the CC bioactivity status of most of these strains was known and the genomes had been sequenced (Introduction 1.1.3). All strains analysed contained a single *mcf* gene. Only AR48 and Fg1 contained *mcf* genes with both the *E. typhina mcf* model gene introns as well as the start and stop codons conserved. The remaining strains have either lost the *E. typhina mcf* model gene start codon along with the first intron but have an in frame start codon soon afterwards (Fl1, E2368, and AR37) or contain an out of frame premature stop codon due to acquisition of a mutation, *e.g.* 23-nucleotide deletion (AR1). An alignment of all these genes identified that most of the variation in sequence occurred at the 5' end (Figure 3.12, and Appendix 18).

These gene annotations, identified through the bioinformatics analyses, were used to predict the corresponding protein products (Method 2.4.1). Only AR48 and Fg1 are predicted to produce a protein product that is similar to the *E. typhina* Mcf model protein. Fl1, E2368, and AR37 are predicted to produce proteins without the N-terminal region of the *E. typhina* Mcf model protein, and AR1 is predicted to produce a protein without the C-terminal region of the *E. typhina* Mcf model protein. An alignment of all these proteins identified that most of the variation in sequence occurred at the C-terminal end (Figure 3.12, and Appendix 19).

## 3.2.2 Analysing the distribution and functionality of the mcf gene within the Epichloë genus through bioinformatics

If *mcf* is responsible for CC bioactivity then understanding the distribution and functionality of this gene not just within *E. festucae* species, but also within the *Epichloë* genera could identify other strains of commercial interest. These newly identified strains may have other bioactivities of interest that are not present in AR48, therefore these strains could be more ideal for commercialisation and/or for the commercialization of products with variable bioactivity ranges.

In total, an additional 14 *Epichloë* strains were analysed from 11 *Epichloë* species (Method 2.4.1). No hybrid strains were analysed. Again all strains contained a single *mcf* gene. Both *E. amarillans* strains, *E. aotearoae*, *E. brachyelytri*, one *E. bromicola* AL04262 strain, *E. glyceriae*, and *E. mollis*, had *mcf* genes with both introns of the *E. typhina mcf* model gene as well as the start and stop codons conserved, *i.e.* 50% of the strains. The remaining strains had either an out of frame premature stop codon due to acquisition of a mutation (*E. bromicola* AL0434, *E. elymi, E. gansuensis*, and *E. typhina* subsp. *Poae* E5819), had lost the *E. typhina mcf* model gene stop codon but had an in frame stop codon soon afterwards (*E. baconii*) or lost the start codon corresponding to the *E. typhina mcf* model gene along with the first intron but had an in frame start codon soon afterwards (*E. typhina* ATC 200736). An alignment of all these genes identified variation in sequence throughout the gene (Figure 3.13, and Appendix 20).

*E. amarillans* strains, *E. aotearoae*, *E. brachyelytri*, *E. bromicola* AL04262 strain, *E. glyceriae*, and *E. mollis*, all contain genes annotated like the *E. typhina mcf* model gene and therefore are predicted to produce proteins that are similar to the *E. typhina* Mcf model protein. *E. bromicola* AL0434, *E. elymi*, *E. gansuensis*, and *E. typhina* subsp. *Poae* E5819, are predicted to produce protein products with the N-terminal region of the *E. typhina* Mcf model protein present but with varying degrees of the central and C-terminal regions of the *E. typhina* Mcf model protein product that is very similar to the *E. typhina* Mcf model protein but with a slight extension at the C-terminal end. Finally, *E. typhina* ATC 200736 is predicted to produce a protein product without the N-terminal region of the *E. typhina* Mcf model protein product without the N-terminal region of the variation in sequence occurred at both the N- and C- terminal ends (Figure 3.13, and Appendix 21).

101

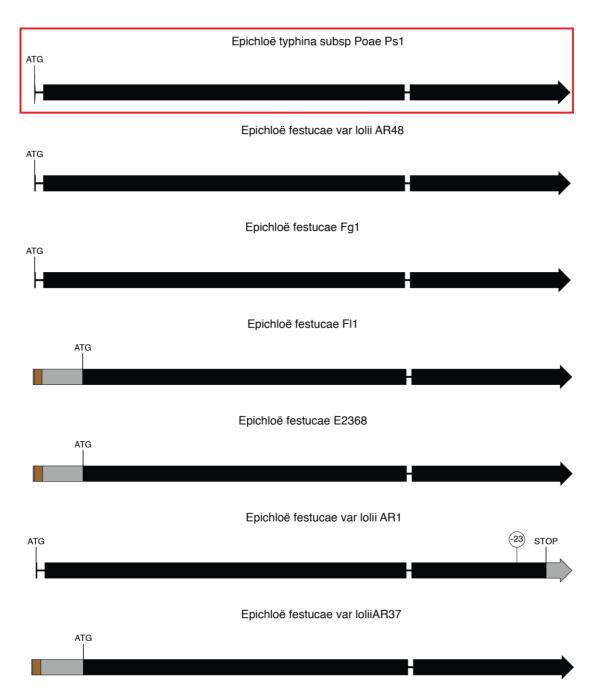
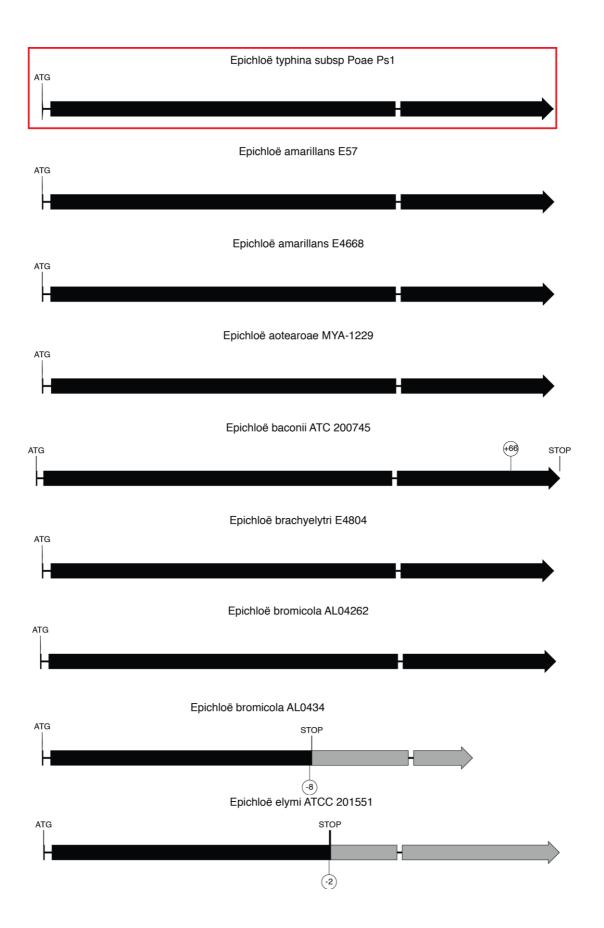


Figure 3.12: Analysis of the predicted makes caterpillars floppy (*mcf*) gene structure in a range of *Epichloë festucae* strains. The *E. typhina* gene model is highlighted in a red box (Ambrose *et al.*, 2014). The gene is represented by a solid line, with transcript overlaid as an arrow with gaps as introns, black sections as translated regions and grey sections as not translated regions. The brown boxes highlight sequences conserved with intron 1 in *E. typhina* model gene but no longer function as an intron due to mutations. Nucleotide changes are represented by circles, with the number as well as insertion (+) or deletions (-) inside the shape. The start codons are annotated.



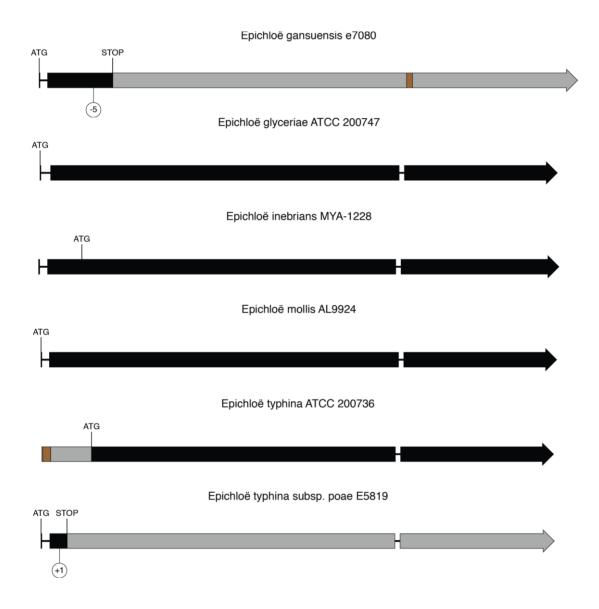


Figure 3.13: Analysis of the predicted makes caterpillars floppy (*mcf*) gene structure in a range of *Epichloë* species. The *E. typhina* gene model in highlighted in a red box (Ambrose *et al.*, 2014). The gene is represented by a solid line, with transcript overlaid as an arrow with gaps as introns, black sections as translated regions and grey sections as not translated regions. The brown boxes highlight sequences conserved with the intron in *E. typhina* model gene but no longer functional as an intron due to mutations. Nucleotide changes are represented by circles, with the number as well as insertion (+) or deletions (-) inside the shape. The start codons are annotated.

### 3.2.3 Analysing the location of the mcf gene within the Epichloë genus through bioinformatics

The expression of only the *E. typhina mcf* model gene under an inducible promoter in *E. coli* successfully established CC bioactivity (Ambrose *et al.*, 2014). The bioactive is therefore produced by a single gene *i.e.* does not require additional genes for synthesis and/or is not modified by additional genes. This is also observed for the bacterial Mcf proteins however these genes are associated/cluster with other genes involved in transportation and/or transcription regulation (Daborn *et al.*, 2002, Péchy - Tarr *et al.*, 2008, Waterfield *et al.*, 2003). Bioinformatics analysis of the *mcf* gene genomic location was therefore performed on all strains analysed in this study to investigate whether the *Epichloë mcf* gene is within a cluster or associated with other important genes (Method 2.4.1)

The completely assembled Fl1 genome, which has been annotated using the E2368 version 3 gene models (EfM3.0) as well as RNA sequencing data from a range of wild type (WT) and mutant strains, was used as the genome reference (Winter *et al.*, 2018, Schardl *et al.*, 2013c). The BLASTx analysis provided by the online database (Schardl *et al.*, 2013c) was used to identify the genes surrounding *mcf* as encoding for a cytokinesis inhibitor, tRNA methyltransferase, extracellular protein, cell wall protein, tRNA release factor, and hypothetical protein respectively (Figure 3.14).

The location of these Fl1 *mcf* surrounding genes was then analysed in the *Epichloë* strains used in this study (Results 3.2.1 and 3.2.2). All strains contained all of the surrounding genes, although in some genomes the genes were spread across up to three scaffolds. This is due to fragmentation of the genome assemblies. Within each scaffold the orientation and order was conserved. The predicted functionality of each individual gene was not analysed (Figure 3.15).

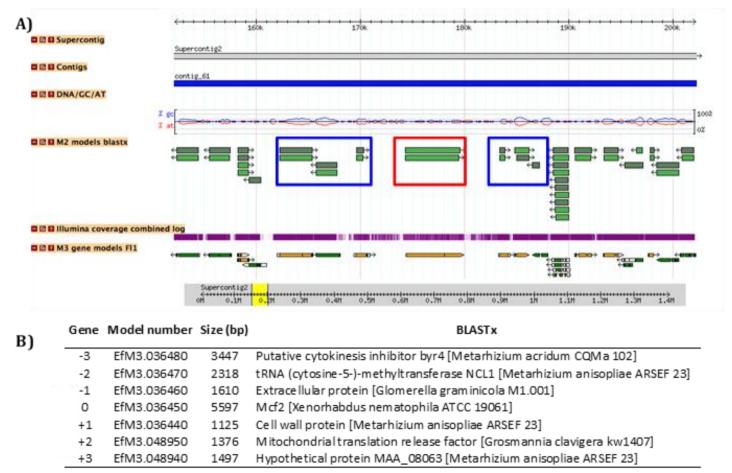


Figure 3.14: Identification of genes surrounding makes caterpillars floppy (*mcf*) within the *Epichloë festucae* Fl1 genome of chromosome 7. A) Screenshot of a 50 kb genomic area around *mcf* EfM3.036450 (red box) with the following characteristics highlighted; GC content, BLASTx genes, Illumina RNAseq reads, and M3 models. The genes selected for further analysis are -3 EfM3.036480, -2 EfM3.036470, -1 EfM 3.036460, +1 EfM3.036440, +2 EfM3.048950, and +3 EfM3.048940 (blue boxes). B) Table of the BLASTx hits and gene sizes of the selected genes in (A).

### A)

Species	Strain	Chromosome/Contig/Sca	ffold (genes)	
E. festucae	Fl1	7(-3,-2,-1,0,+1,+2,+3)		
E. festucae	E2368	85(-3,-2,-1,0,+1)	132(+2,+3)	
E. festucae	Fg1	151918(-3, <b>-2</b> )	149961(-1, 0,+1,+2,+3)	
E. festucae var Iolli	AR48	266(-3,-2,-1)	344(0,+1,+2,+3)	
E. festucae var Iolli	AR1	6(-3,-2,-1,0,+1,+2,+3)		
E. festucae var Iolli	AR37	203244(-3, <b>-2</b> )	200652(-1,0)	200958(+1, +2,+3)
E. amarillans	E57	80(-3,-2,-1,0,+1,+2,+3)		
E. aotearoae	MYA-1229	147(-3, <b>-2</b> ,-1)	267(+1,+2,+3)	
E. baconii	ATC 200745	34(-3,-2,-1,0,+1,+2,+3)		
E. brachyelytri	E4804	396(-3, <b>-2</b> ,-1,0,+1)	570(+2,+3)	
E. bromicola	AL04262	242(-3,-2,-1)	237(0)	204(+1,+2,+3)
E. elymi	Atcc 201551	187(-3, <b>-2</b> ,-1)	187(-1, 0,+1,+2,+3)	
E. gansuensis	e7080	43(-1, <b>-2</b> ,-3)	41(0,+1, +2,+3)	
E. glyceriae	ATCC 2000747	43(-3, <b>-2</b> ,-1,0,+1,+2, <b>+3</b> )		
E. inebrians	MTA-1228	350(-1, <b>-2</b> ,-3)	361(0)	716(+1,+2, +3)
E. mollis	AL9924	3(-3,-2,-1,0,+1,+2,+3)		
E. typhina	ATCC 200736	463(-1, <b>-2</b> ,-3)	262(0,+1)	626(+2,+ <b>3</b> )

10K

tik

126

196

20K

218

Figure 3.15: Analysis of makes caterpillars floppy (*mcf*) syntony in *Epichloë*. A) Schematic of the gene structure around *mcf* in *Epichloë festucae* Fl1 genome of chromosome 7. B) Analysis of syntony from a range of *Epichloë* strains with chromosome/contig/scaffold indicated followed by gene present and order in brackets with orientation indicated by bold genes being on the reverse strand. Genes (-3 EfM3.036480, -2 EfM3.036470, -1 EfM 3.036460, 0 EfM3.036450 (mcf), +1 EfM3.036440, +2 EfM3.048950, and +3 EfM3.048940)

## 3.2.4 Analysing the distribution, functionality, and location of the mcf gene outside the Epichloë genus through bioinformatics

Outside *Epichloë, mcf/P. fluorescens insect toxin (fitD)* is commonly identified in bacterial species such as *Photorhabdus, Xenorhabdus, and Pseudomonas* (Ruffner *et al.*, 2015). To understand the full extent of the *mcf* gene distribution and functionality, bioinformatics was used to identify and then predict the functionality of *mcf* gene/s outside the *Epichloë* genus but within the fungal kingdom (Method 2.4.1).

NCBI searches using the model *E. typhina mcf* model gene and protein sequences did not identify any *mcf* genes in fungal genomes apart from *Epichloë*. However, Dupont (2017) identified four other fungal species with *mcf* genes: *Aspergillus flavus, Metarhizium* species, *Neonectria ditissima*, and *Phlebiopsis gigantean*. Unfortunately, the paper did not provide gene or genome reference identifiers and so the genes where identified by repeating their methodology *i.e.* the listed species were searched against the JGI MycoCom database to identify available strains, and then the *E. typhina* Mcf model protein sequence was analysed by tBLASTn against these strains. Only *N. ditissima* R0905 (NCBI accession KPM41439.1), and *P. gigantea* 11061\_1 CR5-6 (NCBI accession KIP08609.1) genomes had *mcf* gene hits while *A. flavus* NRRL3357, *M. robertsii ARSEF 23* and *M. acridum* CQMa 102 searches did not result in an obvious *mcf* gene hits (Figure 3.16).

These *mcf* genes from *N. ditissima* and *P. gigantea* were then annotated and corresponding proteins predicted (Method 2.4.1). The *N. ditissima mcf* gene is slightly larger than the *E. typhina mcf* model gene (6,145 bp) at 6,334 bp with one intron, which is not conserved in *E. typhina mcf* model gene, and produces a 2,090 aa protein. The *P. gigantea mcf* gene is also slightly larger than *E. typhina mcf* model gene (6,145 bp), though smaller than *N. ditissima mcf* gene (2,090 bp), at 6,264 bp with two introns, which are both conserved in *E. typhina mcf* model gene, and produces a 2,048 aa protein. When the two *mcf* genes and predicted proteins were aligned to the *E. typhina mcf* model gene and Mcf model protein sequences, *P. gigantea* and *E. typhina* model had the highest gene and protein sequence identities (55.3% and 39.5% respectively) then *N. ditissima* and *E. typhina* model (52.0% and 28.8% respectively).

The *N. ditissima* gene and protein sequence was more similar to *E. typhina* model gene then *P. gigantea* (Figure 3.16, and Appendix 22).

The same bioinformatics analysis was performed using the six genes that have been identified surrounding *mcf* in the *Epichloë* to indicate if syntony has been conserved outside *Epichloë* genus (Method 2.4.1, and Results 3.2.3). *A. flavus* and *M. robertsii* both contained genes -3, -2, +2, and +3, with *M. robertsii* containing +1 in addition. The coverage (%) and identity (%) for all of these identified genes were at least 94% for both parameters, except for +2 *A. flavus* at 74% coverage and an E values of 0.0. For *N. ditissima* and *P. gigantean*, searches did not result in any obvious gene hits (Table 3.5).

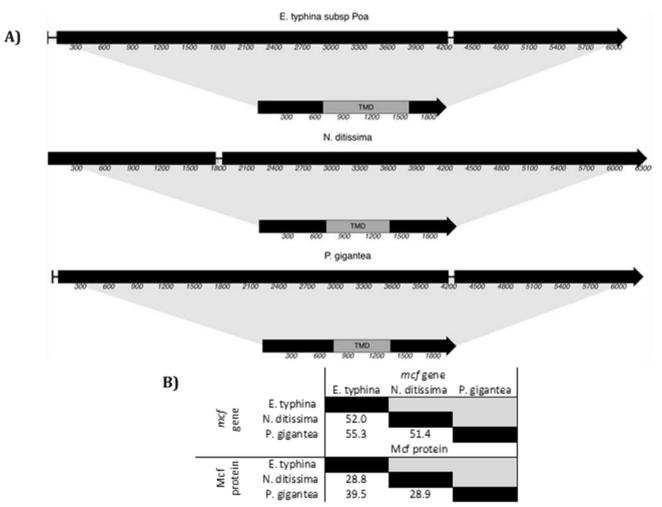


Figure 3.16: Identification of other fungal makes caterpillars floppy (*mcf*) genes outside *Epichloë*. A) Gene annotated for exons (black arrow) and introns (gaps) and translated protein (below) with domains predicted by InterPro scan (grey box). B) Pair wise alignments between *mcf* and Mcf like sequences with identity (%). Black box is an alignment between the same gene and grey box is a repeat alignment.

Table 3.5: Location of makes caterpillars floppy (*mcf*) in the genomes of fungi outside the *Epichloë* genus using reference genes as location markers

						/		
Strain	Gene	Size (bp)	Location	Orientation		Coverage (%)	Identity (%)	E value
	-3	3,004	AAIH02000400.1	N/A	Aspergillus flavus NRRL3357 cytokinesis regulator (Byr4), putative (AFLA_007210), partial mRNA	98	100	0.0
A. flavus ^	-2	2,256	AAIH02000035.1	N/A	Aspergillus flavus NRRL3357 S-adenosylmethionine- dependent methyltransferase superfamily domain- containing protein (AFLA_024870), partial mRNA	100	100	0.0
A. fl	+2	1,597	AAIH02000334.1	N/A	Aspergillus bombycis peptide chain release factor 1 (ABOM_011014), partial mRNA	74	93	0.0
	+3	1,197	AAIH02000344.1	N/A	Aspergillus bombycis hypothetical protein (ABOM_009699), partial mRNA	91	94	0.0
	-3	3,096	ADNJ02000001.1	+ve	Metarhizium robertsii ARSEF 23 cytokinesis regulator mRNA	100	100	0.0
<	-2	1,980	ADNJ02000001.1	-ve	Metarhizium brunneum ARSEF 3297 tRNA (cytosine-5- )-methyltransferase NCL1 partial mRNA	100	99	0.0
M. anisopliae	+1	885	ADNJ02000002.1	N/A	Metarhizium robertsii ARSEF 23 cell wall galactomannoprotein Mp2/allergen F17-like protein mRNA	100	100	0.0
ž	+2	1,253	ADNJ02000004.1	+ve	Metarhizium robertsii ARSEF 23 Peptide chain release factor class I/class II mRNA	96	100	0.0
	+3	1,413	ADNJ02000004.1	-ve	Metarhizium robertsii ARSEF 23 hypothetical protein mRNA	94	100	0.0
N. ditissima *	0	2,090	-	N/A	Makes caterpillars floppy protein [Epichloe typhina subsp. poae]	89	31	0.0
P. gigantea *	0	2,048	-	N/A	Makes caterpillars floppy protein [Epichloe typhina subsp. poae]	97	40	0.0

BLASTn(^)/BLASTp(\*)

Note: Genes (-3 efM3.036480, -2 efM3.036470, -1 efM 3.036460, 0 efM3.036450 (mcf), +1 efM3.036440, +2 efM3.048950, and +3 efM3.048940)

### 3.2.5 Analysing domain composition of Mcf/FitD proteins through bioinformatics

The *mcf* gene is present in all strains analysed throughout the *Epichloë* genera and in two other fungal species *N. ditissima* and *P. gigantean* as well as the bacterial species *Photorhabdus, Xenorhabdus, and Pseudomonas* (Results 3.2.1, 3.2.2, and 3.2.4) (Dowling & Waterfield, 2007). However, there are slight variations in gene annotations and therefore corresponding predicted protein products. To understand the functionality of these Mcf proteins, the domain composition from a selection of *Epichloë* strains as well as *N. ditissima* and *P. gigantean* were analysed using online tools that utilize either the primary (Pfam or InterPro Scan) or secondary (Phyre2 or Hhpred) protein structure (Method 2.4.1). In addition, a range of Mcf/FitD proteins from literature were included in this analysis as controls (Table 3.6).

Species	Bacteria (B)/ Fungus (F)	Protein	Size (a.a)	Domains	Reference
Fungi					
Epichloë festucae var lolii AR48	F	Mcf	1,992	CPD1/CPD2 /TMD	This study
Epichloë festucae Fl1	F	Mcf	1,844	CPD1/CPD2 /TMD	This study
Neonectria ditissima	F	Mcf	2,090	CPD1/CPD2 /TMD	This study
Phlebiopsis gigantean	F	Mcf	2,048	CPD1/CPD2 /TMD	This study
Epichloë typhina subsp Poae Ps1	F	Mcf	1,996	TMD	(Ambrose, Koppenhöf er et al. 2014)
Bacteria					
Photorhabdus luminescens	В	Mcf1	2,929	BH3/TMD/ RTX	(Dowling, Waterfield et al. 2007)
Photorhabdus luminescens	В	Mcf2	2,388	HRM/TMD/ RTX	(Waterfield , Daborn et al. 2003)
Pseudomonas fluorescens	В	FitD	3,003	GT/TMD	(Péchy□ T arr, Bruck et al. 2008)
Vibrio vulnificus	В	MCF-like domain	367	CPD/TMD	(Agarwal, Agarwal et al. 2015)

### Table 3.6: Characteristics of published Mcf/FitD proteins

GT: Glycosyltransferase domain; CPD1/2: Cysteine protease domain 1 or 2; TMD: Transmembrane domain, Mcf: makes caterpillars floppy, and fitD; P. <u>fluorescens</u> insect toxin Pfam and InterPro scan both predicted the presence of a central transmembrane domain (TMD) similar to the *Clostridium difficile* TcdA and TcdB TMD (pfam12920 and IPR024769) for all Mcf/FitD protein sequences, and at similar locations between each sequence and between tools. No other domains were predicted using either tool (Table 3.7 and Figure 3.17).

Table 3.7: Makes caterpillars floppy (Mcf)/ P. fluorescens insect toxin (FitD) protein domain predictions using online tools that predicts using primary protein structure.

Endophyte	Protein	Domain	Range (a.a)	Accession	E value
Pfam					
<i>E. festucae</i> var	Mcf	TcdA_TcdB_pore	703-1324	pfam12920	1.22E-174
lolii AR48					
E. festucae Fl1	Mcf	TcdA_TcdB_pore	555-1176	pfam12920	2.24E-173
E.typhina subsp	Mcf	TcdA_TcdB_pore	701-1319	pfam12920	5.44E-151
Poae Ps1					
N. ditissima	Mcf	TcdA_TcdB_pore	707-1328	pfam12920	2.46E-179
P. gigantea	Mcf	TcdA_TcdB_pore	706-1346	pfam12920	1.56E-120
P. luminescens	Mcf 1	TcdA_TcdB_pore	1616-2249	pfam12920	0.00E+00
P. luminescens	Mcf 2	TcdA_TcdB_pore	1011-1648	pfam12920	0.00E+00
P. fluorescens	FitD	TcdA_TcdB_pore	1618-2250	pfam12920	0.00E+00
Interpro					
<i>E. festucae</i> var	Mcf	TcdA_TcdB_pore	702-1353	IPR024769	N/A
lolii AR48					
E. festucae Fl1	Mcf	TcdA_TcdB_pore	554-1205	IPR024769	N/A
E.typhina subsp	Mcf	TcdA_TcdB_pore	706-1357	IPR024769	N/A
Poae Ps1					
N. ditissima	Mcf	TcdA_TcdB_pore	710-1383	IPR024769	N/A
P. gigantea	Mcf	TcdA_TcdB_pore	705-1354	IPR024769	N/A
P. luminescens	Mcf 1	TcdA_TcdB_pore	1617-2276	IPR024769	N/A
P. luminescens	Mcf 2	TcdA_TcdB_pore	1012-1675	IPR024769	N/A
P. fluorescens	FitD	TcdA_TcdB_pore	1619-2277	IPR024769	N/A

Both Phyre2 and Hhpred predicted the presence of the same central TMD as Pfam and InterPro Scan (c4r04A/4r04A) in all Mcf/FitD proteins, and at similar locations between each sequence and between tools. In addition, Phyre2 also predicted the presence of two N-terminal cysteine protease domains (CPD1 and CPD2) (d1hq0a and d1ukfa respectively) for all proteins, except for *E. festucae* Fl1 Mcf and *P. fluorescens* FitD which did not have a CPD1 predicted to be present. These domains are in similar

locations between each protein sequence. The only other domain predicted to be present was a signalling domain (SD) (*Epichloë:* c3ilaG and *Photorhabdus:* c4rswB) that was identified at the C-terminus for *E. festucae* AR48 Mcf, at the C-terminus for *E. typhina* Mcf model, and at the N-terminus for *P. luminescens* Mcf2 (Table 3.8 and Figure 3.17).

Only the TMD was predicted for the control strains; *E. typhina* Mcf model, *P. fluorescens*, and *P. luminescens*. Literature also predicts BH3 (Bcl-2 domain three) domain, HrmA domain, RTX-like domains (Repeat-in-toxin), and glycosyltransferase domain for these proteins (Dowling & Waterfield, 2007). None of these domains were predicted during this bioinformatics analysis. However, the CPD1 and CPD2 were predicted to be present for these proteins and at a similar position as the literature predicted domains, except for *P. fluorescens* which did not have the CPD1 predicted to be present (Table 3.6, and 3.8, and Figure 3.17).

Table 3.8: Makes caterpillars floppy (Mcf) and P. fluoresces insect toxin (FitD) protein domain predictions using online tools that predicts
using secondary protein structure.

Endophyte	Protein	Domain	Range (a.a)	Model number	Confidence (%)	Coverage( %)	E value
Phyre2							
E. festucae var Iolii AR48	Mcf	Clostridium difficile toxin a (tcda)	690-1597	c4r04A_	100	N/A	N/A
		Cysteine proteinases	546-632	d1ukfa_	96.9	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	14-137	d1hqOa_	85.1	N/A	N/A
		Signaling protein	1945-1966	c3ilaG_	48.6	N/A	N/A
E. festucae Fl1	Mcf	Clostridium difficile toxin a (tcda)	542-1449	c4r04A_	100	N/A	N/A
		Cysteine proteinases	399-484	d1ukfa_	96.8	N/A	N/A
E.typhina subsp Poae Ps1	Mcf	Clostridium difficile toxin a (tcda)	688-1601	c4r04A_	100	N/A	N/A
		Cysteine proteinases	551-643	d1ukfa_	96.4	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	34-136	d1hqOa_	93.2	N/A	N/A
		Signaling protein	1949-1968	c3ilaG_	56.7	N/A	N/A
N. ditissima	Mcf	Clostridium difficile toxin a (tcda)	675-1642	c4r04A_	100	N/A	N/A
		Cysteine proteinases	598-647	d1ukfa_	95.6	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	137-173	d1hqOa_	66.9	N/A	N/A
P. gigantea	Mcf	Clostridium difficile toxin a (tcda)	694-1598	c4r04A_	100	N/A	N/A
		Cysteine proteinases	560-623	d1ukfa_	96.3	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	103-142	d1hqOa_	89.2	N/A	N/A
P. luminescens	Mcf 1	Clostridium difficile toxin a (tcda)	1609-2532	c4r04A_	100	N/A	N/A
		Cysteine proteinases	1407-1551	d1ukfa_	91	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	1011-1050	d1hqOa_	67.3	N/A	N/A
P. luminescens	Mcf 2	Clostridium difficile toxin a (tcda)	1004-1931	c4r04A_	100	N/A	N/A
		Signaling protein	44-294	c4rswB_	100	N/A	N/A
		Cysteine proteinases	801-946	d1ukfa_	95.9	N/A	N/A
		CNF1/YfiH-like putative cysteine hydrolases	405-444	d1hqOa_	65.1	N/A	N/A
P. fluorescens	FitD	Clostridium difficile toxin a (tcda)	1611-2537	c4r04A_	100	N/A	N/A
		Cysteine proteinases	1465-1560	d1ukfa_	100	N/A	N/A
Hhpred							
E. festucae var Iolii AR48	Mcf	Toxin A; Glucoslytransferase	438-1425	4R04_A	N/A	100	1.10E-10
E. festucae Fl1	Mcf	Toxin A; Glucoslytransferase	290-1277	4R04_A	N/A	100	1.60E-10
E.typhina subsp	Mcf	Toxin A; Glucoslytransferase	442-1429	4R04_A	N/A	100	2.30E-10
Poae Ps1							
N. ditissima	Mcf	Toxin A; Glucoslytransferase	470-1459	4R04_A	N/A	100	3.80E-1
P. gigantea	Mcf	Toxin A; Glucoslytransferase	439-1429	4R04_A	N/A	100	1.30E-10
P. luminescens	Mcf 1	Toxin A; Glucoslytransferase	1285-2344	4R04_A	N/A	100	6.30E-13
P. luminescens	Mcf 2	Toxin A; Glucoslytransferase	679-1743	4R04_A	N/A	100	4.90E-1
		HopA1; signaling protein	31-291	4RSW_A		99.9	5.30E-2
P. fluorescens	FitD	Toxin A; Glucoslytransferase	1287-2348	4R04 A	N/A	100	2.10E-13

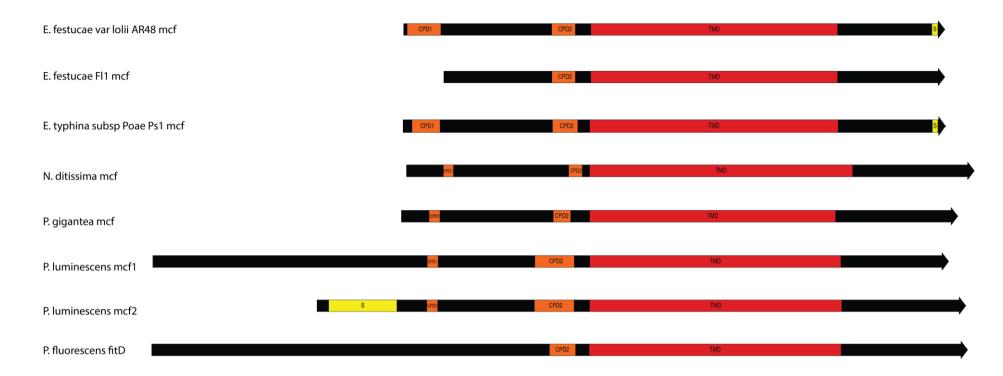


Figure 3.17: Domain analysis of makes caterpillars floppy (Mcf) and P. <u>f</u>luoresces <u>insect toxin</u> (FitD) proteins using online tools (Pfam, InterPro Scan, Phyre2 or Hhpred) in a range of species. Black arrow is the protein. Red box is domain/s predictions identified by all online tools. Orange box is domain/s predictions identified by some of the online tools. Yellow box is domain/s predictions identified by one online tool. CPD1/2: Cysteine protease domain 1 or 2, TMD: transmembrane domain, S: Secretion signal.

# 3.2.6 Analysing the potential bioactivity of Mcf through reverse genetics of Epichloë festucae Fg1

From the bioinformatics analysis, both AR48 and Fg1 appear to be the only *E. festucae* strains analysed in this study that contain an equivalent *E. typhina mcf* model gene and therefore produce an equivalent *E. typhina* Mcf model protein. This *Epichloë* allele will be referred to as AR48 allele. The remaining analysed *E. festucae* strains have either an allelic variant, this *Epichloë* allele will be referred to as Fl1 allele, and/or a non-functional *mcf* gene therefore the bioactivity potential of the produced Mcf protein is unknown (Results 3.2.1). In addition, AR48 has confirmed presence of CC bioactivity, while AR1 and AR37 have confirmed absence of CC bioactivity; these strains have AR48 possibly non-functional allele or Fl1 possibly non-functional respectively (Introduction 1.1.3). There is therefore a correlation between the presence of a functional AR48 *mcf* gene/Mcf protein and CC bioactivity. In addition, Mcf has been directly linked to CC bioactivity through an artificial CC trial (Ambrose *et al.*, 2014).

Biological activity is typically linked to specific genes by first deleting the gene of interest (gene deletion,  $\Delta$ ), which should result in abolishment of bioactivity, and then reintroducing the gene back into the  $\Delta$  (complementation, COMP), which should result in re-establishment of bioactivity (Scott *et al.*, 2012). Although AR48 is the logical strain of choice because of the demonstrated bioactivity against CC it is very slow growing and intractable to genetic manipulation (Introduction 1.1.3). Therefore the fast growing strain Fg1, which shares a close common ancestor to AR48 and has the same alkaloid profile as AR48 was used (Results 3.1).

Two different plasmids were used in an attempt to delete the *mcf* gene in Fg1 through homologous recombination (Method 2.4.26 and 2.4.27). pTM03 was designed to delete the whole Fg1 *mcf* gene *i.e.* approximately 6 kb. However mis-annotation of AR48/Fg1 *mcf* meant that the start of the gene was still present in the Fg1 genome after the recombination event *i.e.* the first few amino acids either side of the first intron (Appendix 23). pTM05 was designed to delete approximately 2 kb from the start of the Fg1 *mcf* gene removing the start of translation. This second plasmid was designed as deletions of smaller regions typically occur at higher rates. In this second

construct both the mis-annotated and gene model start sites will be deleted (Figure 3.19, and Appendix 24). Plasmid pTM03 or pTM05 was linearized with the restriction enzyme Smal1 and gel purified before being transformed in WT Fg1 fungal protoplast. Transformants were selected by plating the transformed protoplast mixture on hygromycin. Hygromycin resistant colonies were subsequently nuclear purified to generate homokaryons for molecular analysis. Gene deletions were identified from ectopic integrations through PCR screening across the deleted region, which results in difference in product size, typically a smaller WT ectopic band and a larger  $\Delta mcf$  band. Putative  $\Delta mcf$  are then confirmed using Southern analysis (Method 2.3.4, 2.4.9, 2.4.17, 2.4.21, 2.4.22, 2.4.25, and 2.4.31 to 2.4.37).

For the pTM03 transformation 86 transformants were screened using multiplex PCR as the deleted region is much larger than the replacement resistance gene (Method 2.4.21). The first set of primers amplified outside and across the hygromycin resistance gene and the second set of primers amplified within the deleted region. All transformants produced both products indicating that the resistance gene was ectopically integrated. For the pTM05 transformation 323 transformants were screened using PCR. This time the deleted region was small enough to distinguish  $\Delta mcf$ strains through amplification outside and across the hygromycin resistance gene (Figure 3.18 and 3.19).

One heterokaryon transformant (Fg1 TM05 #117) was identified *i.e.* both smaller  $\Delta mcf$ and larger WT products produced. This transformant was purified through spore purification (Method 2.4.10). PCR products were successfully amplified outside and across both borders and no product was produced when amplified within the deleted region. Finally, the transformant was confirmed as a knock-out (KO) through Southern blotting. When the Fg1 WT genomic DNA was digested using *Bam*H1 and probed with the digoxigenin (DIG)-11-dUTP labelled linearized pTM05, a 6 kb band was observed. The creation of the gene deletion adds a *Bam*H1 cut site as well as increases the size of this region. The #117 transformant is therefore a "true"  $\Delta mcf$  strain as 5.2 kb and 1.1 kb bands were present as a result of the added cut site. The 6.3 kb band was present as a result of incomplete digestion, however this could occur for both WT and  $\Delta mcf$ 

digests. The 3.4 kb band was universal for both WT and  $\Delta mcf$  as this region is not affected by the homologous recombination event. The two additional bands between sizes 2.7 kb and 2.8 kb are most likely ectopic integrations as the bands are not present in the WT and are not larger than the inserted site *e.g.* 6.3 kb, which would result from tandem integration (Figure 19 and 20, and Appendix 26).

The in culture morphologies were analysed for the Fg1  $\Delta mcf$  at both the macroscopic and microscopic (Method 2.4.15 and 2.4.16) level. The Fg1 WT strain was used as the control. For in culture macroscopic morphology, Fg1 WT and Fg1  $\Delta mcf$  strains size and morphology was comparable, with both strains forming rough white fluffy filamentous colonies. For in culture microscopic morphology, Fg1 WT and Fg1  $\Delta mcf$  strains again were comparable with both strains forming normal hyphal tips, forming branch points, bundles, coils, and conidia (Figure 21).

# 3.2.7 Analysing the potential bioactivity of Mcf through reverse genetics in Epichloë festucae var Iolii AR1 and Epichloë festucae var Iolii AR37

As an alternative approach, due to the complications in initially making an Fg1  $\Delta mcf$  strain, AR1 and AR37 were both transformed with a functional AR48 mcf gene (Method 2.4.9, and Results 3.2.6). AR1 has an AR48 possibly non-functional allele while AR37 has a Fl1 possibly non-functional allele, and both have confirmed absence of CC bioactivity (Introduction 1.1.3, and Results 3.2.1). The advantage of complementation is that the plasmid integrates ectopically through non-homologous recombination that occurs at a much higher rate than homologous recombination. However, the plasmid integrates at random loci and multiple times. This affects the overall transformed gene expression level and can cause disruption of other important genomic regions.

Protoplasts of AR1 and AR37 were transformed with pTM04 using the same method used to create gene deletions (Results 3.2.6). pTM04 was designed to contain the AR48 *mcf* coding region as well as 2 kb extensions at both the 5' and 3' ends as promoter and terminator respectively. As this plasmid did not encode for a selectable maker, the protoplasts were co-transformed with pDB48 that contains the hygromycin resistance gene. The AR1 and AR37 transformants were screened using PCR. To target the

introduced AR48 *mcf* gene, and not the AR1/AR37 WT gene that was still present, primers were designed to amplify across the borders between the backbone and gene of the ectopically integrated pTM04. (Method 2.3.4, 2.4.21, 2.4.22, and 2.4.28, and Appendix 23, 27, and 28).

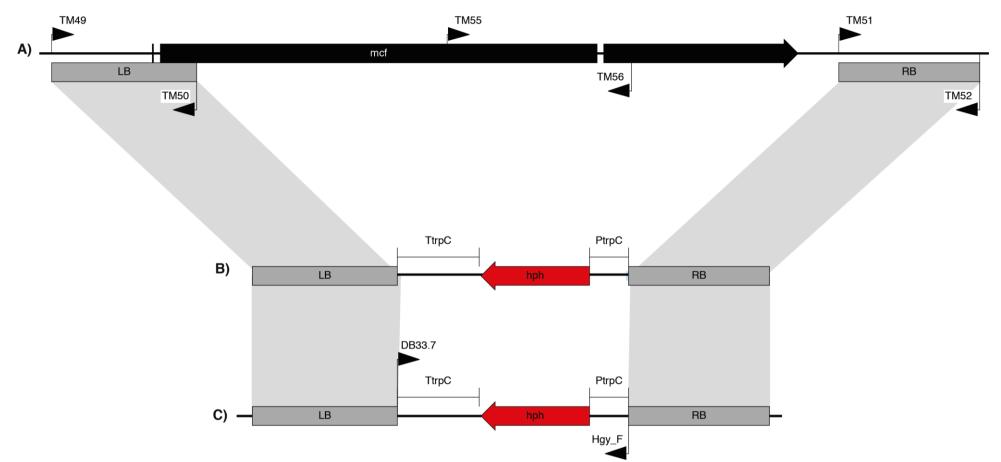


Figure 3.18: Strategy for deletion of the whole *Epichloë festucae* Fg1 makes caterpillars floppy (*mcf*) gene. (A) Physical map of wild type *mcf* genomic locus. (B) Physical map of the linear insert of the *mcf* replacement construct pTM03. (C) Physical map of the  $\Delta mcf$  deletion mutant locus. The black box is *mcf* with introns as gaps. The homologous regions are in light grey boxes. The red box is the hygromycin (hph) selectable marker, and the promoter/terminator are in brackets.

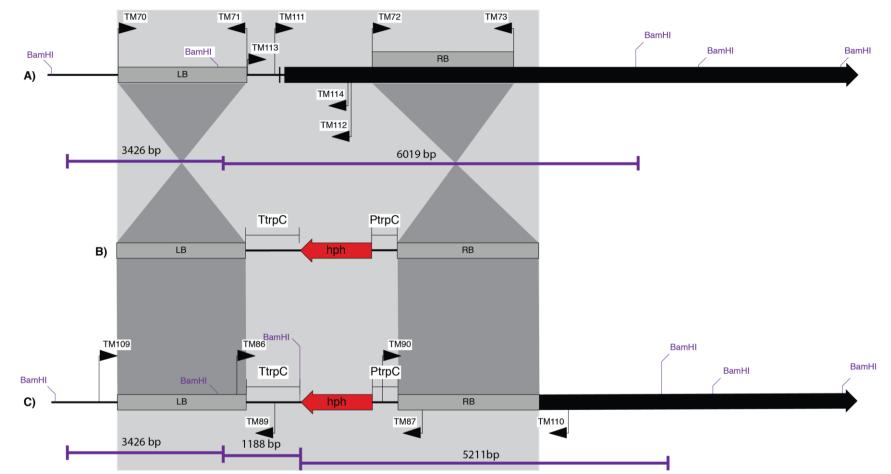


Figure 3.19: Strategy for deletion of the 5' end of the *Epichloë festucae* Fg1 makes caterpillars floppy (*mcf*) gene. (A) Physical map of wild type *mcf* genomic locus. (B) Physical map of the linear insert of *mcf* replacement construct pTM05. (C) Physical map of the  $\Delta mcf$  deletion mutant locus. The black box is *mcf* with introns as gaps. The homologous regions are in dark grey boxes. The regions where the digoxigenin (DIG)-11-dUTP labelled linear pTM05 insert probe binds are in light grey boxes. The red box is the hygromycin (hph) selectable marker, and the promoter/terminator are in brackets. The restriction cut sites for the *Bam*H1, used for the Southern blot, are indicated in purple with corresponding fragments with sizes in purple lines below.

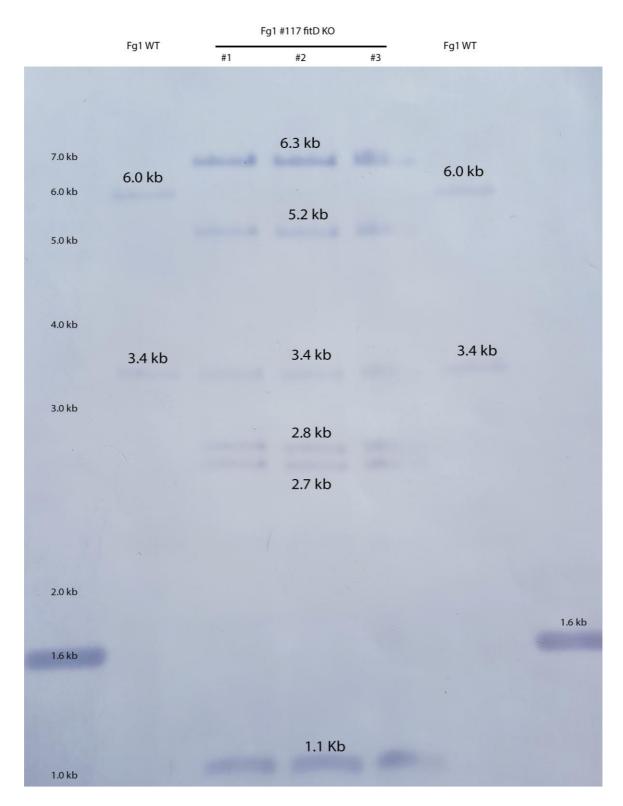


Figure 3.20: NBT/BCIP strained Southern blot of *Bam*H1 genomic digest (1 µg) of *Epichloë festucae* Fg1 wild type (WT) and makes caterpillars floppy (*mcf*) gene deletion ( $\Delta mcf$ )(#117) strains probed with digoxigenin (DIG)-11-dUTP linear pTM05 insert probe. The ladder is 1 kb plus with the 1.6 kb band as a reference. 1#, 2#, and 3# refer to three spores isolated from the same transformant (#117). WT complete digest with *Bam*H1: 6.0 kb and 3.4 kb.  $\Delta mcf$  complete digest with *Bam*H1: 5.2 kb, 3.4 kb, and 1.1 kb.

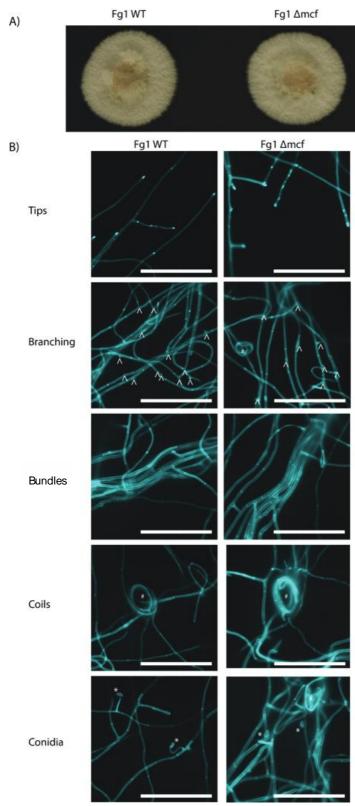


Figure 3.21: Culture phenotype of *Epichloë festucae* Fg1 wild type (WT) and makes caterpillars floppy (*mcf*) gene deletion ( $\Delta mcf$ ) strains. A) Colony morphology of Fg1 WT and  $\Delta mcf$  strains grown on 2.5% PD agar at 22°C for 7 days. B) Fluorescent images captured by inverted microscopy of hyphae grown for 7 days on 2% water agar plates and stained with Calcofluor white. Bar= 20 µm. Arrows indicate branch points, stars indicate conidia and hash-tags indicate coils.

# 3.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass

A comprehensive analysis of the alkaloid profile of both AR48 and Fg1 identified two compounds that might be the bioactives responsible for stem boring fly (SBF-*Cerodontha australis*) and/or cutworm moth caterpillar (CC-*Agrotis ipsilon*) bioactivity observed in AR48 infected plants (Introduction 1.1.3). The first compound is the new indole-diterpene (IDT) compound three that is unique to AR48 and Fg1 and was identified from an analysis of IDTs in grass samples infected with these strains (Results 3.1). The second product is an endotoxin encoded makes caterpillars floppy (*mcf*), which was previously identified by Ambrose *et al* (2014) to be present in *Epichloë* and shown to have CC bioactivity. Out of the *E. festucae* strains analysed in this study, only AR48 and Fg1 genomes contained functional *mcf* gene that were the same *mcf* allele as Ambrose *et al* (2014) bioactive *mcf* gene. Both products are therefore unique to AR48 and Fg1, in relation to the *E. festucae* strains analysed, and therefore good candidates for the unique insect bioactivity. Insect trials involving endophyte infected ryegrass material and insects captured from the New Zealand environment were carried out to investigate the bioactive potential of these two products.

# 3.3.1 Design and perform a stem boring fly (Cerodontha australis) whole plant choice bioactivity trial on a range of wild type Epichloë-infected ryegrass

During an Argentine stem weevil (ASW-*Listronotus bonariensis*) field trial in New Zealand, which involved a range of *Epichloë* infected ryegrass plants, SBF protection was observed only in ryegrass plants infected with AR48 or AR47 that presented with fewer insect damaged tillers and/or insect killed tillers then the other endophyte infected plants (Introduction 1.1.3). This SBF bioactivity was confirmed in subsequent SBF trials (Alison Popay, AgResearch; personal communication). A SBF whole plant choice (SWPC) trial was set up at Grasslands AgResearch Palmerston North to confirm these results (Method 2.5.3). The strains tested in this SBF whole plant choice (SWPC) trial were AR48 and the closest related strain (by simple sequence repeat (SSR) analysis) AR47, the two commercial strains AR1 and AR37, and the control strain common toxic (CT) and endophyte–free (nil) (Results 3.1.1). In preparation for the

SWPC trial, 20 infected plants of each endophyte strain were trimmed to a length of approx. 15 cm, and grouped into replications with one endophyte strain infected plant per replication. The replications were then randomised, and covered with a netted bag. At the start of the trial, SBF adults were captured from surrounding AgResearch Grasslands (Palmerston North) fields using a net, sorted and sexed at Massey University Drosophila Laboratory, and then added to the netted plants with 20 male and 20 female flies per replication. The plants were then bottom watered for 6 weeks over one breeding cycle to give time for eggs to be laid in the tillers, hatch, and the emerging larva to feed on the inner tissue before pupating at the base. The plants were then uncovered and assessed for damage based on the presence of mines (tiller damage), presence of pupae, and pupae collected to assess fly emergence (Figure 3.22).

Both the control strains CT and nil plants had the presence of mines and pupae, with pupa successfully emerging when collected into Eppendorf tubes. For both strains approximately 40% of the tillers per plant had insect damage in the form of a mine with typically one mine per tiller. Again for both strains, nearly 100% of the mines contained a pupa, and approximately 80% of the collected pupae emerged. Both commercial strains AR1 and AR37 infected plants sustained insect damage at similar levels as the CT and nil plants, and the remaining parameters tested were also at similar levels as the CT and nil plants. AR48 and AR47 infected plants sustained little to no insect damage *i.e.* approximately 2% of tillers per plant had insect damage. This difference was statistically significant. For AR47 the tillers with mines did have pupae present at similar levels to CT, nil, AR1 and AR37, and the pupae present did emerge at similar levels to CT, nil, AR1 and AR37. AR48 had statistically fewer pupae per mine than all other strains and statistically fewer emerging pupae per mine then all other strains apart from AR47 (Figure 3.23).

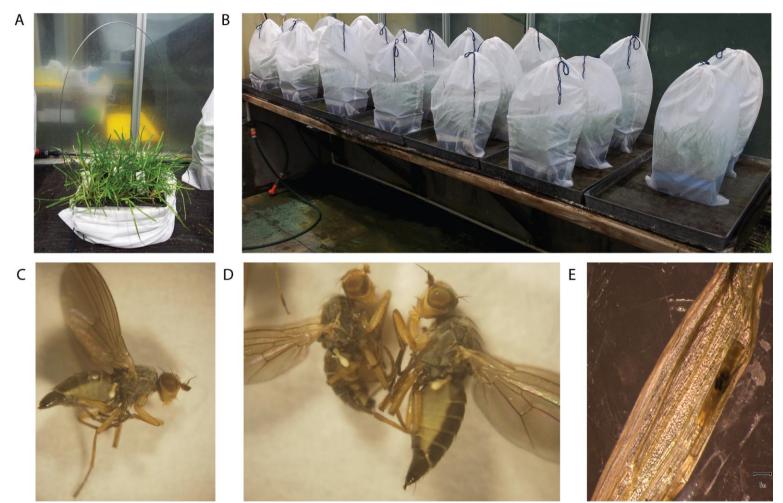


Figure 3.22: Stem boring fly (SBF-*Cerodontha australis*) whole plant choice trial (SWPC) set up. A) One replication containing 6 plants covered with netting that is supported by a wire. B) All 20 replications in the glasshouse. C) Side view of a SBF. D) Male SBF (Left) and SBF fly (Right). E) Larva/caterpillar in ryegrass tiller with surrounding leaf damage (mine).

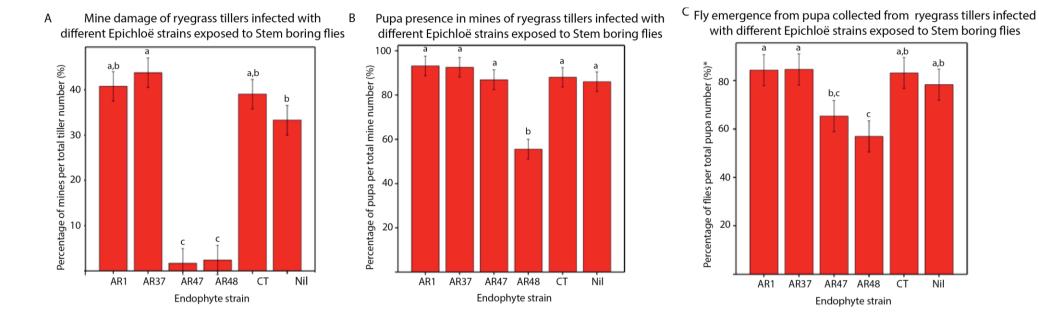


Figure 3.23: Stem boring fly (SBF-*Cerodontha australis*) whole plant choice trial (SWPC) results. A range of *E. festucae* strains infected into ryegrass were assessed for bioactivity by exposing the 20 plants of each endophyte type, one plant of each endophyte per replication with 20 male and 20 female SBF added to each replication, over the breeding season for 6 weeks in a glasshouse. A) Tiller damage inflected by feeding larvae that form mines. B) The presence of pupae at the base of each mine. C) The ability of the adult fly to hatch from the pupae, assessed by collecting the pupae in Eppendorf tubes (\*only the first 10 replications were assessed). Statistics were done by one-way analysis of variance (ANOVA) using GenStat 17th edition (VSN International). All results presented are averages of 20 replications. CT= Common toxic. Nil= no endophyte present.

# 3.3.2 Design and perform a cutworm moth caterpillar (Agrotis ipsilon) whole plant choice bioactivity trial on a range of wild type Epichloë-infected ryegrass

Two separate laboratory CC trials both conducted at Purdue University in the USA, also identified CC bioactivity in only AR48 and AR47 infected ryegrass (Introduction 1.1.3). Again, a whole plant choice trial was set up at Grasslands AgResearch in an attempt to replicate this CC bioactivity (CWPC) (Method 2.5.6). The same experimental design used for the SWPC trial was used in CWPC trial, but with the addition of *E. festucae* strains Fl1 and Fg1; both of these strains are predicted to have functional copies of *mcf* but with Fg1 containing a AR48 *mcf* allele and Fl1 containing a Fl1 *mcf* allele. In addition, only Fg1 infected ryegrass contains the new IDT compound three (Results 3.1). CC were obtained by catching cutworm moths (CM) using a light trap with hatched caterpillars raised on detached dock leaves. As for SBF, 20 replications were set up with plants prepared in the same manner but with the addition of counting the number of tillers per plant before the start of the trial. To each replication, 40 CC were added and plants bottom watered for 2 weeks to allow for insect damage to occur by the feeding CC. The plants were then assessed for difference in tiller number, and overall plant damage using a visual score system (Figure 3.24).

When the plants were assessed for changes in tiller numbers after CC exposure, the strains clustered into three groups; group 1 which contains strains not statistically significantly different to nil *e.g.* nil, Fg1, AR47, and AR48; group 2 which contains strains statistically significantly different to nil and some but not all of the other *E. festucae* strains *e.g.* CT, AR37, and AR1, and group 3 which contains strains statistically significantly different to all other strains *e.g.* Fl1. Group 1 had the largest tiller loss, and group 3 had the smallest tiller loss or largest tiller gain. This same clustering pattern was observed when the plants were visually scored for damage, however the least damaged plants were group 3 and the most damaged plants were group 1 (Figure 3.25).

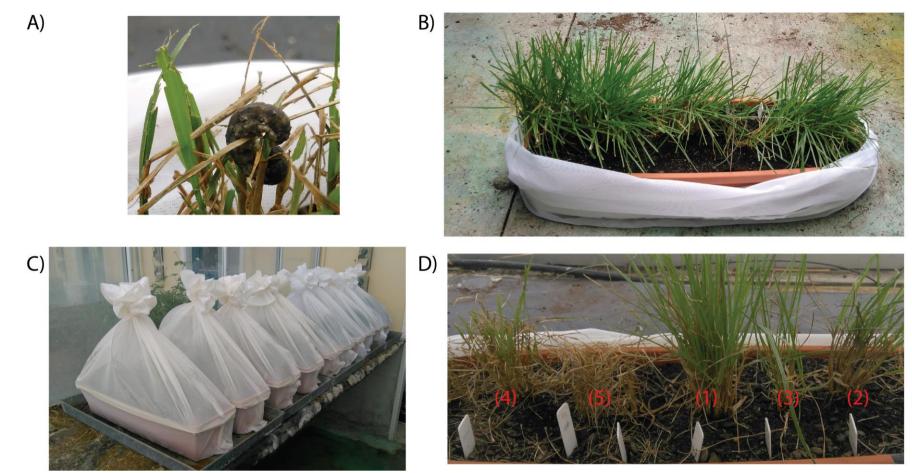


Figure 3.24: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) whole plant choice trial (CWPC) set up. A) CC on a ryegrass tiller. B) One replication before the start of the insect trial containing 6 plants covered with netting that is supported by a wire. C) All 10/20 replications in the glasshouse, with the remaining 10 replication mirrored on other side of glasshouse. D) One replication at the end of the insect trial with example of each visual score value (1= No damage. 2= 10% damage. 3= 50% damage. 4= 80% damage. 5= Dead).

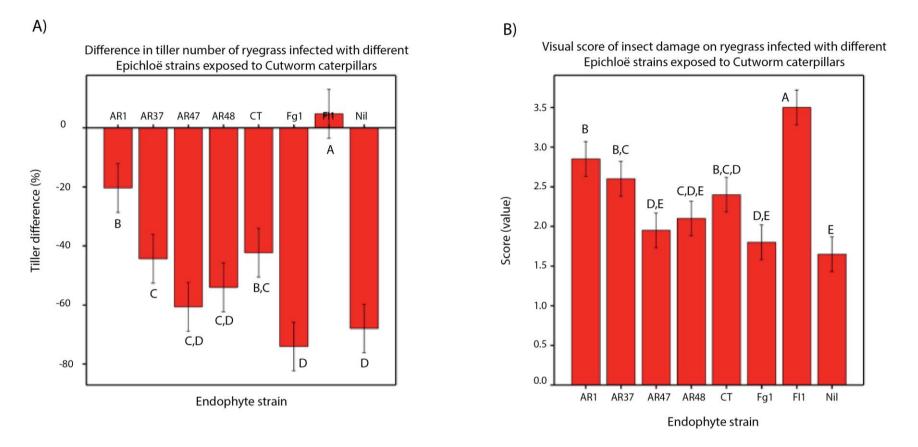


Figure 3.25: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) whole plant choice trial (CWPC) results. A range of *E. festucae* strains infected into ryegrass were assessed for bioactivity by exposing the 20 plants of each endophyte type, one plant of each endophyte per replication with 40 CC added to each replication, for 2 weeks in a glasshouse. A) Tiller difference before and after CC exposure. B) Visual score value given to each plant where 1= Dead. 2= 80% damage. 3= 50% damage. 4= 20% damage. 5= No damage. Statistics were done by one-way analysis of variance (ANOVA) using GenStat 17th edition (VSN International). All results presented are averages of 20 replications. CT= common toxic. Nil= no endophyte present.

# 3.3.3 Design and perform a cutworm moth caterpillar (Agrotis ipsilon) detached tiller no choice bioactivity trial on a range of wild type and mcf deletion Epichloë-infected ryegrass

The trials conducted in Purdue both demonstrated AR48 and AR47 infected ryegrass has bioactivity towards CC (Introduction 1.1.3). However, ryegrass infected with both these strains showed no statistically significant difference to nil ryegrass in the Grasslands CWPC trial (Results 3.3.2). As the USA CC trials were detached tillers, a second experimental design, CC detached tiller no choice (CDTN) trial, was designed based upon the USA trials methodologies (Method 2.5.9). This CDTN trial was therefore set up in an attempt to replicate this bioactivity at Grasslands AgResearch.

For the CDTN trial, the same endophyte strains were used as for the CWPC trial with the exception of AR47 which was removed and Fg1  $\Delta mcf$  (Results 3.2.6) which was included. The plants used in this trial were located in two different glasshouses. A PC1 glasshouse that contained 5 plants each of the following endophyte strains: AR48 wild type (WT), Fg1 WT, AR37 WT, Fl1 WT, CT WT, and nil. A PC2 glasshouse that contained 3 plants each of the following endophyte strains: Fg1 WT and Fg1  $\Delta mcf$ . For the PC1 glasshouse plants, the bioactivity of each endophyte strain was assessed using 5 biological replications *i.e.* 5 different plants labelled 1, 2, 3, 4, and 5, as well as 3 technical replications *i.e.* 3 caterpillars per plant with plant 5 having 4 caterpillars labelled A, B, C, and D, so that a total of 16 caterpillars were used for each endophyte strain *e.g.* 1A, 1B, 1C etc. For the PC2 glasshouse plants, the bioactivity of each endophyte strain was assessed using 3 biological replications *i.e.* 3 different plants, as well as 3 technical replications *i.e.* with 3 caterpillars per plant, so that a total of 9 caterpillars were used for each endophyte strain (Method 2.5.9).

In preparation for the trial, CC were obtained from eggs laid by captured cutworm moths (CM), and then weighed and ranked by weight. From this population, 114 CC were selected with the smallest weight variability between them and endophyte strains were assigned throughout the weight range *e.g.* AR48 plant 1 caterpillar A, Fg1 plant 1 caterpillar A etc. then AR48 plant 1 caterpillar B, Fg1 plant 1 caterpillar B etc. The CDTN trial was performed in an air-conditioned laboratory with CC kept in

containers filled with bark (Figure 3.26). Each day, CC were weighed, the old detached tiller removed and weighed, and new detached tiller weighed and added. At the end of the 8 day trial, the change in CC weight, change in tiller weight, and CC survival rate was then calculated and statistically analyse (Method 2.5.9).

The nil endophyte ryegrass treatment was statistically significantly higher than the none (no ryegrass) treatment, being 75% survival versus 0% survival respectively. For the PC1 glasshouse plants, all strains apart from Fg1 had statistically significantly lower survival rates then nil. While there was large variability in the survival rates between endophyte strains, *i.e.* 15% to 80%, none of the strains were statistically significantly different to each other. For the plants maintained in the PC2 glasshouse, the survival rate of Fg1 and Fg1  $\Delta mcf$  was not statistically significantly different to one another. The survival rate range of the PC2 plant material fed CWPC was much narrower and higher than the PC1 plant material fed CWPC at 80% to 100% in contrast to 15% to 80% respectively (Figure 3.27).

In addition to survival rate, the daily weight change of the CC was assessed. Again, the artificial environment was able to sustain the CC as all CC, apart from the none (no ryegrass) treatment, had a positive weight change trend over the assessed period. This difference between none and the remaining samples was statistically significant. For the plants maintained in the PC1 glasshouse, Fl1 was the only strain that had a gain in weight that was statistically significantly lower in rate than the remaining plant material, *e.g.* nil, AR48, Fg1, AR37, and CT, with Fl1 at 4.5 mg in contrast to 4.75 mg to 5.25 mg (log weight scale) for the remaining strains, on the final day. All other strains did not have statistically significant different daily weight changes compared to nil across the assessed time period. For the plants maintained in the PC2 glasshouse, there was no statistically significant difference in daily weight change for Fg1 and Fg1  $\Delta mcf$  samples. The PC2 CC had a higher daily weight change then the PC1 CC over the assessed time period (Figure 3.28).

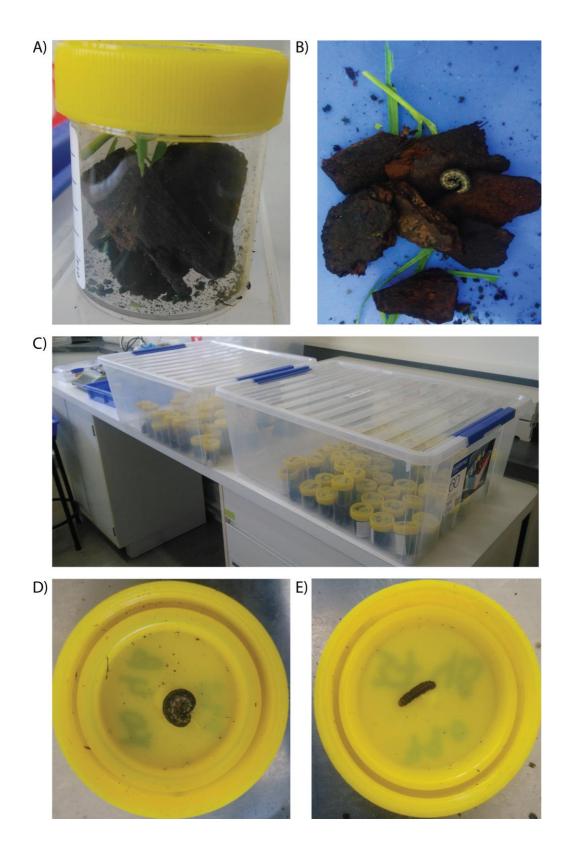
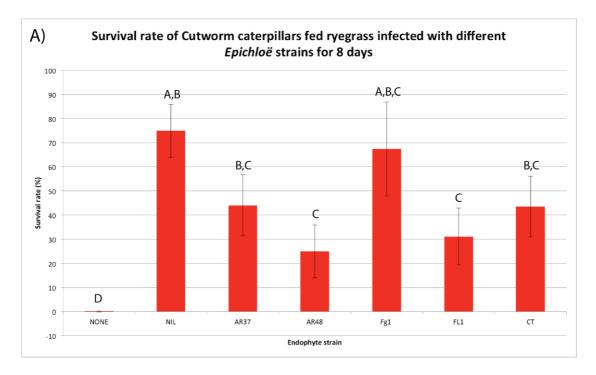


Figure 3.26: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) detached tiller no choice trial (CDTN) set up. A) and B) A single sample containing bark, caterpillar, and detached tiller. C) Layout of trial on laboratory bench. D) Healthy alive CC. E) Dead CC.



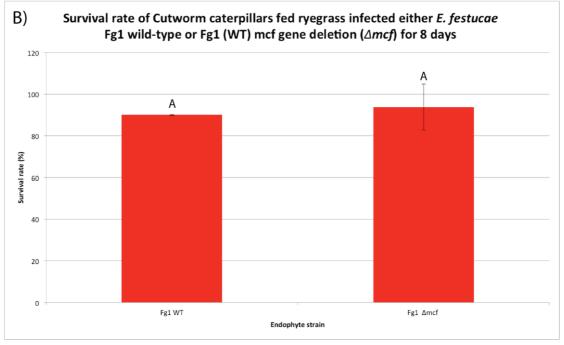


Figure 3.27: Cutworm moth caterpillar (CC-Agrotis ipsilon) detached tiller no choice trial (CDTN) survival rate (%) results. CC, kept in containers in an air-conditioned laboratory, and were fed detached tillers of ryegrass infected with a range of *E. festucae* strains every 24 hours over 8 days. A) Survival rate of caterpillars fed plants maintained in a PC1 Glasshouse containing a range of *Epichloë* strains. All values are averages of 16 replications. B) Survival rate of CC fed plants maintained in a PC2 glasshouse containing WT and delta-mcf FG1. Statistics were done by one-way analysis of variance (ANOVA) using GenStat 17th edition (VSN International). All PC1 data presented as an average of 16 replications and PC2 data presented as an average of 9 replications. CT= common toxic. WT= wild type. Nil= no endophyte. None= no ryegrass.

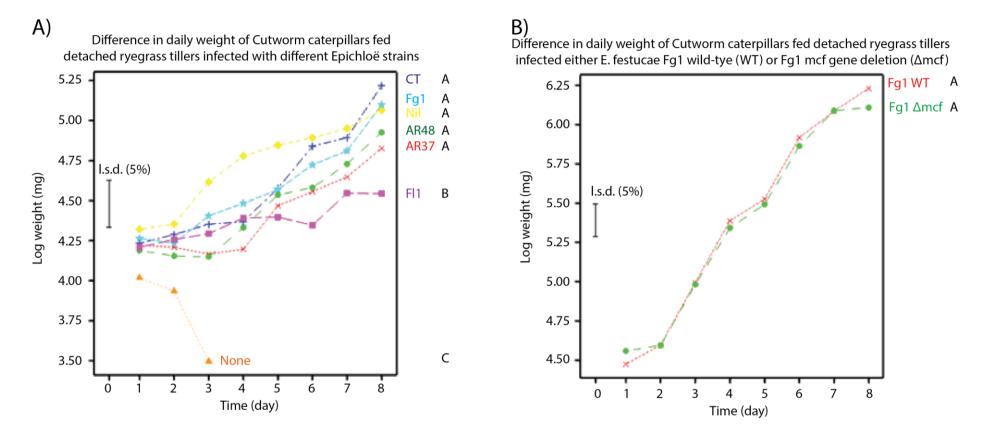


Figure 3.28: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) detached tiller no choice trial (CDTN) daily weight change results. CC, kept in containers in an air-conditioned laboratory, and were fed detached tillers of ryegrass infected with a range of *E. festucae* strains every 24 hours over 8 days. A) Daily weight change of CC fed plants kept at a PC1 glasshouse. B) Daily weight change of CC fed plants kept at a PC2 glasshouse. Statistics were done by repeated measurements linear mixed model using GenStat 17th edition (VSN International). All PC1 data presented as an average of 16 replications and PC2 data presented as an average of 9 replications. CT= common toxic. WT= wild type. Nil= no endophyte. None= no ryegrass.

# 3.3.4 Design and perform a porina caterpillar (Wiseana spp) detached tiller no choice bioactivity trial on a range of wild type Epichloë-infected ryegrass

Porina (*Wiseana* spp) is a major ryegrass pest in New Zealand, and *Epichloë* infected ryegrass is known to be bioactive against the porina caterpillar (PC) most likely through the IDT class *epoxy*-janthitrems that are produced by a set of *idt* genes including *janD* (Hennessy *et al.*, 2016) (Sarah Finch, AgResearch; personal communication). The absence of *janD* in both genomes of AR48 and Fg1 suggest that these strains cannot synthesise *epoxy*-janthitrems and this was validated by mass spectrometry (MS) analysis of AR48 and Fg1 infected ryegrass (Results 3.1). An overseas trial has shown AR48 to have bioactivity against the CC, which is yet to be repeated in New Zealand. It is therefore possible that the bioactive that targets CC is also able to target other species of Lepidoptera such as the PC (Introduction 1.1.3, and Results 3.3.2 and 3.3.3).

A porina caterpillar detached tiller no choice (PDTN) trial was therefore set up to test this bioactivity at Grasslands. The same method used for the CDTN trial was used for the PDTN trial, except the PC obtained from porina moths (PM) caught using a light trap, were raised on an artificial diet instead of dock leaves. The strains tested for this trial were AR48, Fg1, Fl1, AR37, and nil, and all the endophyte infected plants were maintained in the same PC1 glasshouse prior to detachment of tiller. Each endophyte strain had 9 replications; 3 plants and 3 caterpillars per plant. The PDTN trial was conducted in a temperature controlled growth chamber at 18°C (Method 2.5.9).

The PDTN trial was successful as the PC fed on the nil treatment had a positive weight gain trend over the entire trial ending at approximately 32.5 mg, while the PC fed AR37 (which is known to affect PC) had a negative weight loss trend ending at approximately 26.0 mg. This difference was statistically significant. Of the remaining strains, AR48 was the only strain that was statistically significantly different to nil, being not statistically significantly different to AR37. The amount of detached tiller consumed over the entire trial was also analysed for each strain. All endophyte-infected detached tillers were statistically significantly different to nil, with less detached tiller consumed, *e.g.* approximately 0.5 mg to 1.20 mg for endophyte infected in comparison to 1.60 mg for endophyte free, respectively. Fl1 was statistically significantly different to AR37 but not AR48 and Fg1, and AR37 was statistically significantly different to Fl1 but not AR48 and Fg1 (Figure 3.29).

- A) Daily caterpillar weight of Porina caterpillars fed artifical diet containing freeze-dried ryegrass tillers infected with different Epichloë strains
- B) Total amont of diet consumed by Porina caterpillars fed artifical diet containing freeze-dried ryegrass tillers infected with different Epichloë strains for 10 days

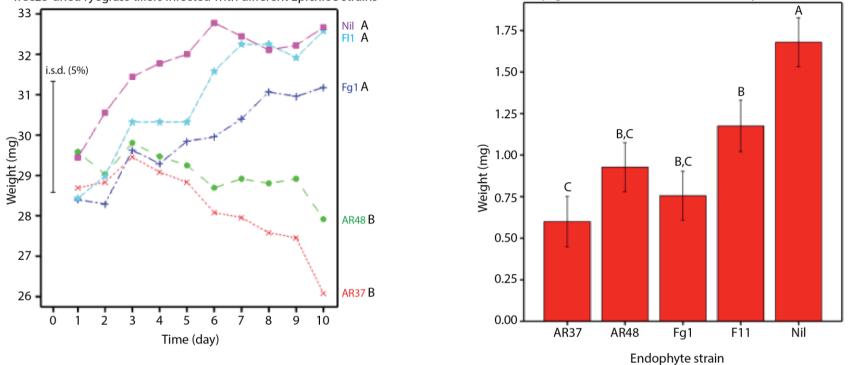


Figure 3.29: Porina caterpillar (PC-*Wiseana* spp) detached tiller no choice trial (PDTN) results. PC, kept in containers in a temperaturecontrolled chamber, were fed a detached tillers infected with different *E. festucae* strains over 10 days. A) Daily caterpillar weight. B) Total detached tiller weight consumed over 10 days. All values are averages of 9 replications. Statistics were done by either repeated measurements linear mixed model (A) or One-way analysis of variance (ANOVA) (B) using GenStat 17th edition (VSN International). Nilno endophyte present.

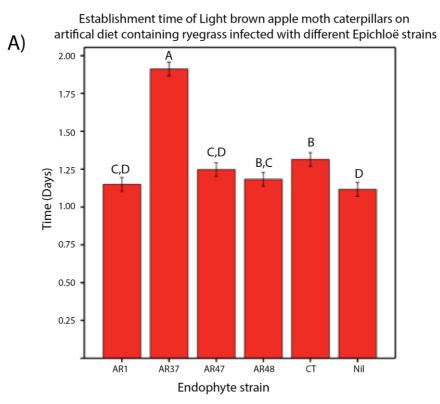
# 3.3.5 Design and perform a light brown apple moth caterpillar (Epiphyas postvittana) bioactivity trial on a range of wild type Epichloë-infected ryegrass

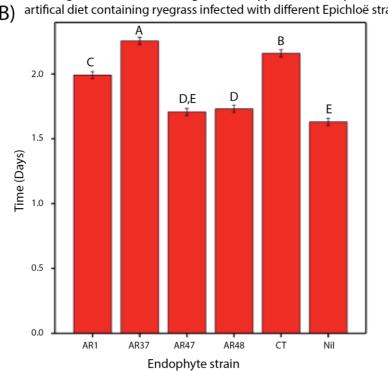
The light brown apple moth caterpillar (LBAM-Epiphyas postvittana) is not a pest of ryegrass but is currently being developed at AgResearch as a "universal" insect bioactivity indicator in a LBAM artificial diet no choice (LADN) trial. The LBAM, provided by Plant and Food New Zealand, can be easily and quickly bred under laboratory conditions in large numbers throughout the year, making the insect an ideal model system (Alison Popay, AgResearch; personal communication). The LBMA were raised on an artificial diet in clear screw top containers filled 2/3 with bark in a temperature-controlled room and fed artificial diet containing different endophyte infected ryegrass. One restriction of the artificial diet is that the caterpillars can only be raised up to the final moult and therefore do not form pupae and emerge into moths *i.e.* approximately 2 to 3 weeks before naturally dying off. Over this time period, the caterpillars are observed for the timing of establishment, identified when the caterpillars produce a silk cocoon, as well as the timing of the first, second, and third moults, identified by size change and lighting of the head. The testing agent can be deemed as being/containing an insect bioactive when the caterpillars do not establish or do not moult, have reduced establishment and/or moulting times, and/or die prematurely. All times are in relation to a blank control *i.e.* nil endophyte infected ryegrass or the suspension reagent of the purified compound (Method 2.5.12).

This trial was performed with freeze-dried ryegrass containing: AR48 and AR47 to test the robustness of the "universal" insect bioactivity indicator as well as whether AR48/AR47 have bioactivity against other Lepidoptera species outside grass pests. AR37 was included as a positive control as previous trials with this strain have shown large increases in both the time taken to establish and moulting. Nil was included as a negative control as there is no effect on establishment or moulting of LBAM (Alison Popay, AgResearch; personal communication).

For establishment time, only AR37 had a statistically significant increased time at approximately 1.90 days in relation to all the other strains at approximately 1.25 days. For the remaining strains, AR48, AR47, AR1, CT, and nil, only AR48 and CT were

statistically significantly different to nil. However, AR48 was not statistically significantly different to both AR47 and AR1. For the timing of the first moult, AR37, CT, and AR1 were statistically significantly different in relation to each other at approximately 2.25 days, 2.20 days and 2.00 days respectively, as well as to the remaining strains AR47, AR48, and nil which were at approximately 1.75 days). For the remaining strains, only AR48 was statistically significantly different to nil but not to AR47. For the timing of the second moult, AR37 and CT were both statistically significantly different to all other strains but not to each other at 2.25 days. AR1 was statistically significantly different to all strains at 2.20 days. For the remaining strains at approximately 2.15 days, again only AR48 was statistically significantly different to nil but not to AR47. Finally for the timing of the third moult, only AR48, AR47, and AR1 underwent a third moult within the assessed period of 16 days. This timing was not statistically significantly different between these strains (Figure 3.30).





Timing of the first moult of Light brown apple moth caterpillars on B) artifical diet containing ryegrass infected with different Epichloë strains

8

6

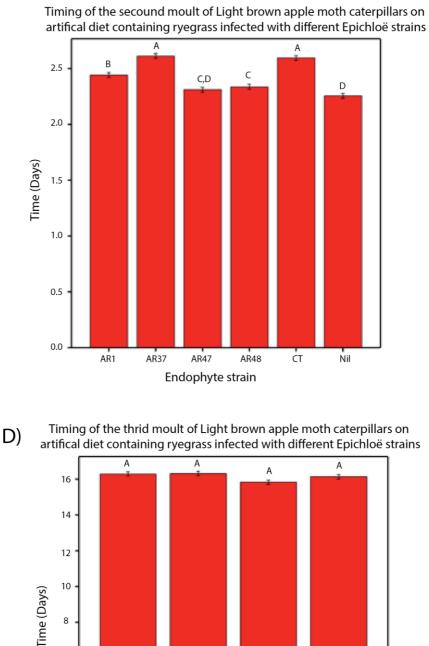
4

2

0

AR1

C)



Timing of the thrid moult of Light brown apple moth caterpillars on

Endophyte strain

AR47

Figure 3.30: Light brown apple moth caterpillar (LBAM-Epiphyas postvittana) artificial diet no choice trial (LADN) results. LBAM, kept in containers in a temperature controlled room, were fed an artificial diet containing carrot juice, agar, and freeze-dried ryegrass infected with different strains of *E. festucae* strains over 16 days. A) Time taken to establish a silk cocoon. B) Time taken to undergo first moult. C) Time taken to undergo second moult. D) Time taken to undergo third moult. All values are averages of 25 replications. Statistics were done by oneway analysis of variance (ANOVA) using GenStat 17th edition (VSN International). Nil= no endophyte present.

AR48

Nil

# **4.0 DISCUSSION**

# 4.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1

### 4.1.1 Fg1 as a model AR48 strain

For *Epichloë*, a common technique used to assess the role of a candidate gene in a specific process is through targeted gene deletion and resultant phenotype assessment *i.e.* reverse genetics, *e.g. noxA* deletion in *E. festucae* Fl1 strain, when inoculated into ryegrass seedlings, results in loss of the maintenance of the plant symbiotic interaction (Tanaka *et al.*, 2008). The sexual *E. festucae* strains in general are easier to create targeted gene deletions then the asexual *Epichloë festucae* var *lolii* strains. The reason for this is currently unknown but attempts to generate targeted gene deletions in *E. festucae* var *lolii* AR37 have to date been unsuccessful (Richard Johnson, AgResearch; personal communication), whilst a range of targeted gene deletions in *E. festucae* Fl1 have been successful (Scott *et al.*, 2012, Tanaka *et al.*, 2008, Charlton *et al.*, 2012, Becker *et al.*, 2015, Johnson *et al.*, 2013b). In addition, the sexual strains have a faster growth rate then the asexual strains, making an already lengthy process manageable, *e.g.* 5-7 days for sexual and 2-3 weeks for asexual, for regeneration of colonies.

The potential stem boring fly (SBF-*Cerodontha australis*) and cutworm moth caterpillar (CC-*Agrotis ipsilon*) bioactivity of makes caterpillars floppy (*mcf*) was investigated using reverse genetics. However AR48 is an asexual strain and therefore before target gene deletion could be performed an appropriate sexual model strain needed to be identified. Fg1 has an identical deletion in *perA* as AR48 (Young *et al.*, 2005) suggesting a close evolutionary relationship, which was then supported through simple sequence repeat (SSR) analysis that showed AR48 and Fg1 were in the same clade. Bioinformatics analysis followed by mass spectrometry (MS) identified that AR48 and Fg1 have identical profiles for both known *Epichloë* bioactive secondary metabolite (SM) pathways *i.e.* no ergot alkaloid or loline genes, a non-functional peramine gene, and all indole-diterpene (IDT) genes but non-functional *idtK* and *idtJ* and sequence diverged *idtP* and *idtQ*, as well as functional *mcf* gene. However, Fg1 in ryegrass does not have CC bioactivity, and the SBF bioactivity has currently not been assessed. Fg1 is

#### **Chapter four: Discussion**

therefore not an entirely equivalent AR48 model system. The lack of observed bioactivity in Fg1 could be due to the strain being present in a non-native host, which is known to affect endophyte gene expression levels (Leuchtmann *et al.*, 2000, Christensen *et al.*, 1997). The expression levels of the *mcf* gene (or Mcf protein) were not measured, and so additional experiments are required to assess this hypothesis.

The sexual model FI1 strain does have CC bioactivity whereas the SBF bioactivity is unknown. However, both the known *Epichloë* bioactive SM pathways and the *mcf* gene differ between FI1 and AR48, making FI1 a poor model for AR48. It is possible that FI1 infected ryegrass has a different CC bioactive present that is not present in AR48 infected ryegrass, which could lead to misinterpretation of *mcf* reverse genetic analysis, *e.g.* ergot alkaloids are suggested to have CC bioactivity and FI1 has a complete ergot alkaloid pathway (Potter *et al.*, 2008, Schardl *et al.*, 2013a).

# 4.1.2 AR48 and Fg1 in planta insect bioactivity potential predicted through bioinformatics

At the start of the study, the complete profiles of the known bioactive SM pathways in AR48 and Fg1 were unknown. The most effective method for assessing the potential capabilities of AR48 and Fg1 infected plants for the associated bioactivities of these pathways is to perform a bioinformatics analysis of the genes present in these pathways, followed by MS analysis of the corresponding compounds produced by these pathway genes. Although this does not directly assess AR48 and Fg1 in planta bioactivity towards these target insects, it does provide strong evidence for potential bioactivity, *i.e.* if the gene *perA* is predicted by bioinformatics analysis to be absent or non-functional and peramine is not detected in endophyte infected material by MS, then that endophyte infected plant most likely does not have Argentine stem weevil (ASW-*Listronotus bonariensis*) bioprotection. Of course the bioactivity can only be confirmed with an insect trial, but these types of experiments take a lot of time and can only be performed at certain times of the year.

The bioinformatics analysis of AR48 and Fg1 genomes identified the presence of all the peramine and lolitrem B pathway genes, and the absence of all the loline and ergot

#### Chapter four: Discussion

alkaloid pathway genes. The perA gene, although present, was non-functional and although all IDT genes were present only a partial lolitrem B pathway was detected in AR48 and Fg1 plant material, which agrees with the bioinformatics idt gene predictions (Young et al., 2009). In addition, the AR48 and Fg1 idt genes coincide with idt gene clusters in other IDT producing Epichloë strains, i.e. diversity is present in the presence and absence as well as functionality of the genes, but with the cluster order and gene order within each cluster generally well conserved (Young et al., 2009). However, idtP is isolated on a short single contig in the genome of AR48, but this is most likely a result of the fragmented AR48 genome assembly. Therefore *idtP* is expected to group with *idtQ*, *idtF*, *idtC*, and *idtB* in cluster 2 (Young *et al.*, 2009). Also due to the fragmented genome assemblies for both AR48 and Fg1, the order between each cluster as well as the distance between the clusters is unknown. AR48 does have an additional 5920 bp between *idtM* and *idtK* in cluster 1 in comparison to Fl1 and Fg1 (Young *et al.*, 2009), but this region does not contain additional genes. The only discrepancy in the IDT MS profile was that the Fg1 infected plant material contained late-pathway compounds in the lolitrem B pathway derived from IdtE and IdtJ, e.g. lolitrem K and lolitriol (Young et al., 2009) and these compounds were not identified in AR48 infected material. Given that both AR48 *idtE* and AR48 *idtJ* genes are 100% identical to the Fg1 *idtE* and Fg1 *idtJ* genes, it is assumed that these compounds are present in AR48 infected material but at levels below the limit of detection.

Based on the absence of the ergot alkaloid pathway and an inability to synthesise lolitrem B, AR48 and Fg1 infected plant material would be unlikely to have the mammalian bioactivity associated with the presence of ergovaline and lolitrem B, *e.g.* summer slump and fescue foot, and ryegrass staggers (Tor-Agbidye *et al.*, 2001), respectively. Conversely based upon a non-functional *perA* gene (peramine), and an inability to synthesise *epoxy*-janthitrems, these strains would not be expected to have the insect bioactivity associated with these compounds, *e.g.* ASW (Rowan *et al.*, 1990) and PC (Hennessy *et al.*, 2016) protection, respectively. In contradiction to this, AR48 infected ryegrass appears to have PC bioactivity, so this is therefore likely to be due to the presence of another bioactive compound with activity towards PC, possibly Mcf that is known to target a range of Lepidoptera caterpillars (Daborn *et al.*, 2002,

Waterfield *et al.*, 2003, Péchy - Tarr *et al.*, 2008). This highlights the limitation of the above screening method.

## 4.1.3 AR48 and Fg1 new indole-diterpene compound three structure predicted through mass spectrometry

During routine IDT MS analysis using targeted multiple reaction monitoring (MRMs) four novel IDT peaks, which are not normally present in the FI1 model IDT profile (Wade Mace, AgResearch; personal communication), were identified only in AR48 and Fg1 infected plant material. These peaks had characteristic IDT fragments and the same MRMs compared to known IDT compounds, but different retention times, and for some of the compound's different overall fragmentation patterns. The new IDT compounds with the same overall fragmentation patterns are most likely stereoisomers of known IDT compounds, *i.e.* the change in retention time is due to differences in the spatial arrangement of the same atom arrangement. This could be confirmed by comparing these new IDT compounds with stereoisomer standards of the corresponding known IDT compound and/or nuclear magnetic resonance (NMR) structure confirmation. While the new IDT compounds with different overall fragmentation patterns are most likely constitutional isomers, *i.e.* the change in retention time is due to differences in the arrangement of the same atoms. This could only be confirmed by NMR structure confirmation. Note that that stereochemistry of the standards used is not known.

The new IDT compound one has the same MRMs ratio as terpendole E but comes off the column 0.2 mins earlier and has a different fragmentation pattern then terpendole E. This peak is most likely intermediate 1 that is slightly less hydrophobic than terpendole E, though commonly not observed in the Fl1 IDT profile (Wade Mace, AgResearch; personal communication). This would be confirmed by comparing the new IDT compound one's fragmentation pattern and retention time with an intermediate 1 standard and/or intermediate 1 detectable strain. The new IDT compound two has the same MRMs as 13-desoxypaxilline but comes off the column 0.3 mins earlier and has a different fragmentation pattern. This peak is a true new IDT peak as there is no known IDT compound with this profile. The new IDT compound

three has the same MRMs as paxitriol but comes off the column 1 min later and has a different fragmentation pattern. This peak is also a new IDT peak as there is no known IDT compound with this profile. Finally, the new IDT compound four has the same MRMs and fragmentation pattern as terpendole I but comes off the column 0.1 mins earlier. This peak is most likely a stereoisomer of terpendole I around the hydroxyl groups, resulting in a change in retention time.

The structure of the new IDT compound three (paxitriol-like peak) was further investigated due to the large change in retention time and fragmentation pattern in comparison to paxitriol and terpendole E, which both have the same MRMs. This new IDT compound three contains three OH groups on the 6-ring IDT core structure, as supported by fragmentation of both the new compound three and paxitriol into 420 m/z, 402 m/z, and 384 m/z ions, in contrast to the fragmentation of terpendole E where a 384 m/z ion is not observed. The new IDT compound three therefore has the same chemical formula as paxitriol of C<sub>27</sub>H<sub>35</sub>NO<sub>4</sub> but appears to be rearranged to form a more hydrophobic structure.

Where the new IDT compound three differs to paxitriol is in the placement of these three OH groups. While paxitriol fragments to generate a 378 ( $MS^2$ ) ion, due to the loss of ( $CH_3$ )<sub>2</sub>CO, this ion is absent for the new IDT compound three. Instead the new IDT compound fragments to generate a 366 ( $MS^2$ ) ion due to the loss of C<sub>4</sub>H<sub>8</sub>O. Given these differences in fragmentation, OH group present on carbon 27 in paxitriol is suspected to be absent in the new IDT compound three. To generate the analogous fragmentation from paxitriol, first the OH group on carbon 27 needs to be removed, at the  $MS^2$  fragmentation level, so that subsequent fragmentation at the  $MS^3$  level generates loss of C<sub>4</sub>H<sub>8</sub>O. These differences support that the new IDT compound three is a constitutional isomer and not a stereoisomer of paxitriol.

The placement of the three OH groups can be inferred through the comparison of paxitriol and terpendole E fragmentation patterns. Terpendole E fragments to generate the unique 350 m/z ion because of the presence of a methyl group on carbon 12. In contrast, paxitriol does not have the methyl group on carbon 12 as it has been

reduced to a double bond between carbons 11 and 12 and does not generate the 350 m/z fragment ion. As the new IDT compound three does not generate the 350 m/z fragment ion, the new IDT compound must therefore have a double bond between carbons 11 and 12, and so the only logical places for the three OH groups are on carbons 10, 13, and 14, given that these sites have been observed to be hydroxylated in other *E. festucae* produced IDT compounds (Figure 4.2).

The results from the MS analysis suggest the new IDT compound three has the chemical formula of C<sub>27</sub>H<sub>35</sub>NO<sub>4</sub>. The new IDT compound three has three sites of hydroxylation at carbons 10, 13, and 14, and a double bond between carbons 11 and 12. The absence of the OH group on carbon 27 would result in the formation of a hydrophobic appendage on the new IDT compound three that would explain the longer retention time observed in relation to paxitriol and terpendole E.

### 4.1.4 AR48 and Fg1 new indole-diterpene compound three synthesis predicted through bioinformatics

All IDT producing fungi contain four core genes which are involved in the first four steps of each pathway; *idtG* encoding a geranylgeranyl pyrophosphate synthase, *idtC* encoding a geranylgeranyl transferase, *idtM* encoding a FAD dependent epoxidase, and *idtB* encodes a cyclase. IdtG converts isopentenyl diphosphate (IPP) and/or dimethylallyl pyrophosphate (DMAPP) and/or geranyl pyrophosphate (GPP) and/or farnesyl pyrophosphate (FPP) into geranylgeranyl pyrophosphate (GGPP). GGPP then undergoes indole condensation by IdtC with indole-3-glycerol phosphate (IGP) to produce 3-geranylgeranyl indole (3-GGI). Finally, 3-GGI then undergoes single or double epoxidation by IdtM depending on the pathway. These epoxidated compounds are then cyclized by IdtB by either Markovnikov or anti-Markovnikov mechanisms (Van de Bittner et al., 2018). Diversity is then achieved through regio- and stereo-specific decorations of these cyclized products. Epichloë strains that contain functional idtG, idtC, idtM, and idtB, IdtM perform either single or double epoxidation/s and IdtB performs anti-Markovnikov cyclisation (Saikia et al., 2006). Single epoxidation with anti-Markovnikov cyclisation leads to emindole SB formation, while double epoxidation with anti-Markovnikov cyclisation leads to paspaline formation (Van de

Bittner *et al.*, 2018). However, for both lolitrem and paxilline synthesis, paspaline is the major intermediate of the pathways not emindole SB (Saikia *et al.*, 2006, Young *et al.*, 2009). Diversity is then achieved through multiple oxidation steps, by the P450 monooxygenases IdtP and IdtQ, from the intermediate paspaline (Figure 4.1) (McMillan *et al.*, 2003, Saikia *et al.*, 2012).

The tentative structure for the new IDT compound three identified from AR48 indicates that the novelty is due to differences in the location of the hydroxyl groups decorated onto the paspaline backbone. IdtP is known to hydroxylate at carbons 10 and 12 while IdtQ is known to hydroxylate at carbons 11 and 13 (Saikia et al., 2012). The synthesis of the new compound would therefore involve the previously documented functions of IdtP and IdtQ, *i.e.* IdtP would hydroxylate carbon 10 and 12, and IdtQ would hydroxylate carbon 13, with the hydroxylation at carbon 14 performed by an unknown mechanism (Saikia *et al.*, 2012). The new IDT compound three is therefore the result of a new OH location combination as well as an OH and backbone combination not previously observed. All IDT compounds synthesised from paspaline in both the paxilline and lolitrem B pathways have a hydroxyl group on carbon 27 (McMillan et al., 2003, Saikia et al., 2012), however this is absent in the predicted structure of the new IDT compound three. This would suggest that the new IDT compound is derived from a precursor without the hydroxylated carbon 27 such as emindole SB, which is present in AR48 and Fg1 infected plant material at a level detectable by MS. However, ring I cannot form without this hydroxyl group. The predicted structure of the new IDT compound three therefore cannot be resolved from our current understanding of the lolitrem B pathway. Finally, the new IDT compound two (13-desoxypaxilline-like peak) might be part of the pathway which involves the new IDT compound three (paxitriol-like peak) given that the known IDT equivalent compounds, *i.e.* 13-desoxypaxilline and paxitriol, can both be modified into paxilline. The role of IdtP and IdtQ in the synthesis of the new IDT compound three was investigated using heterologous idt gene expression in different P. paxilli pax deletion ( $\Delta$ ) backgrounds. The presence of either AR48 *idtP* or *idtQ*, in a *P. paxilli*  $\Delta paxQ$  or ΔpaxP background respectively did not result in the synthesis of the new IDT compound three or other AR48 ldtP or ldtQ complementation specific IDT compounds.

This mirrors the previous findings using *idtP* and *idtQ* genes from Fl1 heterologously expressed in *P. paxilli*  $\Delta paxQ$  or  $\Delta paxP$  backgrounds, respectively (Saikia *et al.*, 2012). The failure to complement may be due to the first step post-paspaline in *Epichloë spp*. involves IdtQ, rather than IdtP as found in *P. paxilli*. This hypothesis would explain the absence of IDT products in the AR48 *idtP* complemented  $\Delta paxP$  background. However, one might expect to find some post paspaline products such as terpendole E in a AR48 *idtQ* complemented  $\Delta paxQ$  background but the competition for substrate from the *P. paxilli* PaxP in this background may result in very low levels of other IDTs (Figure 4.2) (Motoyama *et al.*, 2012).

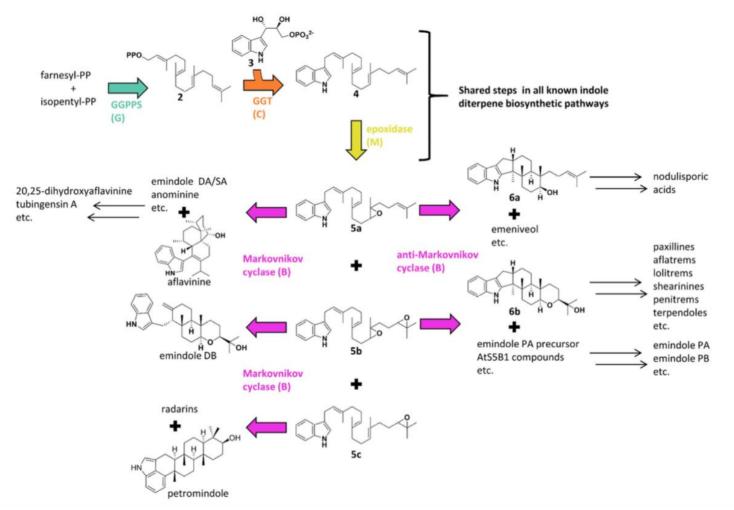


Figure 4.1: Schematic of the diversity of early pathway indole diterpene (IDT) compounds produced by the four core genes (G, C, M, and B- coloured) in fungi. Diversity is achieved through either markovnikov or anti-markovnikov cyclisation by M and single or double epoxidation by B (Van de Bittner *et al.*, 2018), taken from with permission from American Chemical Society.

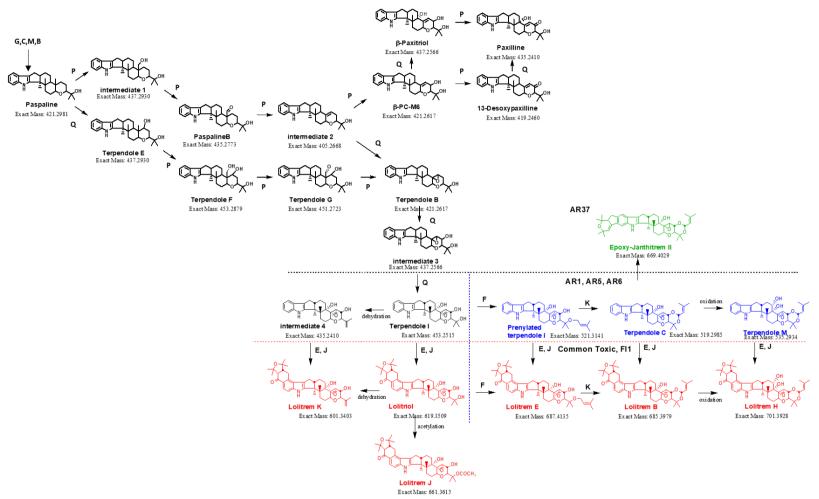


Figure 4.2: Simplified lolitrem B pathway in *Epichloë*. Genes (single letter *e.g.* indole-diterpene (IDT) = *idtP* = P) next to associated reaction. The section containing the end products of a strain is indicated. P/Q derived IDTs (black). E/J derived IDTs (red). F/K derived IDTs (blue/red). Epoxy-janthitrem IDT green).

# 4.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

#### 4.2.1 Mcf gene characterisation in Epichloë

Peramine and lolitrem B are both *Epichloë* bioactive SM however the characteristics of these two compounds are very different. Peramine is synthesised by a single gene *perA*, a non-ribosomal peptide synthetase (NRPS), that is not part of a cluster and is located in a GC rich non sub-telomeric region (Tanaka *et al.*, 2005). Lolitrem B is the end product of a biosynthetic pathway network involving 11 different genes (*idt*). These genes are located in a AT rich sub-telomeric region and group into three sub-clusters (Figure 4.3) (Young *et al.*, 2009, Saikia *et al.*, 2012).

The *perA* gene and the *idt* gene cluster have maintained syntony between and within *Epichloë* species (Tanaka *et al.*, 2005, Young *et al.*, 2009). The *perA* gene is located in all *Epichloë* strains analysed to date with different allelic variations identified with varying degrees of functionality (Tanaka *et al.*, 2005, Berry *et al.*, 2015). The lolitrem B cluster is not always present in *Epichloë* strains, and if present variation is seen in both the presence/absence of genes and functionality of the genes (Young *et al.*, 2009). Both the *perA* gene and *idt* gene cluster are widely distributed in the *Epichloë* genus and are unique to *Epichloë* (Tanaka *et al.*, 2005, Young *et al.*, 2009). Finally, peramine has insect bioactivity that targets specifically ASW (Rowan *et al.*, 1990), while lolitrem B has mammalian bioactivity that targets a range of grazing mammals, *i.e.* sheep and cattle (Gallagher *et al.*, 1981), and the intermediate compounds of this pathway have varying degrees of insect and mammalian bioactivity (Finch *et al.*, 2010).

Bioinformatics analysis of *mcf* within *Epichloë* identified that *mcf* is present in all analysed strains, which covered a wide range of species within the genus, and that there were two allelic variants of the *mcf* gene, with only one of the allelic variants present in each analysed strain but with varying degrees of functionality. The "AR48" allele has two introns and is predicted to contain two N-terminal cysteine protease domain (CPD1 and CPD2) and a central transmembrane domain (TMD). The AR48 allele

is identical to the *E. typhina* subsp *Poae Ps1 mcf* gene that has CC bioactivity (Ambrose *et al.*, 2014). The "Fl1" allele has only the second intron and is predicted to contain only one N-terminal CPD (CPD2), and a central TMD. While the Fl1 allele has been shown to be expressed we do not yet know whether the protein product functions as an endotoxin (Eaton *et al.*, 2015, Schardl *et al.*, 2013c). The presence of two *Epichloë mcf* alleles parallels to *P. luminescens mcf* which also has two *mcf* alleles; *mcf1* and *mcf2* (Dowling & Waterfield, 2007). The *mcf2* allele is slightly shorter than the *mcf1* allele at the N-terminal region (Dowling & Waterfield, 2007), and this reduction at the N-terminal region is also observed between the Fl1 *mcf* and AR48 *mcf* alleles respectively.

The *Epichloë mcf* gene is located in a GC rich region that is non sub-telomeric and has maintained syntony within the *Epichloë* genus in relation to the surrounding genes. These surrounding genes do not have a functional connection to Mcf regulation, synthesis, post-translational modification or transportation. The *Epichloë mcf* gene is therefore not part of a cluster (Figure 4.3). Although the original data (Ambrose *et al.*, 2014) suggested that *mcf* might be present in *Epichloë* as the result of a horizontal gene transfer (HGT) event, recent evidence suggests otherwise (Dupont 2017; Tian et al. 2017). Finally, the protein produced by *mcf* (Mcf), does not require additional genes to be active (Ambrose *et al.*, 2014). Mcf has insect bioactivity which currently has only been tested against CC (Ambrose *et al.*, 2014). The characterisation of *mcf*/Mcf therefore parallels *perA*/peramine and not *idt*/lolitrem B, when comparing the genomic location, functionality, and distribution as well as protein synthesis and bioactivity. A major difference between *perA*/peramine and *mcf*/Mcf is that the former is the metabolic product of an NRPS encoded by *perA* whereas Mcf is the direct translational product of *mcf*.

The *Epichloë mcf* gene and protein characteristics also parallels to the bacterial *mcf*/Mcf, but with some variation to the bacterial *P. fluorescence* insect toxin (*fitD*/FitD). Both *Epichloë mcf* and bacterial *mcf1/mcf2* genes are not part of a cluster, and the synthesis of Mcf1/Mcf2 has been shown to occur through a single gene (Daborn *et al.*, 2002, Waterfield *et al.*, 2003). However, *Mcf1* and *Mcf2* are in genomic

locations that are associated with type IV and type I secretory machinery genes respectively, and the Mcf1/Mcf2 have predicted secretory domains, *e.g.*RTX-like (repeat-in-toxin) exportation domains, that can be recognised by these systems (Daborn et al., 2002, Waterfield et al., 2003). However, there is no direct evidence for secretion of Mcf1/Mcf2 by these transport systems. In contrast to the bacterial mcf genes, the bacterial *fitD* gene is part of a cluster but additional cluster genes are involved in transport (*fitA, fitB*, and *fitC*) and regulation (*fitE, fitF*, and *fitG*), therefore fitD is still solely involved in FitD synthesis (Péchy - Tarr et al., 2008). The Epichloë mcf and bacterial *mcf1/mcf2* are present in all of the respective strains analysed to date and with allelic variants, but only *Epichloë mcf* and bacterial *mcf2* have maintained syntony in the gene genomic location (Dowling & Waterfield, 2007). In contrast, the fitD cluster, which also has fitD allelic variants, is only present in a select few strains, and has only maintained syntony within the cluster but not in the clusters genomic location (Péchy - Tarr et al., 2008). The bioactivity of both the Epichloë Mcf and bacterial Mcf/FitD proteins target insect species within the Lepidoptera family (Péchy - Tarr et al., 2008, Waterfield et al., 2003, Daborn et al., 2002, Ambrose et al., 2014).

#### 4.2.2 Proposed role of Epichloë Mcf

All Mcf-like proteins have the general domain composition of a N-terminal toxin domain, with the specific cytotoxic mechanism variable between strains, a N-terminal CPD, a central TMD and a variable C-terminal region that can be absent of domains or have secretion and/or receptor domains (Dowling & Waterfield, 2007). This general domain composition is also observed for the *C. difficile* TcdA and TcdB toxins (Reineke *et al.*, 2007), in which the Mcf TMD is commonly aligned to when analysed with online tools designed to predict protein domain structure, *i.e.* BLASTp, InterPro Scan, and Phyre2. Overall, the *Epichloë* Mcf protein domain composition has two N-terminal CPD (CPD1 and CPD2 labelled in order from the N-terminus), a central TMD and a variable C-terminal region with no domain predictions except for a secretion domain predicted for only the *E. festucae* Fl1 *mcf* gene. The difference between the two *Epichloë mcf* alleles is that the AR48 variant has both CPD1 and CPD2, while Fl1 variant has only the CPD2. The role of the *Epichloë* Mcf protein in providing insect bioprotection for

endophyte infected ryegrass was therefore proposed by combining the *Epichloë* domain composition, and how this relates to other Mcf like proteins which have experimental evidence for both location, target cells, and cellular mechanism, *i.e. P. luminescens* Mcf1 as well as *C. difficile* TcdA and TcdB toxins.

The *Epichloë mcf* gene is expressed by the endophyte when the endophyte is present in the plant host (Eaton *et al.*, 2015, Winter *et al.*, 2018). The protein has two possible locations; either secreted by an unknown mechanism, as no secretion domain or genes predicted to be involved in secretion were identified, or retained in the cytosol of the mycelia. Mcf is hypothesized to enter the insect's digestive tract passively, *i.e.* through ingestion of endophyte infected grass material that once in the midgut is chemically and physically digested resulting in release of Mcf regardless of the *in planta* location. This contrasts with the active delivery of the bacterial Mcf *i.e.* upon infection, the bacteria are regurgitated from the nematode into the midgut where Mcf is secreted by a type I/IV secretory system out of the bacteria (Dowling & Waterfield, 2007, Forst *et al.*, 1997).

Mcf either targets the midgut epithelial cells (Dowling *et al.*, 2004, Daborn *et al.*, 2002) through receptor mediated endocytosis (Sauerborn *et al.*, 1997), or is passively taken up through the natural absorption of nutrients by the midgut (Takeda, 2012). There is no C-terminal receptor domain predicted to be present in any of the *Epichloë* Mcf proteins, making receptor mediated endocytosis an unlikely mechanism. However, as this domain is most likely involved in the target insect specificity which could be unique to *Epichloë*, and given that the fungal *mcf* gene is not widely distributed or well-studied, this domain might not have been previously annotated and therefore would have been missed in the bioinformatics analysis. Assuming endocytosis of Mcf by the midgut epithelial cells, regardless of mechanism, acidification of the endosome will cause a conformational change resulting in insertion of the TMD into the membrane, and translocation of the N-terminal CPD1 into the cytosol (Qa'Dan *et al.*, 2000). Interaction with a cytosolic component or another stimulus, which could be another point for target insect specificity, will cause another conformational change

resulting in activation of the CPD2 and self-cleavage. This will release the N-terminally located CPD1 into the cytosol (Voth & Ballard, 2005, Jank & Aktories, 2008).

CPD domains are present in bacterial cytotoxic necrotizing factors (CNF) (Boquet, 2001). However, the presence of two N-terminal CPD domains has been predicted to be present in all Mcf proteins analysed in this study, apart from *P. fluorescens* FitD and the *E. festucae* Fl1 Mcf allele that only have CPD2. For CPD1, the bioinformatics prediction therefore might not be specific for the presence of CPD1, but more a prediction for the presence of a "cellular toxin domain" which could vary for each protein, *i.e.* the CPD1 domain overlaps with the BH3 and HrmA for Mcf1 and Mcf2 respectively (Dowling & Waterfield, 2007). The CPD2 is most likely a CPD required for self-cleavage, a key step in the cellular mechanism hypothesised for all Mcf like proteins (Figure 4.4).

The modification of the midgut epithelial cells by the Mcf N-terminal CPD1 (Dowling *et al.*, 2004, Daborn *et al.*, 2002) therefore most likely results in dysfunction of the midgut, the primary organ involved in osmoregulation (Giordana *et al.*, 1985), and this loss of osmoregulation presents as a "floppy'" phenotype (Ambrose *et al.*, 2014). The dysfunctional midgut may become leaky allowing Mcf to enter the haemocoel, and depending on target cell specificity, target a wider range of cell types, *e.g.* haemocytes, which would result in weakening of the insect immune system (Daborn *et al.*, 2002).

#### 4.2.3 Epichloë festucae Fg1 ∆mcf characterisation

To date, the focus of reverse genetic studies in *Epichloë* has typically been on characterising genes involved in the establishment and maintenance of the symbiotic interaction that forms between the endophyte and plant host as well as dissecting secondary metabolism. Analysis of the symbiotic interaction is then assessed through in culture and *in planta* macroscopic and microscopic phenotypes, and/or the secondary metabolism *in planta* profile (Tanaka *et al.*, 2008, Charlton *et al.*, 2012, Scott *et al.*, 2012, Johnson *et al.*, 2013b, Becker *et al.*, 2015, Saikia *et al.*, 2008).

For this project, a *mcf* deletion strain ( $\Delta mcf$ ) was created in Fg1, the genetically malleable AR48 model strain, to investigate the possible role of Mcf in the bioactivities observed in AR48 infected ryegrass. There was no obvious abnormal phenotypic variation between Fg1 wild type (WT) and Fg1  $\Delta mcf$  confirming that deletion of this gene had no effect on growth in culture or *in planta*. This would be expected for a gene encoding a bioprotective SM (Saikia *et al.*, 2012) but also shows that the proposed ectopic integrations have not affected important genes for overall fungal functionality. The Fg1  $\Delta mcf$  infected plants were therefore assessed for changes in insect bioactivity in relation to Fg1 WT infected plants. However, the lack of bioactivity of Fg1 WT infected plants was not known before the creation of the Fg1  $\Delta mcf$  strain or before the Fg1  $\Delta mcf$  infected plant insect bioactivity trial. As such, the Fg1  $\Delta mcf$  strain cannot be used to study the potential role of Mcf in insect bioactivity unless conditions can be established for suitable Mcf expression *in planta*, *e.g. mcf* might be expressed in the native plant host for Fg1 but not in perennial ryegrass.

#### 4.3 Test the insect bioactivity capabilities of AR48 and Fg1 infected

#### ryegrass

#### 4.3.1 AR48 and Fg1 new indole-diterpene potential bioactivity

There is strong evidence to suggest that the new IDT compound three has bioactivity against SBF because the new IDT compound three has been detected in AR48 and AR47 infected plant material and not in AR37 or AR1 infected plant material. This directly correlates with SBF bioactivity, confirmed in three independent feeding trials, presence in AR48 and AR47 infected plant material and absence in AR37 and AR1 infected plant material. The potential for the new IDT compound three to have CC bioactivity cannot be assessed due to the uncertain results obtained in this study from two different CC feeding trials. The lolitrem B pathway intermediate compounds, in which the new IDT compound three is predicted to be included, have a range of nonbioactive, insect bioactive, and mammalian bioactive activities (Parker & Scott, 2004, Saikia *et al.*, 2008, Imlach *et al.*, 2011). The different structures of these bioactive compounds cannot be correlated to the presence or absence of bioactivity, and if bioactive, mammalian or insect target species (Sarah Finch, AgResearch; personal

communication). As such, an insect trial using the purified AR48 new IDT compound three would need to be performed to unequivocally prove that the AR48 new IDT compound three is the causal agent for SBF bioprotection. In general the mechanism through which IDT compounds are bioactive is by binding to cellular ion channels causing disruption of functionality that results in loss of membrane potential and cellular death (Smith et al., 2000). The specificity of the IDT is dependent on the specificity of the ion channel that the compound interacts with, therefore IDT compounds that only disrupt ion channels present in insects and not mammals are ideal for plant bioprotective compounds, e.g. glutamate-gated chloride channels (Wolstenholme, 2012) and not calcium activated potassium channels (Imlach et al., 2011). Alternatively, if the IDT does interact with an ion channel present in both mammals and insects, e.g. sodium channel (Dong, 2007), if the presence of the IDT is high enough in the plant material for insect mortality or deterrence, but low enough in grass associated consumables to elicit no effect on animals, then the IDT could still be considered as a commercial bioprotectant (Bloomquist, 1996). Currently, there is no evidence of the AR48 new IDT compound three mode of action in terms of disruption of ion channels or an alternative mechanism.

#### 4.3.2 Epichloë Mcf infected ryegrass potential bioactivity

There is evidence from multiple experiments performed in independent laboratories that show that both the fungal and bacterial Mcf proteins have CC bioactivity (Ambrose *et al.*, 2014, Daborn *et al.*, 2002), however there is not a strong correlation between the presence of Mcf and the presence of CC bioactivity in this study. For both trials, Fl1 infected plant material had strong CC bioactivity, AR48, AR1, and AR37 infected plant material had no or weak CC bioactivity, and Fg1 infected material had no bioactivity. The Fg1  $\Delta mcf$  infected plants did not have CC bioactivity that is expected given that the Fg1 WT infected plants also do not have CC bioactivity. However, all strains had a *mcf* gene predicted to be functional, with AR48 and Fg1 having the AR48 *mcf* allele (Ambrose *et al.*, 2014), and the remaining strains (Fl1, AR1, and AR37) having Fl1 *mcf* allele. Overall, there was no correlation between presence or absence, functional or non-functional, or AR48 or Fl1 *mcf* alleles, and presence of CC bioactivity.

USA trials. Due to these ambiguous results the AR48 new IDT compound three possible role in CC bioactivity can also not be predicted (Table 4.1).

The major flaw in the experimental design of both CC feeding trials is that *mcf* expression levels and/or Mcf protein amount was not analysed before, during, or after the trials. In addition, the functionality of the different *mcf* allelic variants has never been assessed making it nearly impossible to predict the effect that Mcf has on CC bioactivity. As previously noted, the CC bioactivity observed in Fl1 infected plants may be due to the presence of ergot alkaloids which have been suggested to have CC bioactivity (Potter *et al.*, 2008) adding another variable to take into consideration.

In addition, how CC detached tiller no choice (CDTN) trial was performed could have influenced the outcomes. Although the CDTN trial was based upon the methodology of the USA trials, experiments were performed under non-ideal conditions. This could cause increased background variation resulting in unclear results. The PC1 plants were at different stages of growth as the Fl1 and Fg1 plants were inoculated into seedlings, which retards the initial growth, rather than planted from infected seeds like AR48, AR37, and CT. The PC2 plants were younger than the PC1 plants as the Fg1  $\Delta mcf$  strain was only confirmed just in time for inclusion in this trial, and so were inoculated into seedlings approximately 6 weeks after the PC1 plants. The 2017/2018 summer in New Zealand was both late and short. This delayed the breeding season of the cutworm moth (CM-Agrotis ipsilon) and reduced the window of CM availability. The trial could only have been performed late summer/early autumn, i.e. March/April, instead of mid/late summer, i.e. January/February. This meant that the plants were prepared for the trial too early, 5-6 months old rather than 3-4 months old, apart from the PC2 plants. Factors such as plant senescence, microbial infection, and the presence of additional insects (as the plants cannot be treated with chemical insecticides) might have affected the insect trial. These two factors, early plant preparation and delayed breeding season, were amplified when the Fg1  $\Delta mcf$  strain caused the trial to be further delayed, *i.e.* April/May. The SM levels are known to drop in winter due to reduced sunlight and temperatures, therefore the levels of the bioactive compounds

during the trial might have been at lower levels then present in the peak of summer (Wade Mace, AgResearch; personal communication).

In contrast to the CC whole plant choice (CWPC) and CDTN trials, the porina caterpillar (PC-*Wiseana* spp) detached tiller no choice (PDTN) trial detected PC bioactivity in AR48 infected ryegrass along with AR37 infected ryegrass, but not AR1, Fl1, and Fg1 infected ryegrasses, and as the PDTN trial was performed under more ideal conditions than the CDTN trial (all plants at 3-4 months old in the same glasshouse and during the middle of the porina breeding season), the AR48 PC bioactivity potentially could be due to the presence of Mcf. Although, *mcf* expression levels and/or Mcf amount would need to be assessed. In addition, the absence of PC bioactivity for Fg1 correlates to the hypothesized lack of *mcf* expression providing further evidence for *mcf* being a PC bioactive. Despite these limitations these results do suggest that Mcf has a broader target range that includes other Lepidoptera species (Table 4.1). This is supported by the literature, *e.g.* Mcf has been shown to have tobacco hornworm caterpillar (*M. sexa*) bioactivity (Daborn *et al.*, 2002).

By combining the PC and CC bioactivity results, the Fl1 *mcf* allele does not appear to have bioactivity against PC and therefore inferred lack of CC bioactivity. This is because the CC bioactivity in Fl1 infected ryegrass is most likely due to the presence of ergot alkaloids (Potter *et al.*, 2008) and not the presence of a functional Fl1 *mcf* allele. The ryegrass plants infected with AR37, which also has a Fl1 *mcf* allele, does not have CC bioactivity and does not produce ergot alkaloids. The PC bioactivity detected in AR37 infected ryegrass is due to the presence of *epoxy*-janthitrems, not the presence of a functional Fl1 *mcf* allele, and the absence of PC bioactivity in Fl1 infected ryegrass is due to the absence of *epoxy*-janthitrems (Hennessy *et al.*, 2016). Ryegrass infected with AR1, which has a Fl1 *mcf* allele, does not have CC and PC bioactivities, which correlates to the absence of ergot alkaloids or *epoxy*-janthitrems in AR1 infected material. Ryegrass infected with Fg1, which has the AR48 *mcf* allele, has no bioactivity resulting in changes in gene expression, *e.g.* downregulation of *mcf* (Leuchtmann *et al.*, 2000). Ryegrass infected with AR48, which has the AR48 *mcf* allele, has PC

bioactivity and should have CC bioactivity. Both activities could therefore be tentatively due to the presence of the AR48 Mcf protein.

Finally, AR48, AR37, and AR1 infected ryegrasses, but not AR47 infected ryegrass, did have light brown apple moth caterpillar (LBAM- *Epiphyas postvittana*) bioactivity detected in the LBAM artificial diet no choice (LADN) trial. This trial does not provide information on the bioactive but is designed as a "universal" insect bioactive producing strain identifier. The trial was therefore in most cases successful. Overall, the presence of the AR48 *mcf* allele correlates with the presence of PC bioactivity, and inferred CC bioactivity, in AR48 infected plants. Therefore, Mcf could be a general Lepidoptera caterpillar toxin. However, this hypothesis is very tentative and requires thorough investigation (Table 4.1).

## 4.3.3 Difference in AR48 and Epichloë festucae var Iolii AR47 infected ryegrass bioactivities

The two *E. festucae* var *lolii* strains AR48 and AR47 are very difficult to distinguish by SSR, and therefore are most closely related to each other compared to other strains in the AR48 clade. However, despite this very close relationship differences have been observed in insect bioactivity trials between AR48 and AR47 infected plants. AR48 infected ryegrass has ASW bioactivity whilst AR47 infected ryegrass apparently does not (Alison Popay, AgResearch; personal communication). Differences in bioactivity were also apparent in the insect bioactivity feeding trials performed in this study. AR48 had subtle differences in the SBF pupae survival rate in comparison to AR47, and AR48 had LBAM bioactivity and AR47 did not (Table 4.1). These differences could be due to a range of reasons such as gene expression levels, gene functionality, and presence or absence of additional genes within the genome. Further comparative analysis of AR47 and 48 is therefore warranted.

## 4.3.4 Potential alternative bioactives for AR48 infected ryegrass novel insect bioactivity

The SM profile of each *Epichloë* strain is both extraordinarily complex and exceptionally varied. The strain unique SM profiles account for a large proportion of

the diverse range of naturally occurring characteristics observed in *Epichloë* infected plants. This study has identified a new potential insect bioactive SM (the new IDT compound three) produced by AR48 and Fg1 strains that potentially has SBF. In addition, Mcf was also identified as a potential bioactive for CC and/or PC but with less certainty. However, due to the complexity of the SM profile of each strain, unless definitive evidence for the direct effect of a SM on a specific insect is provided, the insect bioactivity of interest could be due to the presence of other compound/s.

NRPS, polyketide synthase (PKS), and NRPS-PKS hybrids are commonly linked to bioactivity towards a range of organisms including insects. NRPS genes are the most commonly studied of the three. NRPSs have been identified in *Epichloë* as either being directly involved in the synthesis of the bioactive compound alone, *e.g.* Peramine produced by the NRPS *perA*, (Berry, 2011), or as being part of a more complex bioactive synthesis pathway, *e.g. lpsA*, *lpsB*, and *lpsC* which are involved in the production of a range of bioactive ergot alkaloids (Schardl *et al.*, 2012). There appears to be equal numbers of PKS and NRPS but very few NRPS-PKS genes (Schardl *et al.*, 2013c). Currently there is one NRPS-PKS bioactive product identified (Song *et al.*, 2016) and no PKS bioactive products identified in *Epichloë*. However, bioactive PKS products have been identified in other fungal species (TePaske *et al.*, 1992).

The paper published by Song (Song *et al.*, 2016) has detected and solved the structure of a PKS-NRPS compound (dahurelmusin A) produced by *Epichloë bromicola*, which has tentative aphid bioactivity. MS detected the same compound in AR48 infected ryegrass (Wade Mace, AgResearch; personal communication). A bioinformatics analysis identified only one PKS-NRPS gene in the genome of AR48 and therefore this compound is most likely the product of this gene, but further experimental analysis is required. This PKS-NRPS is therefore another potential bioactive which could be responsible for either the SBF or CC bioactivity of AR48 infected ryegrass. This also suggests that AR48 infected ryegrass might have aphid bioactivity.

#### 4.3.5 Comparison between different insect bioactivity trial methodologies

There are three main types of insect bioactivity trials performed in this study; whole plant choice (WPC) trial, detached tiller no choice (DTN) trial, and artificial diet no choice (ADN) trial. Each trial type has different advantages and disadvantages that need to be considered when assessing a specific insect bioactivity. Currently all insects, apart from the LBAM, are sourced from the New Zealand environment, therefore the trials can only be performed at set times during the year and can be heavily affected by seasonal variation in the insect populations (Table 4.2).

For the WPC trial the major advantage is that, apart from the plant preparation and insect preparation (which will vary depending on the bioactivity tested) the trial requires very little work once set up. Also, the WPC trial mimics the natural environment more effectively than the DTN and ADN trials. This is an advantage for assessment of naturally occurring insect pests. The WPC trial is more suited to assessing bioactives that deter insects rather than bioactives that are toxic to insects because the plants rather than the insects are assessed therefore the effect of the bioactive on the different stages of the insect's life cycle can be missed. However, this is dependent on the insect bioactivity assessed *i.e.* the WPC for SBF assessed the complete life cycle, but the WPC for CC did not collect any data on the effect of the bioactive on the CC, however the DTN for CC did (Table 4.2).

Conversely, the DTN and ADN trials can assess and distinguish between deterrent and toxin bioactives, and there is more focus on the insect assessment rather than the plant assessment, resulting in increased sensitivity and robustness. However, both the DTN and ADN preparation as well as the actual trial require a lot of work that can reduce the replication and/or sample numbers assessed in each trial. Both the DTN and ADN trials mimic the scenario encountered in a field planted with mono-cultivars where there is no choice. Therefore, insects that are agricultural pests could better suit these trials. The major disadvantage of both the DTN and ADN trials is that the use of detached tillers or artificial diet can result in inactivation of the bioactive leading to false negatives. Bioactive inactivation is less likely to occur in the DTN trial than the ADN trial as the bioactives are still within the plant material. Also, the detachment of

the tiller before ingestion is mimicked by the feeding behaviour of some insects (including CC and PC) so the corresponding bioactive would be expected to remain functional (Table 4.2).

The major disadvantage of the ADN trial is that each insect requires a specific artificial diet that needs to be designed before the ADN trial can be performed. Also, not all insect bioactives can be assessed using the ADN trial as some insect's natural environment might be hard to artificially recreate, *e.g.* the SBF larvae remain in the leaf tissue during development and so cannot be fed like the CC which develop in the soil. However, if a diet and appropriate artificial environment can be designed, and the added bioactive is not inactivated, the insect bioactivity can be assessed in a neutral chemical background resulting in very accurate bioactivity assessment. In contrast, in both the WPC and DTN trials, multiple potential bioactives are present simultaneously during bioactivity assessment that can lead to misinterpretation of results (Table 4.2).

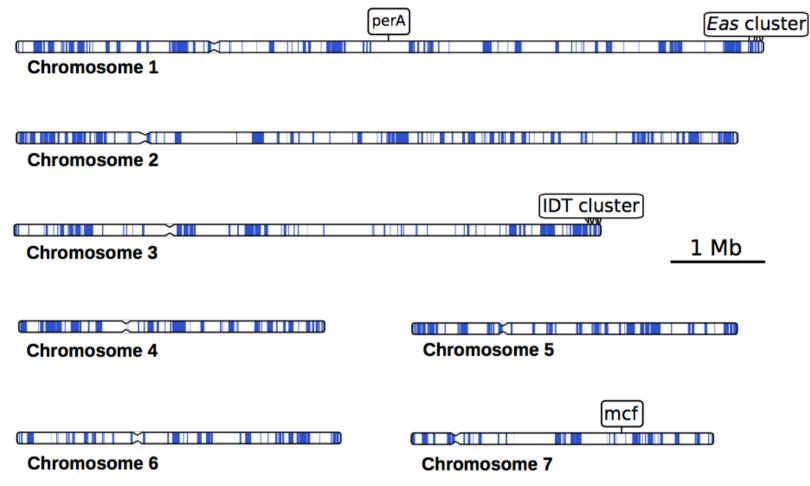


Figure 4.3: Graphical representation of the genomic location of secondary metabolite genes that produce bioactive compounds, in *Epichloë festucae* FI1 complete assembled genome. The gene locations are indicated by a labelled box where *perA* produces peramine, *Eas* cluster produces ergot alkaloids, *IDT* cluster products indole-diterpenes, and *mcf* products Mcf. The AT rich repeat regions are coloured blue, and GC rich regions coloured white.

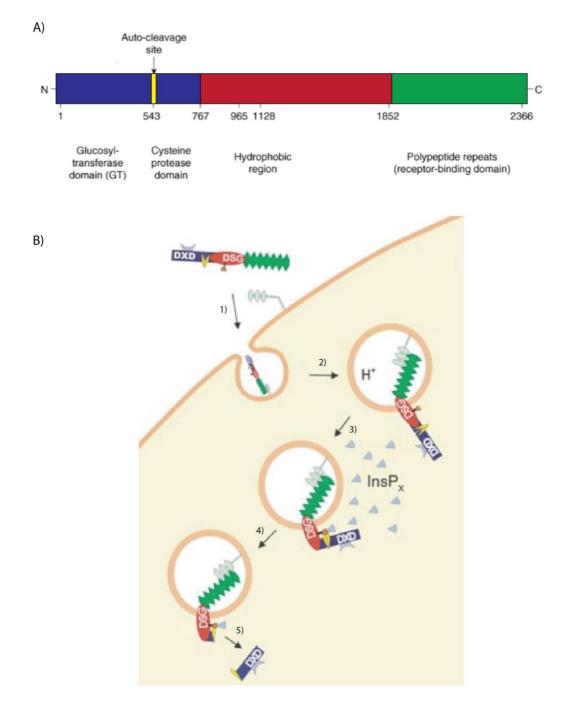


Figure 4.4: Proposed mode of action for the *Epichloë* makes caterpillars floppy (Mcf) protein based on the *Clostridium difficile* TcdA and TcdB toxins. A) Graphical representation of the TcdA/TcdB toxin domain composition. Domains are coloured, with amino acid number and domain labelled below. B) TcdA/TcdB mode of action. 1) Protein enters cell through receptor-mediated endocytosis via the receptor-binding domain (green). 2) Acidification of the endosome causes the first conformational change resulting in inserting of the hydrophobic region (red) and translocation of cytotoxic domain (blue) into the cytosol. 3) Interaction with host components leads to second conformational change resulting auto-cleavage via the cysteine protease domain (orange). 4) The released cytotoxic domain (blue) modifies key host components resulting in cell death via apoptosis. Modified from (Reineke *et al.*, 2007, Jank & Aktories, 2008) with permission from Springer Nature and Elsevier.

Table 4.1: Overall insect bioactivities of *E. festucae* strains against a range of insects using different methodologies.

			Grass		Fruit			
Epichloë	Diptera	-						
festucae	Larva/Caterpillar							
strain	SWC	CWC	CDN	PDN	LAN	4		
AR48	<i>」 」 」 」</i>	×	✓	<i>JJJ</i>	55			
AR47	J J J	×	N/A	N/A	×			
Fg1	N/A	×	×	×	N/A			
FI1	N/A	J J J	$\int \int \int$	×	N/A			
AR37	×	J	1	<i>\ \ \</i>	J			
AR1	×	1	N/A	N/A	1			

1. Plant material targeted by insect. 2. Insect order. 3. Insect life staged targeted by the bioactive. 4. Trial method where SWPC (stem boring fly-*Cerodontha* australis-whole plant choice), CWPC (cutworm moth caterpillar- *Agrotis ipsilon*-whole plant choice), CDTN (cutworm moth caterpillars detached tiller no choice), PDTN (porina caterpillar-*Wiseana* spp-detached tiller no choice) and LADN (light brown apple moth caterpillar-*Epiphyas postvittana*-artificial diet no choice).  $\checkmark \checkmark \checkmark$  strong activity,  $\checkmark \checkmark$  medium activity,  $\checkmark Weak$  activity,  $\times \mathbb{P}$  no activity, and N/A not assessed

#### Table 4.2: Comparison of the different parameters between the insect trials used in this study

	SWC	CWC	CDN	PDN	ALAN
	Sem boring fly whole plant choice	Cutworm moth whole plant choice	Cutworm moth detched tiller no	Porina detched tiller no choice trial	Light brown apple moth artifical
	trial	trial	choice trial		diet no choice trial
Insect					
Туре	Stem boring fly	Cutworm moth	Cutworm moth	Porina	Light brown apple moth
Life stage	Fly	Moth	Moth	Moth	Eggs
Collection	New Zealand environment	New Zealand environment	New Zealand environment	New Zealand environment	Plant and Food
Method	Nets	Light trap	Light trap	Light trap	Ordered
Processing	Sorted and sexed	Sorted	Sorted, sexed, ranked by weight	Sorted, sexed, ranked by weight	None
Trial					
Plant structure	Whole plant	Whole plant	Detched tillers	Detched tillers	Artifical diet
Insect	1 day old fly	3 weeks old caterpillars	3 weeks old caterpillars	3 weeks old caterpillars	1 day old caterpillar
Location	PC0 Glasshouse	PC0 Glasshouse	PC2 Laboratory	PC0 Temperature controlled	PC0 Temperature controlled
				chamber	chamber
Replications	20	20	PC1 16 (3 biological and 5+1	9	25
			technical) PC2 9 (3 biological and 3		
			technical)		
Endophyte strains per	6	8	PC1 7 and PC2 2	5	6
trial					
Endophyte strains per	6	8	1	1	1
replication					
Insects per replication	20 male and 20 female	40 mixed	1	1	1
Duration	6 weeks	2 weeks	8 days	10 days	16 days
Measurements	Mine number, pupa number, fly	Plant tiller difference, plant visual	Caterpillar weight, tiller weight	Caterpillar weight, tiller weight	Establishment time, molt time
	emergence	score			-

SWPC (stem boring fly-*Cerodontha australis*-whole plant choice), CWPC (cutworm moth caterpillar- *Agrotis ipsilon*-whole plant choice), CDTN (cutworm moth caterpillars detached tiller no choice), PDTN (porina caterpillar- *Wiseana spp*-detached tiller no choice) and LADN (light brown apple moth caterpillar-*Epiphyas postvittana*-artificial diet no choice)

#### **5.0 CONCLUSION**

## 5.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1

The known bioactive secondary metabolite (SM) pathways in *Epichloë* were successfully analysed in the *E. festucae* strains AR48 and Fg1. The genomes of both strains were first sequenced, and then analysed for the presence and functionality of each gene involved in the four pathways using alignments with gene models (Fl1 indole diterpene (IDT), ergot alkaloid and peramine gene models as well as E2368 loline gene model) followed by manual annotations. Both the genomes of AR48 and Fg1 do not contain any of the genes involved in the ergot alkaloid and loline pathways. The peramine gene is present but non-functional due to a large deletion that is identical between AR48 and Fg1. All the genes involved in the IDT pathway are present, however *idtK* and *idtF* are non-functional due to SNPs, with the *idtF* SNP identical between AR48 and Fg1. In addition, *idtP* and *idtQ* from AR48 and Fg1 are divergent in sequence identity with the corresponding genes from other *Epichloë* but nearly identical with one another. Given the identical SM profiles, in addition to simple sequence repeat (SSR) analysis, Fg1 was identified as a suitable model experimental system for AR48 as this strain is much more amenable to genetic manipulation.

The IDT profile of AR48 and Fg1 was analysed using IDT extracts from plant material infected with AR48 or Fg1 and analysed using mass spectrometry (MS). All the key IDT compounds predicted to be produced on the basis of the genes identified by bioinformatics were identified in this material. In addition, four new IDT compounds were identified in just AR48 and Fg1 material. The new IDT compound three was further investigated with the structure tentatively solved using MS. The presence of this new IDT compound three is likely due to the modified activity of the divergent IdtP and IdtQ. The possible role of these genes in the production of the new IDT compound three was investigated using heterologous expression in appropriate *P. paxilli* gene deletion strains, however no new intermediate or new IDT compound three products were identified.

# 5.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

The gene *mcf* was identified in all *Epichloë* strains analysed in this study. In addition, two *Epichloë* allelic variants were identified, with approximately half of the genes annotated as functional. Bioinformatics analysis predicted two N-terminal cysteine protease domain (CPD) and a central transmembrane domain (TMD). In preparation for bioactivity analysis by reverse genetics, makes caterpillars floppy (*mcf*) was successfully deleted ( $\Delta$ ) in Fg1 using homologous recombination and the presence of the deletion confirmed by both restriction digest as well as Southern blot analysis. The axenic culture phenotype of this Fg1  $\Delta mcf$  strain did not differ in morphology or growth to the Fg1 wild type (WT) strain, and plants infected with Fg1  $\Delta mcf$  had no distinguishable host interaction phenotype compared to plants infected with Fg1 WT. This lack of a host interaction phenotype is consistent with what has been published for other mutants that are defective in the synthesis of SMs.

#### 5.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass

A stem boring fly (SBF-*Cerodontha australis*) whole plant choice trial (SWPC) successfully identified SBF bioactivity in AR48 and AR47 infected ryegrass and the absence of SBF bioactivity in AR37 and AR1 infected ryegrass. Both cutworm moth caterpillar (CC-*Agrotis ipsilon*) whole plant choice (CWPC) and CC detached tiller no choice (CDTN) trials successfully identified CC bioactivity in Fl1 infected ryegrass while the CC bioactivity of the remaining strains, which included AR48 and Fg1, were inconclusive. The porina caterpillar (PC-*Wiseana* spp) detached tiller no choice (PDTN) trial successfully identified PC bioactivity in AR37 and AR48 infected ryegrass and the absence of PC bioactivity in AR1 and Fl1 infected ryegrass. Finally, the light brown apple moth (LBAM-*Epiphyas postvittana*) artificial diet no choice (LADN) trial identified bioactivity in all strains apart from AR47.

#### Chapter five: Conclusion

#### 5.4 Overall conclusion of the project

In conclusion, while the specific bioactive(s) in AR48 and Fg1 that targets CC and SBF were not identified two strong candidates, a new IDT compound and Mcf (a likely insect toxin) have been characterised. To test whether either, or both, of these candidate bioactives confers specific bioactivity against these insects, feeding experiments using ryegrass infected with deletion mutants of each of these genes singly and together will need to be performed. While the  $\Delta mcf$  strain has been generated, deletions of *idtP* or *idtQ* are yet to be made. The *Epichloë* SBF bioactive was tentatively identified as a new IDT compound three produced in AR48 by the substrate divergent IdtP and/or IdtQ using a newly developed SWPC trial. By combining the CDTN and PDTN trial results, the AR48 Mcf allelic variant was very tentatively identified as the CC bioactive produced by AR48 infected ryegrass. To conclusively identify the new IDT compound and Mcf as the SBF and CC bioactives, respectively, these compounds would need to be assessed for bioactivity using artificial diet insect feeding trials to eliminate potential background compound interference.

Chapter five: Conclusion

#### **6.0 FUTURE EXPERIMENTS**

## 6.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1

#### 6.1.1 AR48 and Fg1 genome assembly

The genomes of both AR48 and Fg1 have been sequenced by Illumina paired-end technology that produces a large number of short reads with high sequence accuracy. This accuracy is very important for bioinformatics analysis in predicting the functionality of genes. However, for *Epichloë*, Illumina sequencing tends to produce fragmented genome assemblies due to the very large numbers of repeats present throughout *Epichloë* genomes. Fragmented genome assembles are problematic as it can prevent analysis of the gene organisation within the genome, *i.e.* gene clusters can be spread across multiple scaffolds and contigs, and genes can be missed *i.e.* genes can be split across scaffolds and contigs. This is apparent for the indole-diterpene (IDT) clusters in AR48 and Fg1. A complete genome assembly could be generated for AR48 and Fg1 by sequencing the genomic DNA using long read platforms such as PacBio technology (Winter *et al.*, 2018). These technologies produce a higher number of very long reads but with a higher sequence error rate. The Illumina reads/scaffolds/contigs, which are very accurate, can then be mapped onto these larger reads, and in most cases, the genome can be fully assembled.

## 6.1.2 Epichloë festucae AR48 and Fg1 new indole-diterpene compound three structure

The structure of the new IDT compound three identified only in AR48 and Fg1 infected ryegrass has been analysed through mass spectrometry (MS). However, it is difficult to completely resolve the structure of metabolites using this technique. To resolve the structure of the new IDT compound three it will be necessary to purify the compound and determine its structure by nuclear magnetic resonance (NMR). This could be achieved though extraction of the IDT fraction from a plant source such as seed where the compound is abundant.

#### 6.1.3 AR48 and Fg1 new indole-diterpene compound three synthesis pathway

The current hypothesis is that the new IDT compound three present only in AR48 and Fg1 infected ryegrass is a result of novel catalytic activities of IdtP and IdtQ acting on the first stable IDT paspaline. The biosynthesis pathway of this new IDT compound three was not successfully recreated when AR48 *idtP* and *idtQ* genes were individually heterologously expressed in the corresponding *P. paxilli* mutant backgrounds, *i.e.* AR48 *idtP* and *idtQ* were expressed in  $\Delta paxP$  and  $\Delta paxQ$  backgrounds, respectively. It is likely that the failure to detect any new intermediates is because IdtQ rather than IdtP uses paspaline as the substrate. A key experiment to do is to introduce *idtQ* into the *P. paxilli*  $\Delta paxP$  mutant background. Generation of a  $\Delta paxP\Delta paxQ$  double mutant would be also very helpful for this analysis. Alternatively, MIDAS (Modular Idempotent DNA Assembly System) can be used to efficiently produce *P. paxilli* strains with different IDT gene combinations that could then be analysed for the resultant IDT compound profile using MS.

#### 6.1.4 AR48 and Fg1 new indole-diterpene compound three phylogenetic distribution

Currently the new IDT compound three has only been identified through MS in AR48, Fg1, and a few AR48-like strains (Wade Mace, AgResearch; personal communication) and not in AR1, AR37, common toxic (CT), and Fl1. The distribution of the new IDT compound three within *Epichloë* can be analysed by identifying other strains that produce the new IDT compound three through IDT seed and/or infected plant material profiling. The seeds can be obtained from the Margo Forde Germplasm centre, and phylogeny through the AgResearch forage endophyte simple sequence repeat (SSR) dendogram (unpublished data). In addition, the role of *idtP* and *idtQ* genes in the synthesis of the new IDT compound three can be supported by analysing the presence and sequence identity of the *idtP* and *idtQ* genes in the corresponding strains from the distribution experiment described above. This could provide a correlation between the presence of the sequence divergent *idtP* and/or *idtQ* genes and the presence of the new IDT compound three.

# 6.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

#### 6.2.1 Epichloë mcf allelic variants

The *Epichloë* makes caterpillars floppy (*mcf*) gene has two allelic variants, the AR48 allele which should have bioactivity (Ambrose *et al.*, 2014) and the Fl1 allele for which the bioactivity status is unknown. Both CC bioactivity trials tentatively suggest that only the AR48 allele has bioactivity against the CC and/or porina (PC-*Wiseana* spp). The same bioactivity trials described in section 5.4 could be used to assess the bioactivity potential of both *Epichloë* Mcf allelic variants. The SWPC and CADN trials would involve a range of strains chosen based on the presence of AR48 *mcf* or Fl1 *mcf* variants, with the addition of the expression levels of the *mcf* allele and/or amount of Mcf protein measured. The CADN trial in addition would require purification of the products of the two Mcf allelic variants. Alternatively, the same artificial trial as described by Ambrose *et al.* (2014) could be performed with *E. coli* and/or *P. paxilli* expressing either the AR48 *mcf* or Fl1 *mcf* genes.

#### 6.2.2 Epichloë Mcf domain functions and bioactivity mechanism

The Mcf proteins are predicted to have a variable bioactive N-terminal toxin domain, a N-terminal cysteine protease domain (CPD), a central transmembrane domain (TMD), and C-terminal receptor/secretory domain (Dowling & Waterfield, 2007). To better understand the functionality of the two *Epichloë mcf* alleles, different *mcf* alleles or *mcf* truncations, with or without a fluorescent tag, could be expressed in *E. coli* and/or *P. paxilli* and then tested for bioactivity using the same artificial insect trial described by Ambrose *et al.* (2014). The insects could then be dissected, and protein location analysed using fluorescent microscopy (Ambrose *et al.*, 2014). In addition, mammalian cell lines expressing these constructs could then be used to analyse the specific cellular mechanism of the bioactivity (Dowling *et al.*, 2004).

#### 6.2.3 Epichloë Mcf in culture, in planta, and target insect localisation

To identify where the Mcf protein is located in *Epichloë* the gene could be tagged with a fluorescent marker and expressed under a constitutively expressing promoter. Total protein content of the mycelia and media could then be extracted separately and analysed for the tagged Mcf protein by a western blot analysis. In addition, *mcf* could also be tagged with a fluorescent marker and expressed under its native promoter. This strain could then be inoculated into seedlings and analysed for Mcf location. This material could then be fed to insects, the insects dissected, and protein location analysed (Ambrose *et al.*, 2014).

### 6.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass

## 6.3.1 Epichloë festucae AR48 and Fg1 new indole-diterpene compound three insect bioactivity

The new IDT compound three could have the potential to have stem boring fly (SBF-*Cerodontha australis*) and/or cutworm moth caterpillar (CC-Agrotis ipsilon) bioactivity, with the strongest evidence towards SBF bioactivity. Another SBF whole plant choice (SWPC) trial could be perform, but using a wider range of strains expressing or not expressing the new IDT compound three, to gather more evidence towards the correlation between SBF bioactivity and the new IDT compound three. Strains would be identified through the previous new IDT compound three distribution analysis described in section 5.4. In addition, or alternatively, purified compound could be assessed for bioactivity either directly (if an artificial diet can be developed for SBF) or indirectly by supplying the compound to the plants. Since SBF larva remain in the plant tissue throughout development this environment could be difficult to replicate for using an artificial diet. The artificial diet could be set into capillary tubes or between two slides to create an artificial tiller for the larva to form a mine down, with the top left empty to form a well. A female fly or egg or larva is then added into the well, and the emerging larva should mine down the artificial tiller. The rate of the mine formation would then be measured to assess bioactivity. For the CC bioactivity potential of the new IDT compound three, a CC detached tiller not choice (CDTN) trial

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could be performed with the same range of strains in the SWPC trial described above. Also, a CC artificial diet no choice (CADN) trial could be performed with the purified new IDT compound three extract. However, an artificial CC diet would need to be developed, but this should be very similar to the porina artificial diet.

### 6.3.2 Epichloë festucae Fg1 mcf reverse genetics

Though Fg1 is a model AR48 strain in relation to the bioactive SM pathway profile, bioactivity towards SFB, CC, and PC have not currently been detected in Fg1 infected plant material. This precludes Fg1  $\Delta mcf$  ( $\Delta$  deletion) being used in a reverse genetics studies to assess Mcf bioactivity. The lack of Fg1 wild type (WT) bioactivity could be due to host incompatibility. By measuring the expression levels of Mcf in a range of different host plant backgrounds (including its native host) inoculated with Fg1 WT, a more suitable association could be identified. Light, temperature, and other factors known to affect gene expression levels could also be analysed to create a high Mcf expressing environment. The Fg1  $\Delta mcf$  could then be assessed for loss of bioactivity.

For most gene functionality analysis, multiple independent gene deletion strains are usually tested to provide validity to the resultant phenotype analysis (Scott *et al.*, 2012). To increase the chance of creating a gene deletion two alternative approaches could be taken; a split marker system (Rahnama *et al.*, 2018), which still relies on homologous recombination, or a gene editing platform such as CRISPR-CAS9 (Ran *et al.*, 2013, Liu *et al.*, 2015), which potentially provides much higher precision and recombination at the target site. Chapter six: Future experiments

### **7.0 LIMITATIONS**

## 7.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1

### 7.1.1 AR48 and Fg1 new indole diterpene three unresolved structure

Mass spectrometry (MS) analysis of the indole diterpenes (IDTs) produced by AR48 and Fg1 infected ryegrass identified four new IDT compounds. The new IDT compound three was chosen for further structural investigation through additional MS analysis, and a tentative structure was obtained. However, given this structure, the placement of the new IDT compound three into the current working IDT pathway was not obvious given the unusual absence of the OH group on carbon 27, which is present on all IDT compounds synthesised after the first stable intermediate paspaline. This structure was resolved at the limitations of the MS machinery and therefore the only way of conclusively solving the structure of the new IDT compound (Future experiments 6.1.2). This is paramount in not only the placement and theoretical synthesis pathway of the new IDT compound three, but also as conclusive evidence that the new IDT compound three is novel and not a stereoisomer of paxitriol, given that the different stereoisomer standards of paxitriol were not avaliable.

### 7.1.2 AR48 and Fg1 new indole diterpene three unresolved synthesis pathway

The synthesis of IDT compounds in *Epichloë* is complex due to the network of pathways catalysed by multiple enzymes. To add complexity each branch point can either be major or minor (shunt). This makes identifying the exact pathways and branches routinely synthesised *in planta* extremely difficult. Bioinformatics analysis identified two genes, *idtP* and *idtQ* (due to sequence identity divergence and in combination with the gene function and predicted new IDT compound three MS structure) as potentially being involved in the synthesis of the new IDT compound three. These two genes were heterologously expressed in *P. paxilli* in an attempt to recreate the new IDT compound three synthesis pathway. However, neither a partial or complete pathway was achieved. The limitation of this approach is that it is nearly impossible to accurately predict the resultant IDT synthesis network of the new gene

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combination, so newly synthesised IDT pathway intermediate compounds can be missed if not accounted for prior to MS analysis.

However, when the new IDT compound three structure is resolved (Future experiments 6.1.2) then more accurate synthesis pathways could be hypothesised reducing the risk of missing pathway intermediate compounds. Also, when the genomes of AR48 and Fg1 are fully assembled (Future experiments 6.1.1), then bioinformatics analysis could be performed to identify any additional AR48 and Fg1 unique genes, in close proximity to the IDT cluster, that might have the potential to be involved in the new IDT compound three synthesis. Finally, MIDAS (Modular Idempotent DNA Assembly System) could be used to efficiently produce *P. paxilli* strains with differing IDT gene combinations with the resultant IDT compound profiles analysed through MS (Future experiments 6.1.3).

# 7.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass

### 7.2.1 Epichloë limited genetic manipulation capabilities

One of the major limitations of this project was producing an *Epichloë festucae* gene deletion strain. This is because *Epichloë* as a system has multiple limitations such as; transformations can only be performed on protoplasts, *Epichloë* has a slow growth rate, the rate of targeted homologous recombination at each site can vary, and to date no targeted homologous recombination event has been successfully achieved in an asexual *Epichloë* strain. However, non-homologous recombination has been achieved *i.e.* gene complementation.

These limitations are why a sexual AR48 model strain was investigated, and although *Epichloë festucae* Fg1 was identified as a candidate based on secondary metabolite (SM) and simple sequence repeat (SSR) profiles, Fg1 does not appear to have the same insect bioactivity *in planta* as AR48. In addition, to obtain a single *mcf* gene deletion in Fg1 required two different plasmid designs and screening of over 500 transformants.

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Finally, as an alternative approach, complementation through non-homologous recombination of *Epichloë festucae* var *lolii* AR1 and AR37, which have potentially non-functional or non-bioactive *mcf* genes, with the AR48 *mcf* gene to gain bioactivity was attempted but these were unsuccessful. Alternative gene deletion methods are yet to be investigated (Future experiments 6.3.2).

### 7.2.2 Epichloë in planta only expressed secondary metabolites

*Epichloë* SMs linked to either mammalian or insect bioactivity, apart from a few exceptions, are only expressed *in planta*. Any assessment of a new SM for bioactivity therefore requires the use of an *Epichloë* infected seed line. If a seed line is not available for the *Epichloë* strain of interest then seedling inoculation is required. This vital step is a major limiting factor due to potentially low infection rate and timing. One way of avoiding the requirement of *in planta* expression is to heterologously express the required genes in a different microorganism that does not require *in planta* expression, and then either incorporating the purified compound or whole inoculum into the insect trial or respective assay. This has currently not been attempted for the Fg1 *mcf* gene. However, *P. paxilli* was used to heterologously express *idtP* and *idtQ* in an attempt to synthesis the new IDT compound three.

## 7.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass

## 7.3.1 Epichloë festucae var lolii complex interaction between bioactive secondary metabolite profiles and insect bioactive capabilities

Identifying the bioactive compound of interest as well as the synthesis pathway is vital for the development of *Epichloë* as a commercialised bioprotectant agent by assisting in the identification of *Epichloë* strains with the best insect bioactive profile Routinely, the initial step is to either use forward or reverse genetics to link a gene/compound with an insect bioactivity. However, this is not trivial due to the complex nature of the *Epichloë* bioactive secondary metabolite profile (Limitations 7.1.2), genetic component/s are typically only expressed *in planta* (Limitations 7.2.2), and each *Epichloë* strain has a complex and unique range of insect bioactive capabilities *i.e.*  redundancy. In an attempt to simplify the interaction between bioactives and the insect of interest, once a gene has been identified, the most accurate method of assessing the insect bioactivity of the resultant product is to purify the compound, either from *Epichloë* plant/seed or an alternative expression system (Limitations 7.2.2), and feed the single compound to the target insect through an artificial diet (Future experiments 6.3.1).

### 7.3.2 Non-model system insect species

Many of the agricultural insect pests studied in the AgResearch *Epichloë* bioprotectant program are non-model systems. The insects are typically only available over the natural breading seasons, and have to be collected from the surrounding environment. This is a major limiting factor when assessing insect bioprotectant capabilities not only in the reduced available time to perform trails but also in synchronisation of the plant preparation with insect availability *i.e.* unforeseeable breeding season variations. For some of the insects an artificial diet has been developed *e.g.* porina, or an easy to obtain plant food source identified *e.g.* cutworm moth caterpillar, therefore the adult insect can be caught and offspring raised extending insect availability. But currently none of the insect pests studied are able to be bread all year round in the laboratory. Other factors to consider when understanding the bioprotectant capabilities of an *Epichloë* stain is that some bioprotectants only effect a particular life stage *e.g.* adult verses larva, redundancy in insect bioprotectants, and the potential of unidentified agricultural pest bioprotectant capabilities resulting in missed *Epichloë* strain potential.

### **8.0 APPENDICES**

idtG		Fl1	E2368	AR48	Fg1	AR5	AR1	AR37
	FI1							
	E2368							
	AR48	99.7						
	Fg1	99.7		100				
	AR5	100		99.7	99.7			
	AR1	100		99.7	99.7	100		
	AR37	99.7		99.4	99.4	99.7	99.7	

idtC

	FI1	E2368	AR48	Fg1	AR5	AR1	AR37
FI1							
E2368	99.4						
AR48	99.7	99.1					
Fg1	99.7	99.1	99.7				
AR5	100	99.4	99.7	99.7			
AR1	99.7	99.1	99.4	99.4	99.7		
AR37	100	99.4	99.7	99.7	100	99.7	

idtM

		Fl1	E2368	AR48	Fg1	AR5	AR1	AR437
	Fl1							
	E2368							
	AR48	99.8						
	Fg1	99.8		100				
	AR5	99.8		99.6	99.6			
	AR1	99.8		99.6	99.6	100		
[	AR37	99.6		99.8	99.8	99.4	99.4	

idtB

		Fl1	E2368	AR48	Fg1	AR5	AR1	AR37
FI1								
E23	58	99.1						
AR4	8	99.6	99.6					
Fg	L	99.6	99.6	100				
AR	5	100	99.1	99.6	99.6			
AR	1	100	99.1	99.6	99.6	100		
AR3	7	100	99.1	99.6	99.6	100	100	

idtP

		FI1	E2368	AR48	Fg1	AR5	AR1	AR37
[	FI1							
	E2368	98.6						
[	AR48	92.8	94					
	Fg1	92.6	93.8	99.8				
[	AR5	99.8	98.4	92.6	92.4			
[	AR1	100	98.6	92.8	92.6	99.8		
[	AR37	100	92.8	92.8	92.6	99.8	100	

idtQ

	FI1	E2368	AR48	Fg1	AR5	AR1	AR437
FI1							
E2368	98.1						
AR48	90.3	91					
Fg1	90.3	91	99.1				
AR5	100	98.1	90.3	90.3			
AR1	100	98.1	90.3	90.3	100		
AR37	100	98.1	90.3	90.3	100	100	

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idtE		Fl1	E2368	AR48	Fg1	AR5	AR1	AR37
	FI1							
	E2368							
	AR48	99.9						
	Fg1	99.7		99.9				
	AR5							
	AR1							
	AR37							

idtJ

		FI1	E2368	AR48	Fg1	AR5	AR1	AR37
	FI1							
	E2368							
	AR48	99						
	Fg1	100		99				
	AR5							
	AR1							
[	AR37							

idtF

	Fl1	E2368	AR48	Fg1	AR5	AR1	AR37
Fl1							
E2368	97.7						
AR48							
Fg1							
AR5	<b>99.5</b>	97.3					
AR1	99.8	97.5			99.8		
AR37	100	97.7			99.5	99.8	

Chapter eight. Annendices

idtK		Fl1	E2368	AR48	Fg1	AR5	AR1	AR37
	FI1							
	E2368							
	AR48							
	Fg1							
	AR5	99.8						
	AR1	100				99.8		
	AR37	99.2				99.1	99.2	
idtS		FI1	E2368	AR48	Fg1	AR5	AR1	AR37
	FI1							
	E2368							
	AR48	99.3						
	Fg1	98.9		99.6				
	AR5	100		99.3	98.9			
	AR1	100		99.3	98.9	100		
	AR37	100		99.3	98.9	100	100	

Appendix 1: Alignment of indole-diterpene (IDT) protein sequences from *Epichloë festucae* species. Protein sequences of the 11 genes involved in IDT synthesis from Fl1, E2368, AR48, Fg1, AR5, AR1, and AR37 strains were aligned to each corresponding gene in each genome using BLASTp tool on Geneious. The sequence identity (%) was analysed.

ldtP	Amino acid	From	То	Non-conserved	From	То
	2	L	F			
	8	Р	L			
	10	G	S			
	12	С	W	✓	Sulfide moiety	Hydrophobic
	23	к	R			
	49	D	G	~	Charged/negative	Uncharged/small
	57	н	Y	~	Charged/negative	Hydrophobic/aromatic
	72	I.	М			
	130	К	R			
	134	т	Α			
	153	G	S			
	161	I.	V			
	177	т	Α			
	185	L	Р	<b>v</b>	Hydrophobic	Aromatic
	191	I.	Н	~	Hydrophobic	Uncharged/small
	208	S	F	~	Polar	Hydrophobic/aromatic
	212	S	Т			
	219	н	Y	✓	Uncharged/small	Hydrophobic/aromatic
	221	F	L			
	226 (Fg1)	S	Р	~	Polar	Aromatic
	230	Е	К	~	Charged/negative	Charged/postive
	299	L	S			
	310	D	Ν	~	Charged/negative	Polar
	311	S	I.	✓	Polar	Hydrophobic
	317	R	Q	✓	Charged/postive	Polar
	318	R	К			
	322	Ν	D	~	Polar	Charged/negative
	352	I.	V			
	374	Y	F			
	395	К	I.	~	Charged/postive	Hydrophobic
	397	S	Р			
	412	G	S			
	415	т	Р			
	488	L	F			
	496	Т	Р	<b>v</b>	Polar	Aromatic
ldtQ	Amino acid	From	То	Non-conserved	From	То
	6	Н	R			
	11	К	Е	~	Charged/postive	Charged/negative
	13	Ν	D	~	Polar	Charged/negative
	15	Α	V			
	20	S	т			
	21	G	S			
	34	Ν	D	~	Polar	Charged/negative

IdtQ Amino acid	From	То	Non-conserved	From	То
40	Ν	D	<b>v</b>	Polar	Charged/negative
46	1	V			
57	1	V			
58	1	М			
62	Н	Y	~	Charged/postive	Hydrophobic/aromatic
76	D	Ν	~	Charged/negative	Polar
88	Е	D			
114	Μ	I			
115	т	Α			
118	F	L			
129	Н	Р	~	Charged/postive	Aromatic
133	I	Т	~	Hydrophobic	Polar
160	S	Α			
162 (Fg1)	S	F	~	Polar	Hydrophobic/aromatic
166	М	I.			
173	М	V			
177 (AR48)	1	S	~	Hydrophobic	Polar
178	Q	E	~	Polar	Charged/negative
179	Y	D	~	Hydrophobic/aromatic	Charged/negative
183	S	L			
185	М	Α	~	Hydrophobic/sulfide	Hydrophobic
189	S	Р	~	Polar	Aromatic
217 (AR48)	т	I.	~	Polar	Hydrophobic
237	Q	R	~	Polar	Charged/postive
242		V			
269	V	L			
270	М	V			
276		Т	~	Hydrophobic	Polar
279	V/I	Α			
306	R	С	~	Charged/postive	Sulfide moiety
311	S/T	G			
313	D	N	~	Charged/negative	Polar
317		V			
321	M/V	Т			
354	L	R	V	Hydrophobic	Charged/postive
358	D	н	V	Charged/negative	Charged/postive
362	E	S	~	Charged/negative	Polar
376	F	K			
411	N	Н			
414	ĸ	R			
425		L	~	Lludua a b c b ! -	Dalar
454	L	Т		Hydrophobic Polar	Polar
470	Т				Hydrophobic
484 522(5a1)	E	A	V	Charged/negative	Hydrophobic
532(Fg1)	R	M			
533	E/K	l (Fg1)/M (AR48)	V	Charged	Hydrophobic
535	Ν	D	<u> </u>	Polar	Charged/negative

Appendix 2: Unique amino acid changes in indole-diterpene (IDT) IdtP and IdtQ from *Epichloë festucae* strains AR48 and Fg1, when compared in an alignment of corresponding genes in *E. festucae* strains Fl1, E2368, AR1, AR37, and AR5.

Chapter	Amino acid	saccharomyc es cerevisiae lanosterol 14- alpha demethylase with2 lanosterol bound	brucei in	crystal structure of human prostacyclin synthase	crystal structure of arabidopsis thaliana allene oxide synthase (aos,2 cytochrome p450 74a, cyp74a) complexed with 13(s)- hod at 1.85 a3 resolution	human cytochrome p450 2e1 in complex with the inhibitor 4- 2 methylpyrazol e	-	crystal structure of allene oxide synthase	structure of cytochrome p450 4b1 (cyp4b1) complexed with octane: an n-2 alkane and fatty acid omega- hydroxylase with a covalently bound heme	crystal structure of sterol 14- alpha demethylase (cyp51) from a2 pathogenic yeast candida albicans in complex with the antifungal drug3 posaconazole	crystal structure of human cyp11a1 in complex with 20,22-2 dihydroxychol esterol
	12										
	49 57							-			-
	185		_	-			~	-		-	v
	191		~				•	~			
	208		~		~			~	~		-
	219		-		-		~	-	-		-
	226 (Fg1)										-
	230				-	-	~				-
	310				-						
	311				-						
	317				-						
	322										
	395			~						~	
	496										

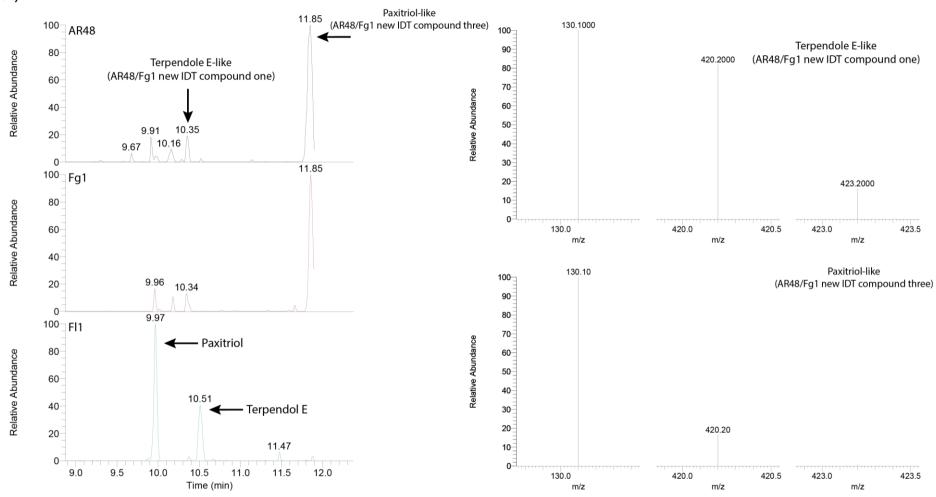
Appendix 3: Phyre2 analysis of *Epichloë festucae* strains AR48 and Fg1 IdtP sequences aligned to the top 10 hits looking at non-conserved amino acid changes in pocket predictions. Underlined amino acid changes are shared with E2368. Impedate the amino acid change is within the predicted pocket and – means the amino acid change is next to an amino acid predicted in the pocket.

Amino acid	saccharomyce s cerevisiae lanosterol 14- alpha demethylase with2 lanosterol bound	crystal structure of human prostacyclin synthase	x-ray structure of cyp51 from trypanosoma brucei in complex2 with posaconazole in two different conformations	crystal structure of human cyp11a1 in complex with 20,22-2 dihydroxychol	albicans in	human cyp7a1	structure of cytochrome p450 4b1 (cyp4b1) complexed with octane: an n-2 alkane and fatty acid omega- hydroxylase with a covalently bound heme	crystal structure of arabidopsis thaliana allene oxide synthase (aos,2 cytochrome p450 74a, cyp74a) complexed with 13(s)-hod at 1.85 a3 resolution	Cytochrom P450	human cytochrome p450 2e1 in complex with the inhibitor 4- 2 methylpyrazol e
<u>11</u>										
13										
34										
40										
62		~	~			~	~	~		
76								~		
129								~	✓	
133							~	~	✓	-
162 (Fg1)							-		-	~
177 (AR48)					~	-		-	-	~
178					-			-	-	-
179								~	✓	
185					~					
189										
217 (AR48)										

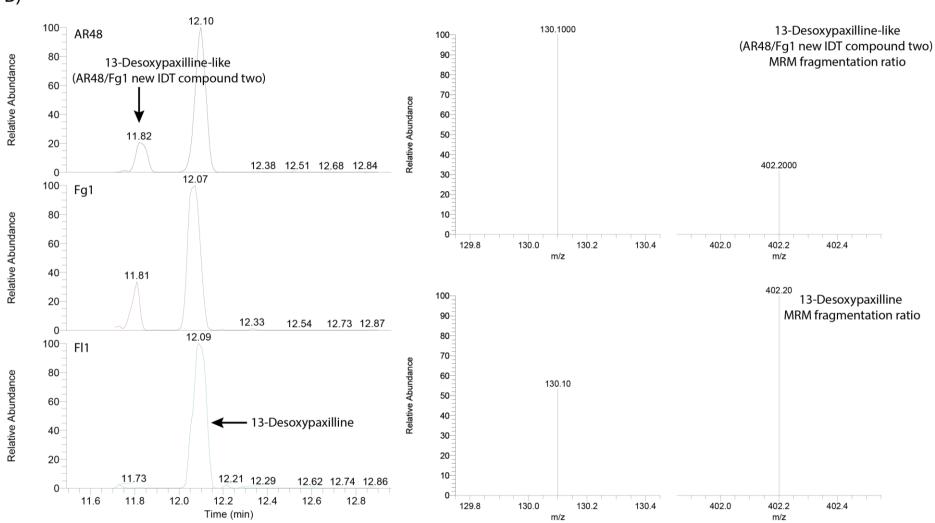
Amino acid	saccharomyce s cerevisiae lanosterol 14- alpha demethylase with2 lanosterol bound	crystal structure of human prostacyclin synthase	x-ray structure of cyp51 from trypanosoma brucei in complex2 with posaconazole in two different conformations	crystal structure of human cyp11a1 in complex with 20,22-2 dihydroxychol esterol	albicans in	crystal structure of human cyp7a1	structure of cytochrome p450 4b1 (cyp4b1) complexed with octane: an n-2 alkane and fatty acid omega- hydroxylase with a covalently bound heme	crystal structure of arabidopsis thaliana allene oxide synthase (aos,2 cytochrome p450 74a, cyp74a) complexed with 13(s)-hod at 1.85 a3 resolution	Cytochrom P450	human cytochrome p450 2e1 in complex with the inhibitor 4- 2 methylpyrazol e
237			-				-		✓	✓
276							✓		~	~
306				-						
313				-						
354										
358										
362					-					
454						-			~	
470	~	-	-	-			-	~	~	-
484	-	~		-		~	-	~	~	
533										
535										

Appendix 4: Phyre2 analysis of *Epichloë festucae* strains AR48 and Fg1 indole-diterpene (IDT) IdtQ sequences aligned to the top 10 hits looking at non-conserved amino acid changes in pocket predictions. Underlined amino acid changes are shared with E2368. Images the amino acid change is within the predicted pocket and – means the amino acid change is next to an amino acid predicted in the pocket.

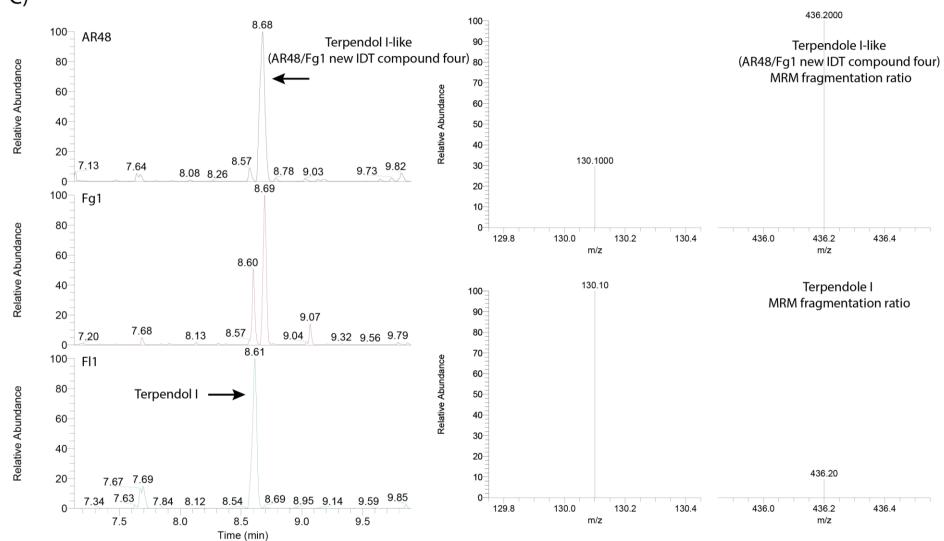
A)

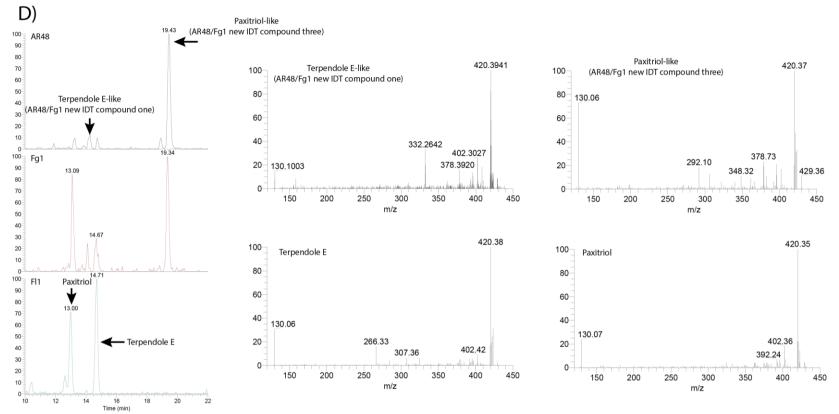


B)

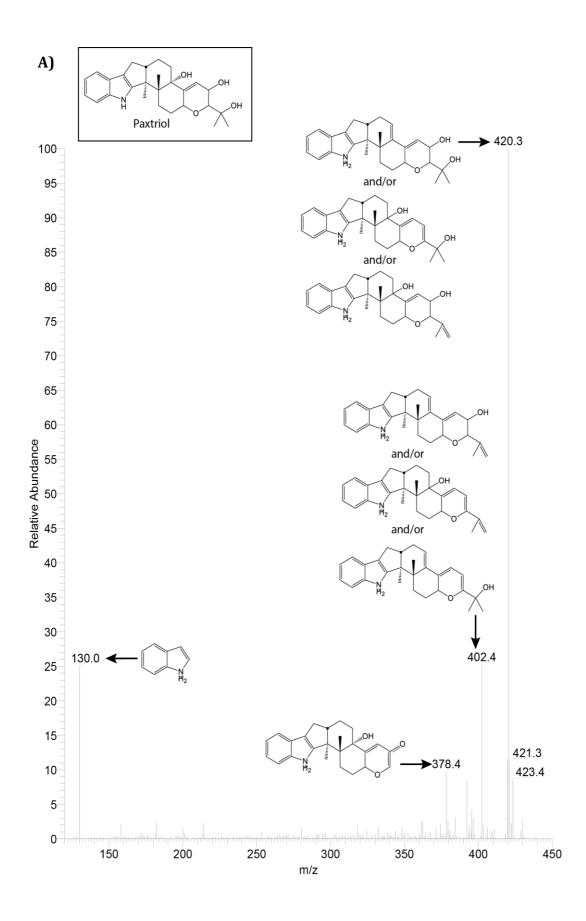


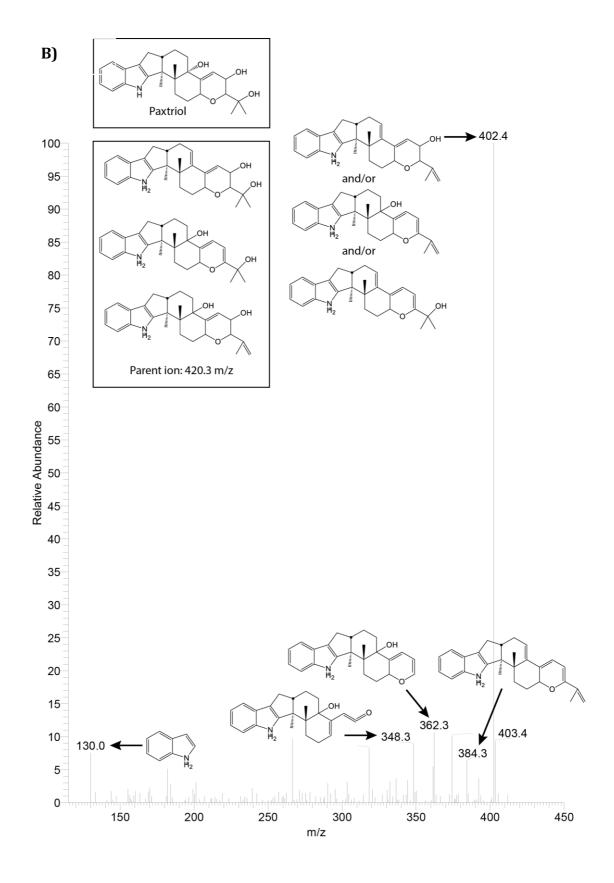
C)

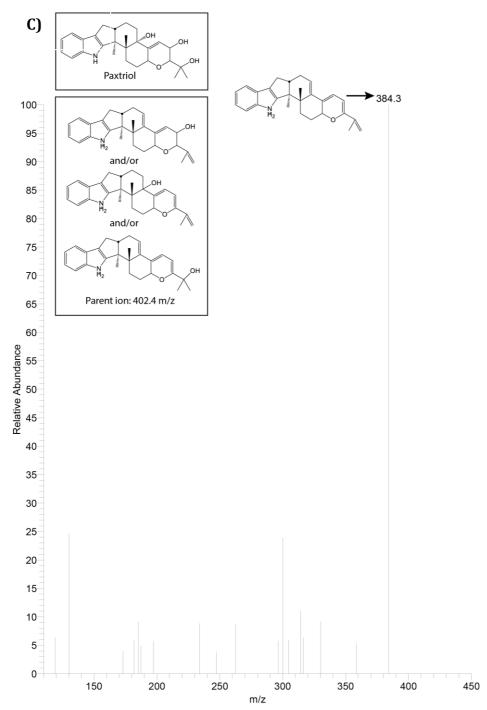




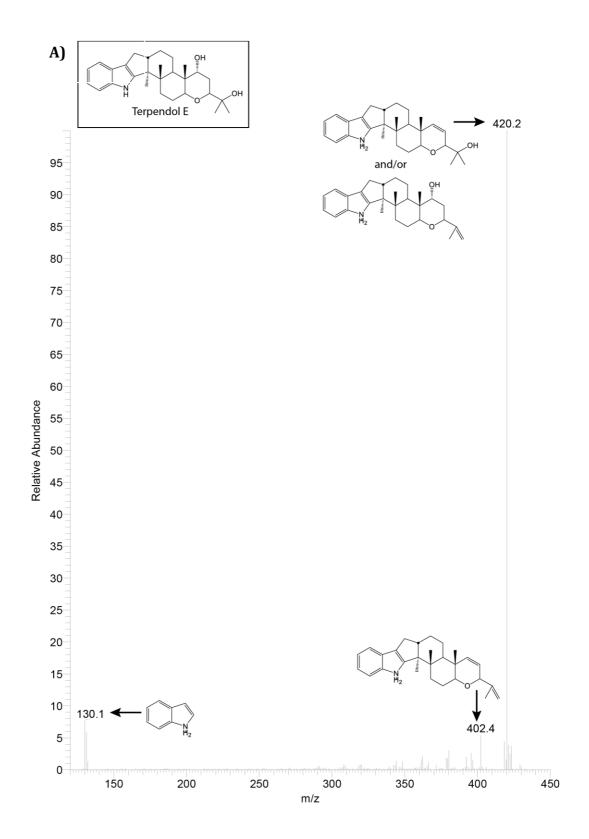
Appendix 5: *Epichloë festucae* strains AR48 and Fg1 mass spectrometry raw data of new indole-diterpene (IDT) peaks. Initial screening was undertaken using the TSQ (triple-quad) with multiple reaction monitoring (MRM) optimised for the indole diterpene products from *Epichloë* endophytes (parent-18 *m/z* and 130 *m/z* core). A) Terpendole E-like peak (AR48/Fg1 new IDT compound one) and Paxitriol-like peak (AR48/Fg1 new IDT compound three). B) 13-desoxypaxilline-like peak (AR48/Fg1 new IDT compound two). C) Terpendole I-like peak (AR48/Fg1 new IDT compound four). D) The linear ion-trap (LTQxI) to obtain a full MS<sup>2</sup> fragmentation spectrum for the paxitriol, terpendole E, paxitriol-like (AR48/Fg1 new IDT compound four). Due to the different chromatographic conditions required for the two instruments (triple-quad and linear ion-trap) the retention times were different (19.4 *c.f.* 11.8)

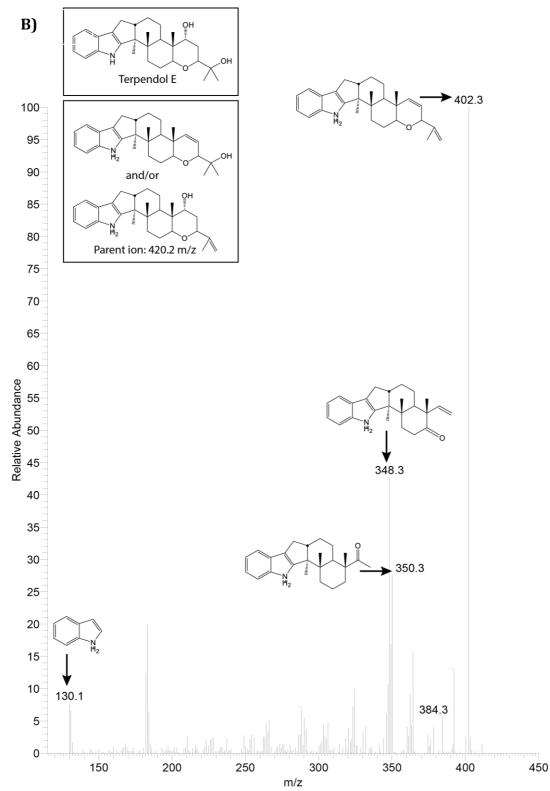






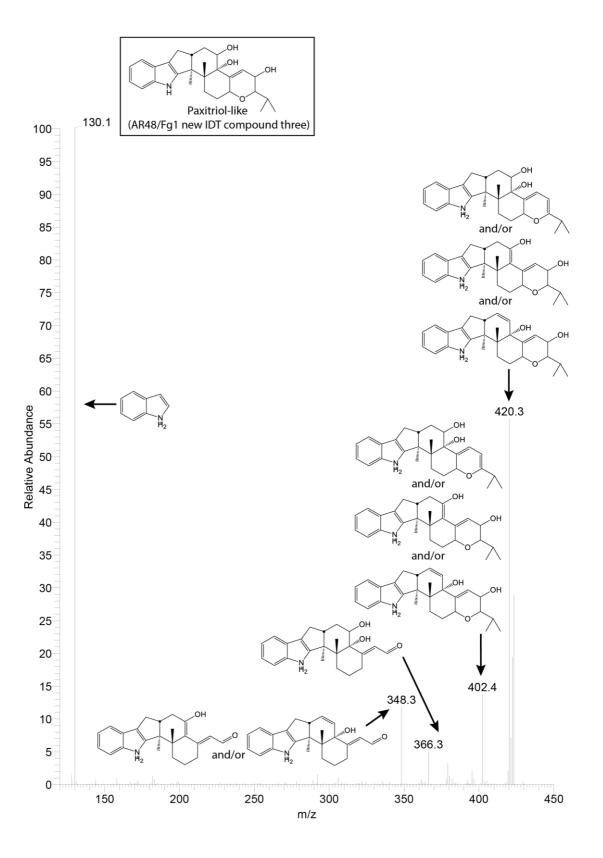
Appendix 6: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of paxitriol from *Epichloë festucae* Fl1 infected ryegrass. A) Collision-inducted fragmentation spectrum of the 438 m/z peak. Key ions are 420 m/z (loss of H<sub>2</sub>O), 402 m/z (loss of two H<sub>2</sub>O), and 378 m/z (loss of C<sub>3</sub>H<sub>7</sub>O). B) Collision-inducted fragmentation spectrum of the 420 m/z peak from the 438 m/z fragmentation spectrum. Key ions are 402 m/z (loss of two H<sub>2</sub>O), 384 m/z (loss of three H<sub>2</sub>O), 362 m/z (loss of H<sub>2</sub>O and C<sub>3</sub>H<sub>6</sub>O), and 348 m/z (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>8</sub>O). C) Collision-inducted fragmentation spectrum of the 402 m/z peak from the 420 m/z fragmentation spectrum from the 438 m/z fragmentation spectrum from the 438 m/z fragmentation spectrum of the 402 m/z peak from the 420 m/z fragmentation spectrum from the 438 m/z fragme

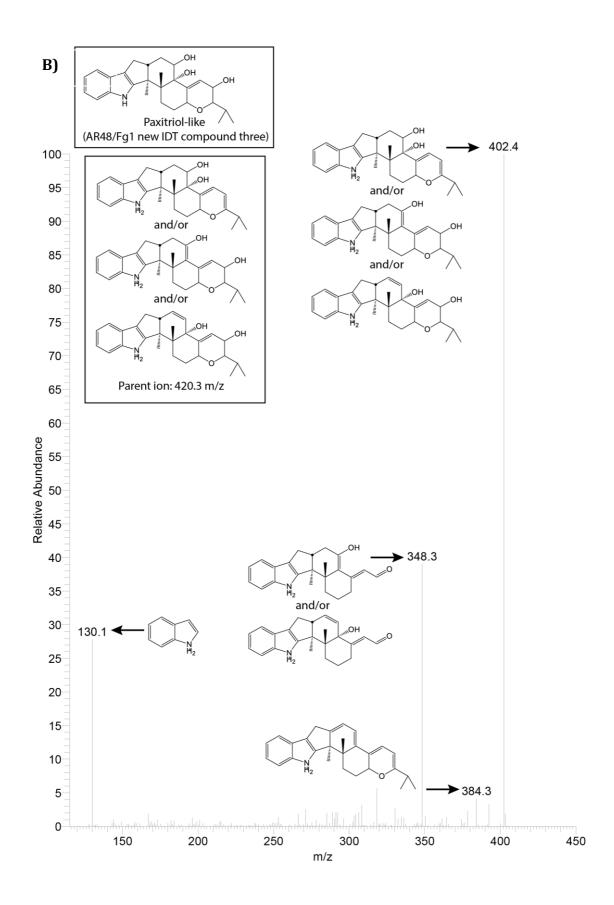




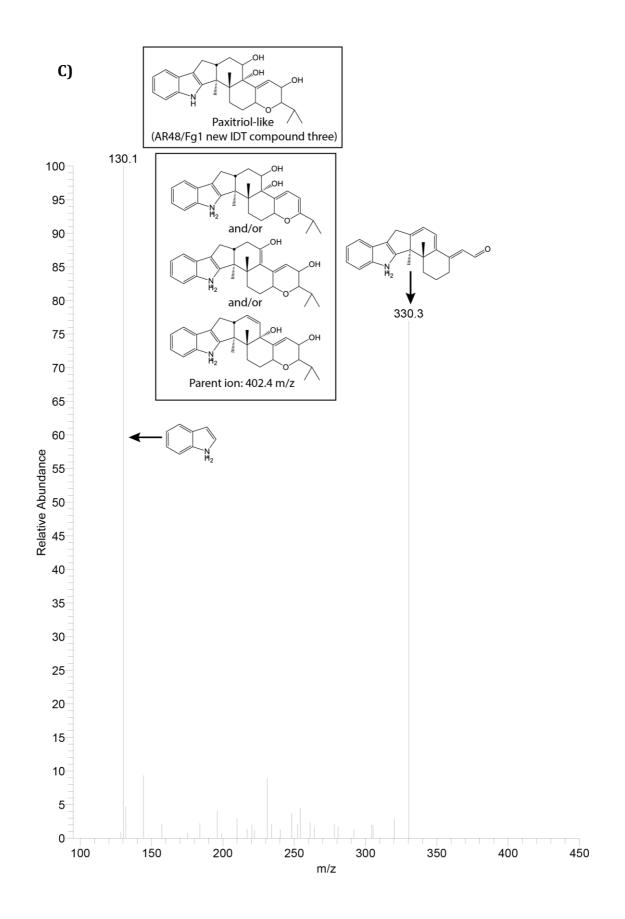
Appendix 7: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of terpendole E from *Epichloë festucae* Fl1 infected ryegrass. A) Collision-inducted fragmentation spectrum of the 438 m/z peak. Key ions are 420 m/z (loss of H<sub>2</sub>O), and 402 m/z (loss of two H<sub>2</sub>O). B) Collision-inducted fragmentation spectrum of the 420 m/z peak from the 438 m/z fragmentation spectrum. Key ions are 402 m/z (loss of two H<sub>2</sub>O). B) Collision-inducted fragmentation spectrum of the 420 m/z peak from the 438 m/z fragmentation spectrum. Key ions are 402 m/z (loss of two H<sub>2</sub>O), 350 m/z (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>7</sub>O), and 348 m/z (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>8</sub>O). The structure of terpendole E and parent ions are inserted into the spectra as reference.

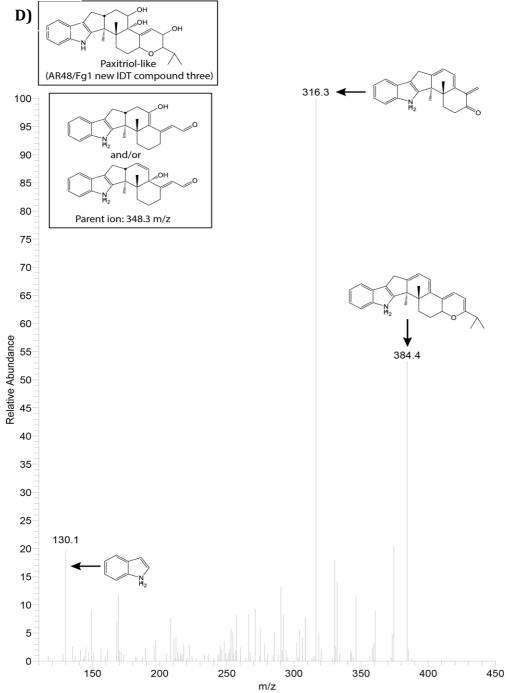
A)



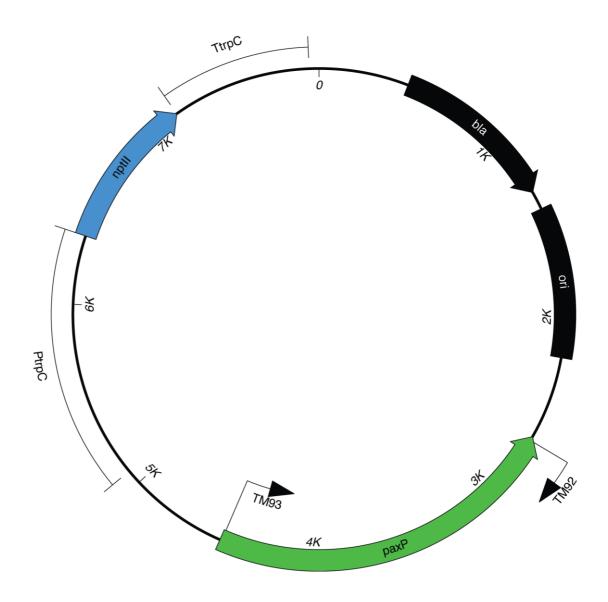


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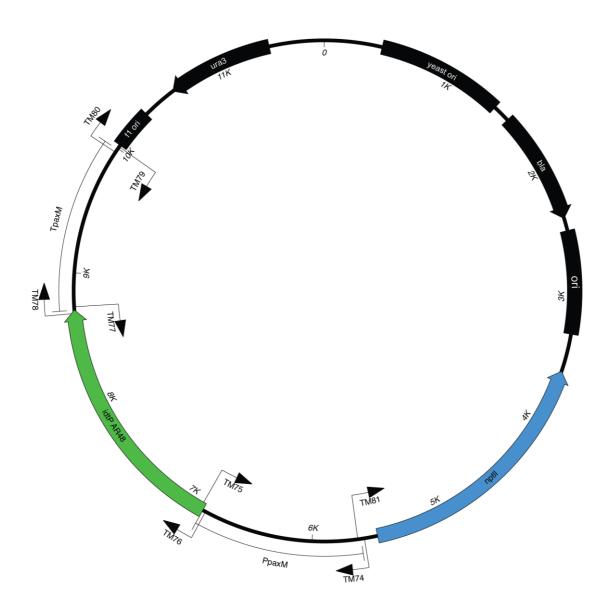




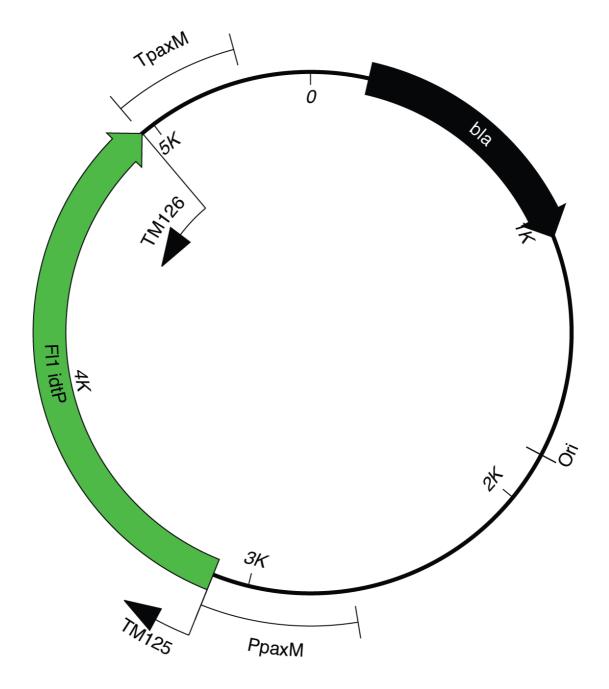
Appendix 8: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of new indolediterpene (IDT) compound three from *Epichloë festucae* AR48 and Fg1 infected ryegrass. A) Collisioninducted fragmentation spectrum of the 438 *m/z* peak. Key ions are 420 *m/z* (loss of H<sub>2</sub>O), 402 *m/z* (loss of two H<sub>2</sub>O), 366 *m/z* (loss of C<sub>4</sub>H<sub>8</sub>O), and 348 *m/z* (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>8</sub>O). B) Collision-inducted fragmentation spectrum of the 420 *m/z* peak from the 438 *m/z* fragmentation spectrum. Key ions are 402 *m/z* (loss of two H<sub>2</sub>O), 384 *m/z* (loss of three H<sub>2</sub>O), and 348 *m/z* (loss of H<sub>2</sub>O and C<sub>4</sub>H<sub>8</sub>O). C) Collisioninducted fragmentation spectrum of the 348 *m/z* peak from the 420 *m/z* fragmentation spectrum from the 438 *m/z* fragmentation spectrum. Key ion is 330 *m/z* (loss of two H<sub>2</sub>O) and C<sub>4</sub>H<sub>8</sub>O). D) Collisioninducted fragmentation spectrum of the 402 *m/z* peak from the 420 *m/z* fragmentation spectrum from the 438 *m/z* fragmentation spectrum. Key ion is 330 *m/z* (loss of two H<sub>2</sub>O) and C<sub>4</sub>H<sub>8</sub>O). D) Collisioninducted fragmentation spectrum of the 402 *m/z* peak from the 420 *m/z* fragmentation spectrum from the 438 *m/z* fragmentation spectrum. Key peaks are 384 *m/z* (loss of three H<sub>2</sub>O), and 316 *m/z* (loss of two H<sub>2</sub>O and C<sub>5</sub>H<sub>12</sub>O). The predicted structure of the novel IDT 3# is inserted into the spectra as reference.



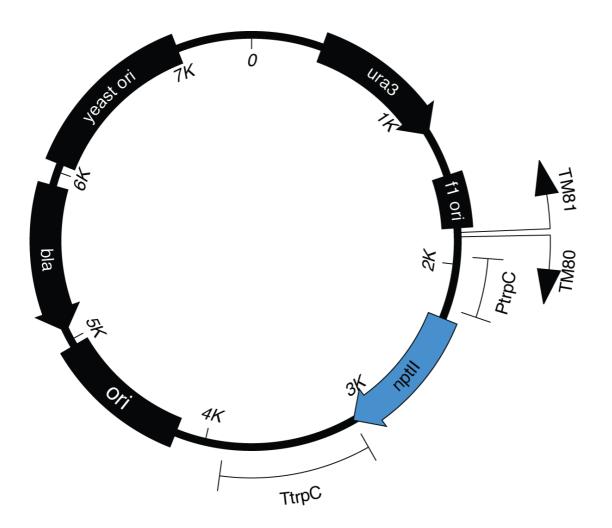
Appendix 9: *Penicillium paxilli paxP* complementation construct (pSS1, 7930 bp). Nptll (geneticin resistance gene). Ori (*E. coli* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtypC (terminator). Primers for screening transformants are indicated by black arrows.



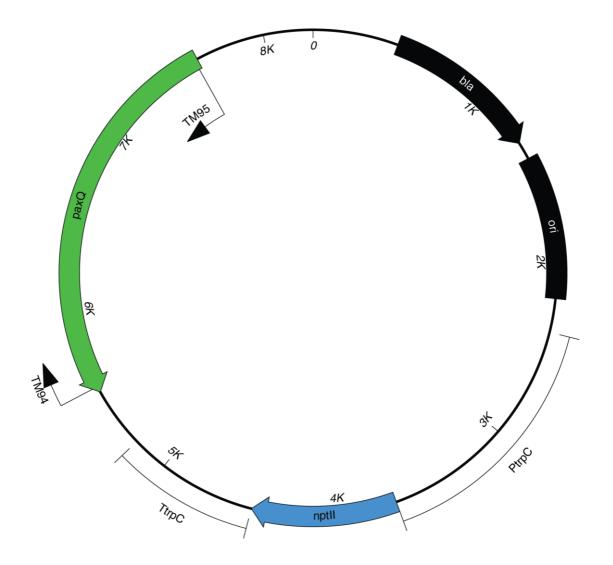
Appendix 10: *Epichloë festucae* AR48 indole diterpene (IDT) *idtP* complementation construct (pTM06, 11818 bp). Nptll (geneticin resistance gene). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PpaxM (promoter). TpaxM (terminator). Primers for screening transformants (TM76/TM77), and for plasmid construction (all) are indicated by black arrows.



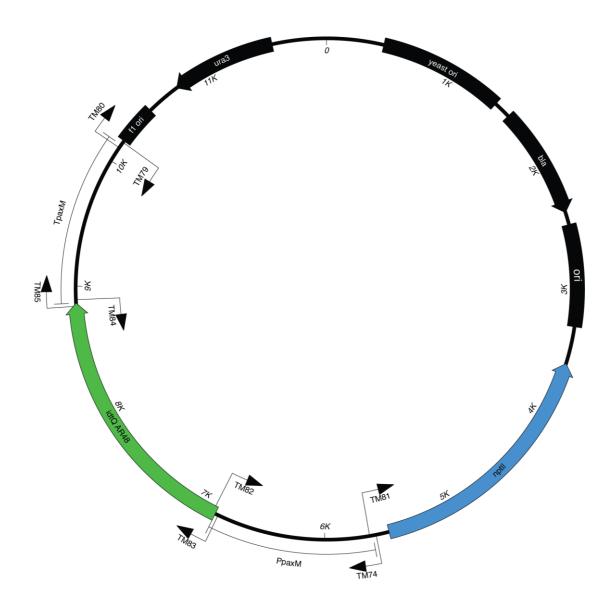
Appendix 11: *Epichloë festucae* Fl1 indole-diterpene (IDT) *idtP* complementation construct (pSS56, 5572 bp). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PpaxM (promoter). TpaxM (terminator). Primers for screening transformants are indicated by black arrows (TM125/TM126).



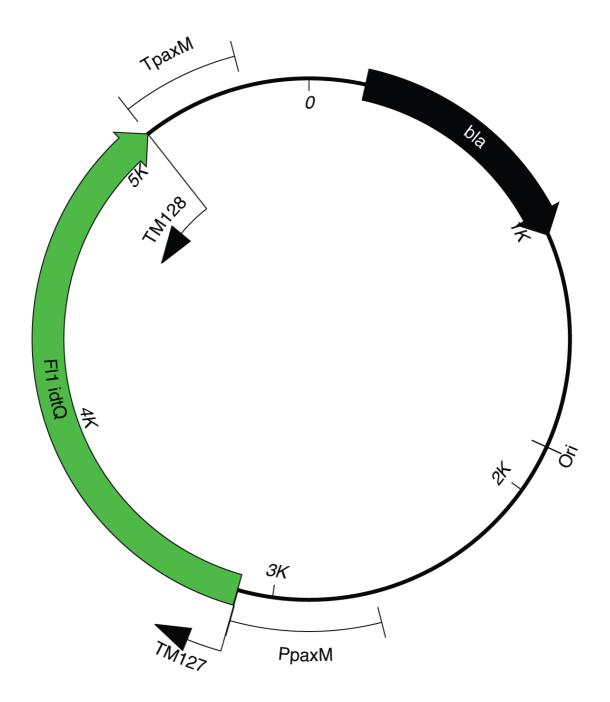
Appendix 12: Geneticin construct (pDB49, 7458 bp). NptII (geneticin resistance gene). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtypC (terminator). Primers for backbone amplification are indicated by black arrows (TM80/TM81).



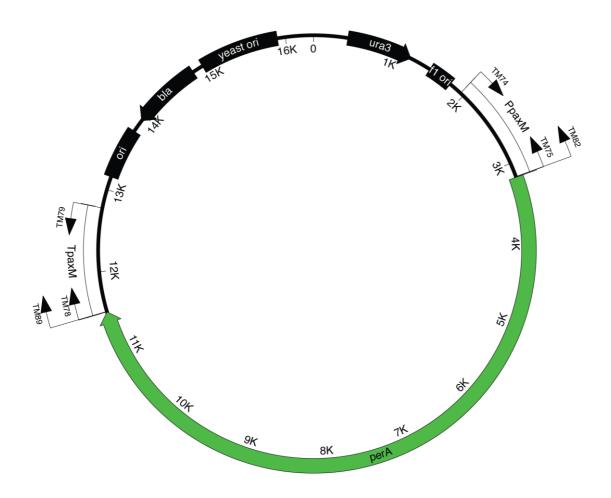
Appendix 13: *Penicillium paxilli paxQ* complementation construct (pSS2, 8270 bp). Nptll (geneticin resistance gene). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtypC (terminator). Primers for screening transformants are indicated by black arrows (TM94/TM95).



Appendix 14: *Epichloë festucae* AR48 indole-diterpene (IDT) *idtQ* complementation construct (pTM07, 11970 bp). Nptll (geneticin resistance gene). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PpaxM (promoter). TpaxM (terminator). Primers for screening transformants (TM74/TM82) and well as plasmid construction (all) are indicated by black arrows.



Appendix 15: *Epichloë festucae* Fl1 indole-diterpene (IDT) *idtQ* complementation construct (pSS58, 5743 bp). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtypC (terminator). Primers for screening transformants are indicated by black arrows (TM127/TM128).



Appendix 16: *Epichloë festucae* Fl1 *perA* complementation construct (pDB05, 16340 bp). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PpaxM (promoter). TpaxM (terminator). Primers for amplifying PpaxM (TM74/TM75 or TM82) and TpaxM (TM78 or TM89/TM79) are indicated by black arrows

# A)

Sample Name	Emind ole SB	Paspaline	Paspaline B	Paxitriol	PC-M6	13-Desoxypaxil line	Paxilline	Prenyl-Paxilline
WT	17918977	37578332	8075647	2927898	32508715	10436887	26170219	935380
∆paxP	3029584	91094265	4209	71901	0	91456	194559	0
PaxP 1	6592671	105294518	500150	853192	64065367	600602	18697037	2038062
PaxP 2	674742	592 60895	501772	472912	19265297	79575	18852009	642213
PaxP 3	16900737	135026875	630529	710940	55319948	2 2 7 5 2 6 6	20472823	1054703
PaxP 4	14099296	123294389	488534	594888	67660262	1761989	18036770	278737
PaxP 5	12891209	103134682	1094406	752215	45541583	5727201	24494919	1140592
AR48 idtP 1	2482041	108142833	6352	935234	1401704	3023137	187247	394236
AR48 idtP 2	1542348	127138139	6923	249436	4064938	13709	847779	0
AR48 idtP 3	532566	92582313	0	460518	1279818	0	261135	4089
AR48 idtP 4	833300	78150456	0	120313	534384	32235	126066	264504
AR48 idtP 5	397293	102 190873	0	7762.47	4297276	21924	1090526	58507
Fl1 idtP 1	26769	145987	0	0	63225	14823	1262358	13514
Fl1idtP 2	9284304	3254345	1892685	299168	2090625	16758196	163431	29825
Fl1 idtP 3	27787	2204310	0	0	0	0	22651	0
Fl1 idtP 4	1899401	38027830	0	12943	1171161	10564	60895	12962
Fl1 idtP 5	473715	59128029	0	84760	1944481	0	231401	0
∆paxQ	13054340	183 57323	1505389	584175	9844740	21689912	58014	0
PaxQ 1	2285600	4432365	35477	216113	3134116	2 2 4 8 6 0	18684353	248886
PaxQ 2	1087373	1880513	2 0 9 0 0	205195	2557373	106461	15900262	113072
PaxQ 3	642404	3002494	116656	143488	3000 385	193580	14824034	84852
PaxQ 4	4798563	17265020	1038662	826374	11393434	522381	22761180	591540
PaxQ 5	767176	1725430	0	30723	424866	21674	3987396	21851
AR48 idtQ 1	24003867	27761677	437569	582158	18870809	18126596	778364	46075
AR48 idtQ 2	17201458	20149840	1529485	646728	15413967	20411402	1446045	167865
AR48 idtQ 3	12818468	11053630	2117029	411201	8540654	19894342	912881	140229
AR48 idtQ 4	7875884	5779140	2429370	416185	7343449	19008538	928543	0
AR48 idtQ 5	295087	619772	1636936	248864	979511	14399058	964380	0
Fl1 idtQ 1	1451588	2982559	693159	4843 03	4286858	12359124	577140	0
Fl1 idtQ 2	0	0	0	0	0	12287	2241	0
Fl1 idtQ 3	2799408	13624157	1385013	540638	7338274	17733240	624483	110034
Fl1 idtQ 4	5317667	7974847	373519	523375	13700297	15927909	101287	0
Fl1 idtQ 5	22827870	34635839	1881418	1396849	19819352	2 5754918	1095717	181800

B)

Sample Name	406_1	406_2	406_3	406_4	406_5	422_1	436_1	438_1	438_2	438_3	452_1	452_2
WT	1517495	305439	11997490	1597872	3607009	358347	14303014	440512	409373	24290	1687018	7352610
ΔpaxP	207263	33292	152788	110097	689456	97187	15006	0	0	1068032	0	11345
PaxP 1	340881	92302	26406486	304214	1045262	258782	12127790	2094979	308268	0	543766	8606249
PaxP 2	163675	37908	3913643	138120	239347	125923	2681550	330691	7241	56674	1583592	5337059
PaxP 3	404234	122056	22714310	387148	2108246	262944	13651948	1764261	425397	1047106	213449	12784720
PaxP 4	547078	72442	16538446	356536	2038552	252445	18073730	2646684	581634	534363	186913	13057553
PaxP 5	366180	105418	19407619	366606	1680068	225122	11427348	1042582	136260	140026	200197	11998505
AR48 idtP 1	135936	35011	485319	227676	240921	129819	863487	70066	15118	212985	1414	285485
AR48 idtP 2	266904	19817	118108	185147	222849	242051	1385273	190437	66755	245226	3023	563015
AR48 idtP 3	162247	59169	155667	76953	92771	184419	887354	82669	50507	150014	1430	169825
AR48 idtP 4	82975	34501	225572	57539	228609	73136	348885	45740	0	97240	798	52317
AR48 idtP 5	408185	58244	109707	189064	130472	232971	2088521	226987	48393	503675	6212	603410
FI1 idtP 1	0	0	55324	0	6184	0	19479	0	0	0	166716	31133
FI1 idtP 2	183751	173088	2091596	143194	750557	351596	450848	11660	527755	0	0	1723321
FI1 idtP 3	2807	0	0	0	15097	0	20987	6547	0	20878	0	10506
FI1 idtP 4	36803	37095	158578	12034	232500	71881	749404	584534	6117	220363	0	512861
FI1 idtP 5	106851	15815	9256	23272	115543	99902	1490575	645053	5648	158483	0	595516
ΔpaxQ	359190	195549	6823101	312732	1126179	372556	2359058	69373	448961	16003	0	4136953
PaxQ 1	208549	72913	1349722	238253	325670	131597	766694	21140	46346	0	3825470	1280787
PaxQ 2	159011	46810	539349	136957	267343	117443	427508	18939	26014	0	3914376	1428075
PaxQ 3	69181	15635	1776571	34841	105715	62533	625674	14799	12487	0	385119	1036072
PaxQ 4	420058	142561	3324042	440887	1080044	316234	2749240	119326	351096	23755	562301	5942380
PaxQ 5	23133	6350	345024	5048	52625	0	222896	5200	4090	0	57293	365944
AR48 idtQ 1	313641	89542	8590519	663534	2657837	198104	4720119	117324	338815	0	0	5682017
AR48 idtQ 2	396487	104225	5461310	401410	1663142	278154	4313133	102557	462922	0	0	4542130
AR48 idtQ 3	300666	133741	5101310	235374	1194673	225154	2133607	47581	659038	0	0	3150312
AR48 idtQ 4	298708	131627	1524459	172874	745873	264641	1715706	34019	497924	0	0	2632723
AR48 idtQ 5	128726	52388	104502	43630	51725	290761	217801	11134	70160	0	0	1318525
FI1 idtQ 1	180707	24421	991075	137972	277420	189691	486232	36114	34622	0	0	3612324
FI1 idtQ 2	0	0	0	0	0	0	0	0	0	0	0	1077
FI1 idtQ 3	192929	65700	4111243	179487	284746	364324	2039514	129379	65325	27464	0	2972341
FI1 idtQ 4	208875	53662	1334895	101097	346883	279005	1837040	120583	25660	35736	0	5791002
FI1 idtQ 5	572774	207127	12390215	485855	1760363	518131	4942215	195392	574664	50722	0	6616585

Appendix 17: *Penicillium paxilli* (pax) complementation raw data of A) known indolediterpenes compound and B) unknown indole-diterpene (IDT)-like compounds. For all transformants; wild type (WT), gene deletions ( $\Delta paxP$  and  $\Delta paxQ$ ) and complementations (paxP, *E. festucae* var *lolii* AR48 *idtP*, or *E. festucae* Fl1 *idtP* into  $\Delta paxP$ ) and (paxQ, AR48 *E. festucae* var *lolli idtQ*, or Fl1 *E. festucae idtQ* into  $\Delta paxQ$ ). The new IDT compounds are labelled with the *m/z* and then the unknown IDT order in that chromatogram.

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Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

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1 ATGGCTCATAAGTAAGAGCTTCCCTCTTTTCTAATTCGTTCTGTCCATGATCGGACTA 60 1 ATGGCTCATAAGTAAGAGCTTCCCTCTTTTTCTAATTCGTTCTGTCCATGATCGGACTA 60 1
61 ACAT GTATCTTTGCAGCGCTAACGAAACGTCCATTGTCGAATTTTCGA 108 61 ACAT GTATCTTTGCAGCGCTAACGAAACGTCCATTGTCGAATTTTCGA 108 17 ACAACCCGAAGTGAGGGGTCAGAACAAAAAAAAGCGAAATTGCAAAGGTCTCTTCTCGT 75 17 ACAACCCGAAGTGAGGGGTCAGAACAAAAAAAAAGCGAAATTGCAAAGGTCTCTTCTCGT 76 61 ACATGTATTTTTGCAGCGCTAACGAAACAAACGTCCATTGTCGAATTTTCGA 108
109 AAGATCCCCTTGGTTTTATCACGAAGGGCGAAAAAGG 145 109 AAGATCCCCTTGGTTTTATCACGAAGGGCGAAAAAGG 145 76 GGCCCTCCGACTCTTCCCGTTTTCACTTCGCCGGCGGCGCGCGC
146       CTATGCCGTC ATCAGCTCCGACAACATTCCTGGACGACG TCCAAATGTTTTTA 198         146       CTATGCCGTC ATCAGCTCCGACAACATTCCTGGACGACG - TCCAAATGTTTTTA 198         136       CGCCGCCGGCCGCAACGCTCCAACGCTCTCGAGGCCGACGCCTTCGGATTGTACAGA 195         137       CGCGGCCGGCGCGCGACCCGGTCCAACGCTCTCCGAGGCCGACGCCTTCGGATTGTACAGA 195         137       CGCGGCCGGCGCGCGACCCGGTCCAACGCTCTCCGAGGCCGAGGCCTTCGGATTGTACAGA 195         146       CTATGCCGTC ATCAGCTCCGACACGCTCTCCGAGGCCTTCGGATTGTACAGA 196         146       CTATGCCGTC ATCAGCTCCGACACACTCCGGACGCCTTCGGATTGTACGAGA 198
199 T C G C A G A C G A A
250 CAGCGT TCG AAATCAGGTACCATGGAGCCGACGCTGGCCCCGACACCGT 298 250 CAGCGT - TCG AAATCAGGTACCATGGAGCCGACGCTGGCCCCGACACCGT 298 253 GACCGTGCTCGGCAACCTCCGCGCCCCTGACGTATAATAATACTATACTAATAATAATAAT 254 GACCGTGCTCGGCAACCTCCGCGCCCCTGACGCTAATAATATACTTATTAATAATAATAAT 254 CACCGT - TCG AAATCAGGTACCATGGAGCCGACGCTGGCCCCGACACCGT 298 250 CAGCGT - TCG AAATCAGGTACCATGGAGCCGACGCCGCCCGACACCGT 298
299 CTCGGCGTATCACTTGGGATACAATGGAGGCGCGCACACGTCTTGGACGCCCGC 352 299 CTCGGCGTATCACTTGGGATACAATGGAGGCGCGCGCACACGTCTTGGACGCCCGC 352 313 AATAACCACGGCTAATTGGTAATAGTATCATAAAGGCATGCACCCCCCT - GCTGCCTAC 370 314 AATAACCACGGCTAATTAGTAATAGTATCATAAAGGCATGCACCCCCCCT - GCTGCCTAC 371 299 CTCGGCGTATCACTTGGGATACAATGGAGGCGCGCACACGTCTTGGACGCCCGC 352
353 C - CAAATCGATATCCCCAAGATAAACCCCGGAA - CATAACTTGCTTTTC ACAGGT - 404 353 C - CAAATCGATATCCCCCAAGATAAACCCCGGAA - CATAACTTGCTTTTC ACAGGT - 404 371 CGCACCTCG - TCTCCAACGGACCTACATGTAATCGTATCGTATGTACGGCTTCTCTGCAGGTGA 429 372 CGCACCTCG - TCTCCAACGGACCTACATGTAATCCGTATGTAGCGCTTCTCTGCAGGTGA 430 353 C - CAAATCGATATCCCCAAGATAAACCCCGGAA - CATAACTTGCTTTTC ACAGGT - 404
405
452 GTACCGTGTCTATCACGACACTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCAT 511 452 GTACCGTGTCTATCACGACACTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCAT 511 490 GTACCGTGTCTATCACGACACTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCAT 549 491 GTACCGTGTCTATCACGACACTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCAT 550 452 GTACCGTGTCTATCACGACACTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCAT 511 1
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571       TCATGCACTATCGCGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       630         571       TCATGCACTATCGCGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       630         609       TCATGCACTATCGCGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       668         610       TCATGCACTATCGCGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       669         571       TCATGCACTATCGTGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       669         571       TCATGCACTATCGTGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       630         74       TCATGCACTATCGCGAAGGTCGCTGGTCTCTGTGTTTCCAGAGGCAAACCCTACTTCAAA       133
631 ATGCACGTGTTGTCCTGTACGATCCGCTGCCGAGAGATGGCCCATCCAT
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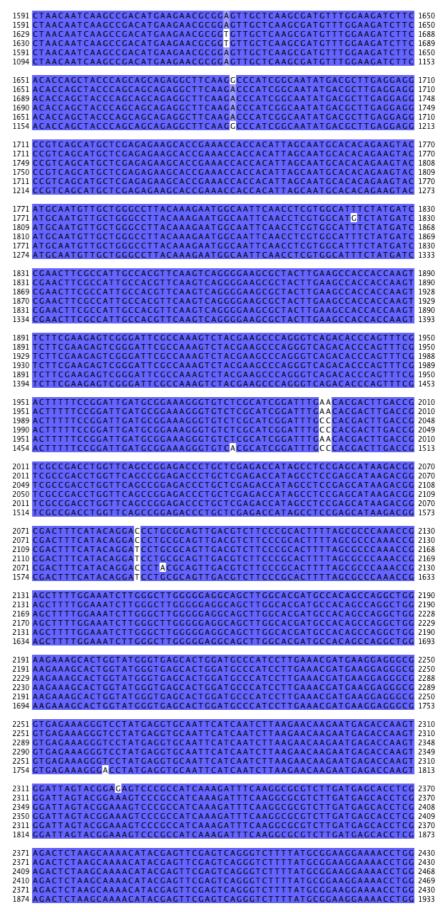
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811 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCA 811 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTG 870 849 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTG 908 850 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTG 909 811 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTG 870 314 ACCCCTTTCATTACGAAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTG 373	
871 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 930 871 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 930 909 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 968 910 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 968 871 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 930 874 ACAATGAAGCCGTTGGCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAG 930	
931 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATCCAAGGAGTTGGTGTCACTCA 990 931 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATGCACAGGAGTTGGTGTCACTCA 990 969 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATGCACAGGAGTTGGTGTCACTCA 102 970 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATGCACAGGAGTTGGTGTCACTCA 102 931 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATGCACAGGAGTTGGTGTCACTCA 990 434 ATAGGCCTTATCCAACTGAACCCAATGCTGATCTCAATGCACAGGAGTTGGTGTCACTCA 493	
991 GCGACCAGCTGTTGCTAAGCGAAAGGTTTGTTAGGCGAACCATGACGTCTTCTCGGAATT 105 991 GCGACCAGCTGCTTCTAAGCGAAAGGTTTGTTAGGCGAACCGTGACGTCTTCTCGGAATT 105 1029 GCGACCAGCTGTTTCTAAGCGAAAGGTTTGTTAGGCGAACCGTGACGTCTTCTCGGAATT 108 1030 GCGACCAGCTGTTTCTAAGCGAAAGGTTTGTTAGGCGAACCGTGACGTCTTCTCGGAATT 108 991 GCGACCAGCTGCTTCTAAGCGAAAGGTTTGTTAGGCGAACCGTGACGTCTTCTCGGAATT 105 494 GCGACCAGCTGTTTCTAAGCGAAAGGTTTGTTAGGCGAACCGTGACGTCTTCTCGGAATT 553	3
1051 ACGACTATACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGGTTTGCAGCAGTCGTGA 1110 1051 ACGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGGTTTGCAGCAGTCGTGA 1110 1089 ACGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGGTTTGCAGCAGTCGTGA 114 1090 ACGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGTTTGCAGCAGTCGTGA 114 1051 ACGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGTTTGCAGCAGTCGTGA 114 554 ACGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAAAGGGGGGTTTGCAGCAGTCGTGA 114	3
1111       GAGAAGGCGAGCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       117         1111       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       117         1149       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       120         1150       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       120         1111       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       120         1111       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       117         614       GAGAAGGCGACCATCGGCAAACACCTCTAGGAGGCACAGCCGGCCAACGTCTCTCCGAAC       673	3
1171 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 123 1171 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 123 1209 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 126 1210 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 126 1171 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 126 1171 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 126 674 AAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATACAATACATATG 733	3
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1471 CAATTCCCGATGAGTATGGCGGCGGCCGTTGCTATCCTCTGGTCTATGCCATGTCAGTGG 153 1471 CAATTCCCGATGAGTATGGCGGCGGCCGTTGCTATCCTCTGGTCTATGCCATGTCAGTGG 153 1509 CAATTCCCGATGAGTATGGCGCGCGCGTTGCTATCCTCTGGTCTATGCCATGTCAGTGG 156 1510 CAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCCATGTCAGTGG 156 1471 CAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCCATGTCAGTGG 153 974 CAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCCATGTCAGTGG 153	) 3 9 )
1531 CACTGGCATCGTCGGATTTCGCCATTGAGCAACTCTGCGCCCAGGTGGTCGGCCTCTCCC 159 1531 CACTGGCATCGTCGGATTTCGCCATTGAGCAACTCTGCGCCCAGGTGGTCGGCCTCTCCC 159 1569 CACTGGCATCGTCGGATTTCGCCATTGAGCAACTCTGCGCCCAGGTGGTCGGCCTCTCCC 162 1570 CACTGGCATCGTCGAATTTCGCCATTGAGCAACTCTGCGCCCAGGTGGTCGGCCTCTCCC 162 1531 CACTGGCATCGTCGGATTTCGCCATTGAGCAACTCTGCGCCCAGGTCGGTC	) 3 9 )

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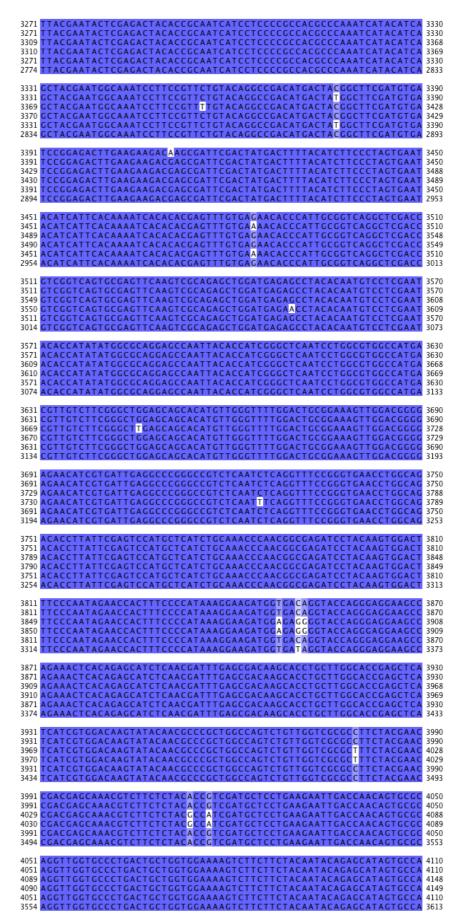
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2431 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 2490 2431 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 2490 2469 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 2528 2470 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 2529 2431 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 259 1934 CGCACGCCGAAGCCATTGATGGCTTGAATGCAATGTTTATTGTGAGAACACTGATAGAGC 2490
2491 ACTTTGCCGGCAAAAAGACTGAAGAGAGCACGACCAACGCCGAGCTCGCAGACGCGCTCA 2550 2491 ACTTTGCCGGCAAAAAGACTGAAGAGAGCACGACCAACGCCGAGCTGGCAGACGCGCTCA 2550 2529 ACTTTGCCGGCAAAAAGACTGAAGAGGAGCACGACCAACGCCGAGCTCGCAGACGCGCTCA 2588 2530 ACTTTGCCGGCAAAAAGACTGAAGAAGAGCACGACCAACGCCGAGCTCGCAGACGCGCTCA 2589 2491 ACTTTGCCGGCAAAAAGACTGAAGAGAGCACGACCAACGCCGAGCTCGCAGACGCCTCA 2550 1994 ACTTTGCCGGCAAAAAGACTGAAGAGAGAGAGCACGACCAACGCCGAGCTCGCAGACGCGCTCA 2559
2551 TGATACACAGCTATCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2610 2551 AGATACACAGCTTTCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2610 2589 TGATACACAGCTATCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2648 2590 TGATACACAGCTATCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2649 2551 TGATACACAGCTATCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2610 2054 TGATACACAGCTATCTCAACCTCACCCAGATTGGTCACCAGACTTTGGGTGACGTAGGGA 2610
2611 AGATGGTCAATCTCGTCAAGGATATGCTTGAGACGGGGCAAGTAGCCAAATCGTCGCTGT 2670 2611 AGATGGTCAATCTCGTCAAGTATAGCTTGAGACGGGGCAAGTGGCCAAATCGTCGCTGT 2670 2649 AGATGGTCAATCTCGTCAAGGATATGCTTGAGACGGGGCAAGTAGCCAAATCGTCGCTGT 2708 2650 AGATGGTCAATCTCGTCAAGGATATGCTTGAGACGGGGCAAGTGGCCAAATCGTCGCTG 2709 2611 AGATGGTCAATCTCGTCAAGGATATGCTTGAGACGGGGCAAGTGGCCAAATCGTCGCTGT 2670 2114 AGATGGTCAATCTCGTCAAGGATATGCTTGAGACGGGGCAAGTGGCCAAATCGTCGCTGT 2173
2671 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2730 2671 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2730 2709 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2768 2710 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2769 2671 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2730 2174 CAACTCTCGTCAAAGGTCTTGGTAATGCCTCAGAAGGCTTCGGCGTGCTCCTTGGCGGTG 2233
2731 CCAACGTTATCCTCGACGCCTACGAGCTTGCCCACAGGGACAATGATGCCCAGAGAGCCG 2790 2731 CCAACGTTATCCTCGACGCCTACGAGGTTGCCCACAGGACAATGATGCCCAGAGAGCCG 2790 2769 CCAACGTTATCCTCGACGCCTACGAGCTTGCCCACAGGGACAATGATGCCCAGAGAGCCG 2828 2770 CCAACGTTATCCTCGACGCCTACGAGCTTGCCCACAGGACAATGATGCCCAGAGAGCCG 2829 2731 CCAACGTTATCCTCGACGCCTACGAGCTTGCCCACAGGACAATGATGCCCAGAGAGCCG 2829 2734 CCAACGTTATCCTCGACGCCTACGAGCTTGCCCACAGGACAATGATGCCCAGAGAGCCG 2829
2791 TCTTTGGCACACAGTTGGCTTTTGATTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTTG 2791 TCTTTGGCACACAGTTGGCTTTTGATTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTTG 2859 TCTTTGGCACACAGTTGGCTTTTGATTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTTG 2830 TCTTTGGCACACAGTTGGCTTTTGATTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTTG 2791 TCTTTGGCACACAGTTGGCTTTTGATTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTTG 2850 2294 TCTTTGGCACACACGTTGGCTTTTGGTTCGGTCACGTTCCTAGCAAGTGTTGGTACCGTGGTGTGGTACGTTGGTACCGTTGGTACGTTGGTACCGTTGGTACGTTGGTACCGTTGGTACGTTGGTACGTTGGTACCGTTGGTACGTTG
2851       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2910         2851       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2910         2889       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGTCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2948         2890       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGCCGTCGTGGAGGTGTCAGTGTTATTC       2949         2891       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2949         2851       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2910         2354       GTGCCGGACTCTTAGGAGCCACTACCGTCGTCGCCGTCGTGGGAGGTGTCAGTGTTATTC       2413
2911 TCGGAGGACTTGCCTTTGGCGTTGGCGCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 2970 2911 TCGGAGGACTTGCCTTTGGCGTTGGCGCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 2970 2949 TCGGAGGACTTGCCTTTGGCGTTGGCGCCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 3008 2950 TCGGAGGACTTGCCTTTGGCGTTGGCGCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 20970 2911 TCGGAGGACTTGCCTTTGGCGTTGGCGCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 2970 2414 TCGGAGGACTTGCCTTTGGCGTTGGCGCTCTGGCATCCGGCTTTGCACAAATTGCCGCCA 2473
2971 AAGCACAGGGCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 3030 2971 AAGCAGAGGCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 3030 3009 AAGCAGAGGCCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 3068 3010 AAGCAGAGGCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 3069 2971 AAGCAGAGGCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 3030 2474 AAGCAGAGGCTGTGGGTCGCTATTTCGGTGACGCCGACGAGGCCTACAAAGCTGGAGGAT 2533
3031 TCAAGTACGATGAGAACCATAAGATTCTTGTTCCTCTGTCTG
3091 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 3150 3091 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 3150 3129 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 3188 3130 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 3183 3091 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 3150 2594 TCGACGCGGCCGGTAACGTCAAATTCGACAGCCAATACATCTATCGCACCTGGCACGGTG 2653
3151       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3210         3151       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3210         3189       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3248         3190       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3249         3190       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3249         3151       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3249         3151       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3210         2654       CCACAGGGTCGGGCAGAATCAACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATGATCC       3210
3211 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3270 3211 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3270 3249 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3308 3250 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3309 3211 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3270 2714 ACGATAAGTCCCAGGCTATTAATGTGCGAGAAGGCATTCGAGCCCCGGCCAGCGGAAAAC 3270

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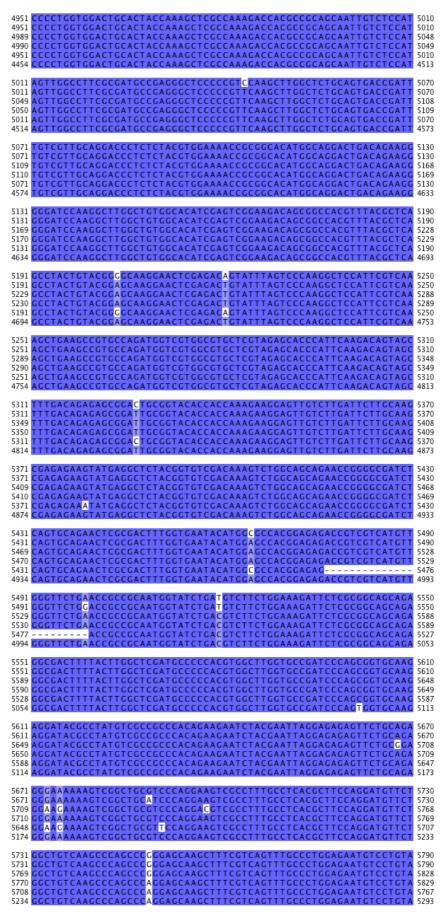
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4171 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4230 4171 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4230 4209 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4268 4210 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4269 4171 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4269 4171 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 4230 3674 TTTCTAAAAGAACCCTTCAACGCGTCTGGGTAGATGGTAGGTGGAATTATTTCATACAAG 3733
4231 TAGCGTACCTGTACCTCTGTCACTAATTGTTTATCCAGTGGACAATCAGATTCACGCTCT 4290 4231 TAGCGTACCTGTACCTCTGTCACTAATTTTTTATCCAGTGGACAATCAGATTCACGCTCT 4290 4269 TAGCGTACCTGTACCTCTGTCACTAATTGTTTATCCAGTGGACAATCAGATTCACGCTCT 4328 4270 TAGCGTACCTGTACCTCTGTCACTAATTGTTTATCCAGTAGACAATCAGATTCACGCTCT 4329 4231 TAGCGTACCTGTACCTCTGTCACTAATTGTTTATCCAGTGGACAATCAGATTCACGCTCT 4329 3734 TAGCGTACCTGTACCTCTGTCACTAATTGTTTATCCAGTGGACAATCAGATTCACGCTCT 3793
4291 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 4350 4291 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 4350 4329 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 4388 4330 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 4389 4291 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 4350 3794 TTTCCGCCACCAGTTGAGCGAGGATTATTTCGGAACTCTCAACTATATCCTCACCGCCGA 3853
4351 TAGCATGAACCTCGTTGCCATGGTTGGAGGCCCTGCCTTGCTTG
4411 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGCGA 4411 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGGGA 4449 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGGGA 4450 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGGGA 4411 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGGGA 3914 AGACAAGTGGACAGGCGAGGCTACACTTCTACTGGAAGACTACTACACTGGGCCGAGCGA 3973
4471 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGGAT 4471 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT 4509 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT 4510 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT 4471 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT 4580 3974 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT 4590 3974 GCTTATGCTCAAGCTCAAGCCATTCGAAGCCCTAGCTGGAGCCGATATCGATGCGGCGAT
4531 AGATCCTCATTTGATTTTTGTGCTGGGACCCAAAGGCAAAGATCGTTACTTTTACCATCA 4590 4531 AGATCCTCATTTGATTTTTGTGCTGGGACCCAAAGGCAAAGATCGTTACTTTTACCATCA 4590 4569 AGATCCTCATTTGATTTTTGTGCTGGGACCCAAAGGCAAAGATCGTTACTTTTACCATCA 4628 4570 AGATCCTCATTTGATTTTTGTGCTGGGACCGAAAGGCAAAGATCTCTACTTTTACCATCA 4628 4531 AGATCCTCATTTGATTTTTGTGCTGGGACCCAAAGGCAAAGATCTCTACTTTTACCATCA 4590 4034 AGATCCTCATTTGATTTTTGTGCTGGGGACCCAAAGGCAAAGATCGTTACTTTTACCATCA 4993
4591 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4650 4591 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4650 4629 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4688 4630 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4688 4591 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4680 4094 ATTCTGGATACGAATGTCCGACCGAACTGTTATCAAACCTCATCCTGACATTCACCTTAA 4650
4651       TTACCTGCTTGTCGGAACCATTGCTTCGACAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4710         4651       TTACCTGCTTGTCGGAACCATTGCTTCGACAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4710         4689       TTACCTGCTTGTCGGAACCATTGCTTCGACAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4748         4690       TTACCTGCTTGTCGGAACCATCGCTTCGGCAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4748         4691       TTACCTGCTTGTCGGAACCATCGCTTCGGCAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4749         4651       TTACCTGCTTGTCGGAACCATCGCTTCGGCAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4749         4651       TTACCTGCTTGTCGGAACCATCGCTTCGGCAGATGGCAGTGGAACTGTTTTGCTTCTT       4710         4154       TTACCTGCTTGTCGGAACCATTGCTTCGACAGATGGCAGTGGTGAACTGTTTTGCTTCTT       4213
4711 CTCATACAATCAACACAAGCTCGTCGTCCAACGAGGAAACGGCAAACAGGCAGAAGAACC 4770 4711 CTCATACAATCAACACAAGCTCGTCGTCGTCCAACGAGGAAACGGCAAACAGGCAGAAGAACC 4770 4749 CTCATACAATCAACACAAGCTCGTCGTCCAACGAGGAAACGGCAAACAAGGCAGAAGAACC 4808 4750 CTCATACAATCAACACAAGCTCGTCGTCCAACGAGGAAACGGCAAACAGGCAGAAGAACC 4809 4711 TTCATACAATCAACACAAGCTCGTCGTCCAACGAGGAAACGGCAAACAGGCAGAAGAACC 4770 4214 CTCATACAATCAACACAAGCTCGTCGTCCAACGAGGAAACGGCAAACAGGCAGAAGAACC 4273
4771 AAGGCCTGTCACAATTTCCCCCGAGCTCGGAACCATCTCAAACTTCTTCTGCGCCAACAA 4830 4771 AAGGCCTGTCACAATTTCCCCCGAGCTTGGAACCATCTCAAACTTCTTCTGCGCCAACAA 4830 4809 AAGGCCTGTCACAATTTCCCCCGAGCTCGGAACCATCTCAAACTTCTTCTGCGCCAACAA 4868 4810 AAGGCCTGTCACAATTTCCCCCGAGCTCGGAACCATCTCAACTTCTTCTGCGCCAACAA 4869 4771 AAGGCCTGTCACAATTTCCCCCGAGCTCGGAACCATCTCAAACTTCTTCTGCGCCAACAA 4830 4274 AAGGCCTGTCACAATTTCCCCCGAGCTCGGAACCATCTCAAACTTCTTCTGCGCCCAACAA 4833
4831 TAACTTGTTTTCCATTACGAATGCGGGTTTCATTCTCCGCTTGACAAGCCACTGTACACT 4890 4831 TAACTTGTTTTCCATTACGAATGCGGGTTTCATTCTCCGCTTGACAAGCCACTGTACACT 4890 4869 TAACTTGTTTTCCATTACGAATGCGGGTTTCATTCTCCGCTTGACAAGCCACTGTACACT 4928 4870 TAACTTGTTTTCCATTACGAATGCGGGGTTTCATTCTCCGGCTTGACAAGCCACTGTACACT 4929 4831 TAACTTGTTTTCCATTACGAATGCGGGTTTCATTCTCCGGCTTGACAAGCCACTGTACACT 4890 4334 TAACTTGTTTTCCATTACGAATGCGGGTTTCATTCTCCGCTTGACAAGCCACTGTACACT 4890
4891       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4950         4891       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4950         4929       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4988         4930       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4988         4831       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4988         4930       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4989         4891       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4989         4891       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4989         4891       CGCTCTGGAAGCCGTCAACCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4989         4891       CGCTCTGGAAGCCGTCAACCATCATCAGTGGCTCGAGCACTACGAAAAGGACGTAGACGGCGG       4950

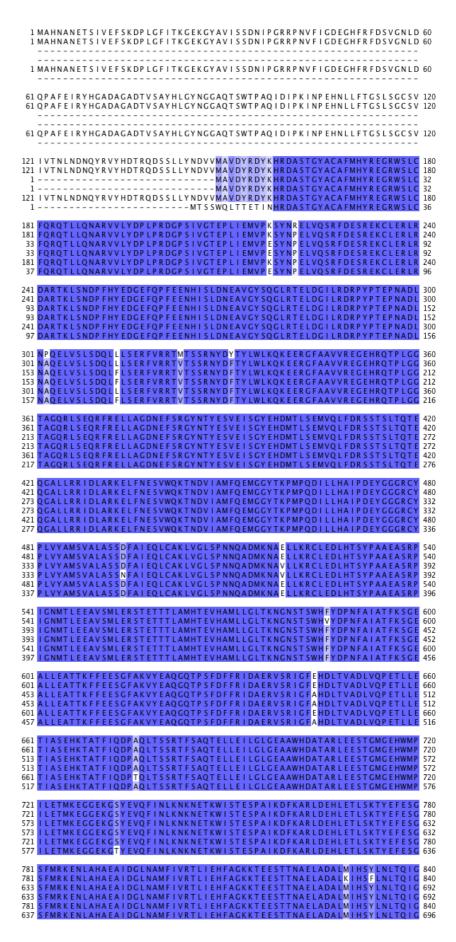
Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_FJ1 Epichloë\_festucae\_FJ1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Épichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloë festucae AR48 Epichloë\_festucae\_RR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Épichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_RR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë festucae Fl1 Epichloë\_festucae\_F2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë festucae AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37



Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	5791 TACCATACTGTCTCAATTTGAGGGCGATACTAGCTTCATGGAGTGTGTTGTTCCGAGCT 5791 TACCATACTGTCTCAATTTGAGGGCGATACTAGCTTCATGGAGTGTGTTGTTCCGAGCT 5829 TACCATACTGTCTCAATTTGAGGGCGATACTAGCTTCATGGAGTGTGTGT	C 5850 C 5888 C 5889 C 5827
Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	5851 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTCAGA 5851 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTCAGA 5889 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTAAGA 5890 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTCAGA 5828 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTCAGA 5354 CAGCTGGGAGTCCTTGACAGGACTTGTCATTGAGTGGAAGGATCAGGGTCAAGTTCAGA	T 5910 T 5948 T 5949 T 5887
Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	5911 TGAGGGTTCTACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACG 5911 TGAGGGTTCTACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACG 5949 TGAGGGTTCCACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACG 5950 TGAGGGTTCTACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACG 5888 TGAGGGTTCTACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACG 5414 TGAGGGTTCTACGAGGCATCCGAGACCGGCTGACTCGTTTCTCGGAAAAAGATTGGACG	A 5970 A 6008 A 6009 A 5947
Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	5971 CGATCTCATAATGATGGAAAT <mark>A</mark> TCGACTGGTCGCTTCTTAAAAATTTCCAGAGGACTCG 5971 CGATCTCATAATGATGGAAATCTCGACTGGTCGCTTCTTAAAAATTTCCAGAGGACTCG 6009 CGATCTCATAATGATGGAAATCTCGACTGGTCGCTTCTTAAAAATTTCCAGAGGACTCG 6010 CGATCTCATAATGATGGAAATATCGACTGGTCGCTTCTTAAAAATTTCCAGAGGACTCG 5948 CGATCTCATAATGATGGAAATCTCGACTGGTCGGTTCTTAAAAATTTCCAGAGGACTCG 5474 CGATCTCATAATGATGGAAATCTCGACTGGTCGGTTCTTAAAAATTTCCAGAGGACTCG	T 6030 T 6068 T 6069 T 6007
Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	6031 TATGGATCCTTCGTGTGTGATTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA 6031 TATGGATCCTTCGTGTGTGATTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA 6069 TATGGATCCTTCGTGTGTGACTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA 6070 TATGGATCCTTCGTGTGTGATTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA 5080 TATGGATCCTTCGTGTGTGATTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA 5334 TATGGATCCTTCGTGTGTGATTCGGTTCACTGATAAGCTACTGACCGTGGGCAATGGGA	G 6090 G 6128 G 6129 G 6067
Epichloë_festucae_AR48 Epichloë_festucae_Fg1 Epichloë_festucae_F11 Epichloë_festucae_E2368 Epichloë_festucae_AR1 Epichloë_festucae_AR37	6091 CAATGAAATTCAGTAG 6091 CAATGAAATTCAGTAG 6129 CGCTGGAAATCAGTAG 6130 CAATGAAATTCAGTAG 6068 CAATGAAATTCAGTAG 5594 CAATGAAATTCAGTAG	6106 6106 6144 6145 6083 5609

Appendix 18: Alignment of makes caterpillars floppy (*mcf*) genes from a range of *Epichloë festucae* strains using the alignment function of Geneious.

Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë festucae AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë festucae AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Épichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë festucae AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë festucae AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë festucae AR48 Epichloë\_festucae\_RR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë festucae FI1 Epichloë\_festucae\_F12 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë festucae AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37



Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_Z368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37

841 HQT LGDVGKMVNLVK DML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 900 841 HQT LGDVGKMVNLVK YML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 900 693 HQT LGDVGKMVNLVK DML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 752 693 HQT LGDVGKMVNLVK DML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 752 841 HQT LGDVGKMVNLVK DML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 900 697 HQT LGDVGKMVNLVK DML ET GQVAK S S L ST LVK G LGNA S EG F GV LLGGANV I LDAY E LAH 752
901 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 960 901 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 960 753 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 812 753 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 812 901 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 960 757 TDNDAQRAVFGTQLAFDSVTFLASVGTVGAGLLGATTVVAVVGGVSVILGGLAFGVGALA 816
961 SGFAQIAAKAQAVGRYFGDADEAYKAGGFKYDENHKILVPLSGAVISEVDAAGNVKFDSQ 1020 961 SGFAQIAAKAEAVGRYFGDADEAYKAGGFKYDENHKILVPLFGAVISEVDAAGNVKFDSQ 1020 813 SGFAQIAAKAEAVGRYFGDADEAYKAGGFKYDENHKILVPLFGAVISEVDAAGNVKFDSQ 872 961 SGFAQIAAKAEAVGRYFGDADEAYKAGGFKYDENHKILVPLFGAVISEVDAAGNVKFDSQ 872 961 SGFAQIAAKAEAVGRYFGDADEAYKAGGFKYDENHKILVPLFGAVISEVDAAGNVKFDSQ 1020 817 SGFAQIAAKAEAVGRYFGDADEAYKAGGFKYDENHKILVPLFGAVISEVDAAGNVKFDSQ 876
1021 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 1080 1021 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 1080 873 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 932 873 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 932 1021 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 932 877 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 932 1021 Y I Y RTWHGATG SGR I NY F FWV GDK P RM I HDK SQA I NVR EG I RAPASGK LTNTR DYTA I I L 932
1081 PATPKSYISYEWQILPFCTGRHDYGFDVIRRLEEDKRFDYDFYIFPSEYIIHKITHEFVR 1140 1081 PATPKSYISYEWQILPFCTGRHDYGFDVIRRLEEDERFDYDFYIFPSEYIIHKITHEFVR 1140 933 PATPKSYISYEWQILPFCTGRHDYGFDVIRRLEEDERFDYDFYIFPSEYIIHKITHEFVR 992 933 PATPKSYISYEWQILPFCTGRHDYGFDVIRRLEEDERFDYDFYIFPSEYIIHKITHEFVR 992 1081 PATPKSYISYEWQILPFCTGRHDYGFDVIRRLEEDERFDYDFYIFPSEYIIHKITHEFVR 992
1141 TPIAVRLDRR SVRVQVAELDESLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGWSSTCWV 1200 1141 TPIAVRLDRRSVRVQVAELDESLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGWSSTCWV 1200 993 TPIAVRLDRRSVRVQVAELDESLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGUSSTCWV 1052 993 TPIAVRLDRRSVRVQVAELDENLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGWSSTCWV 1052 1141 TPIAVRLDRRSVRVQVAELDESLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGWSSTCWV 1200 997 TPIAVRLDRRSVRVQVAELDESLHNVLEYTIYGAGANYTIGLNPGVAMTLSSGWSSTCWV 1056
1201 LDCGKLDGENIVIEARAVSISGFRVNLADTLFESMLICKPNGEILQVDFPNRTTFPIKED 1260 1201 LDCGKLDGENIVIEARAVSISGFRVNLADTLFESMLICKPNGEILQVDFPNRTTFPIKED 1260 1053 LDCGKLDGENIVIEARAVSISGFRVNLADTLFESMLICKPNGEILQVDFPNRTTFPIKED 1112 1053 LDCGKLDGENIVIEARAVSISGFRVNLADTLFESMLICKPNGEILQVDFPNRTTFPIKED 1260 1057 LDCGKLDGENIVIEARAVSISGFRVNLADTLFESMLICKPNGEILQVDFPNRTTFPIKED 1260
1261 GDR YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYTVDA 1320 1261 GDR YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYTVDA 1320 1113 G EG YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYA I DA 1172 1113 G EG YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYA I DA 1172 1261 GDR YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYA I DA 1320 1117 GDR YQGG SQK LT EHLNDL SDKHLLGT ELI I VDKYTTP AGQ SVGRAFY EPT SKR LLYTVDA 1320
1321 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1380 1321 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1380 1173 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1232 1173 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1232 1321 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1380 1177 PEELTNSAQVGALTAGGKVFFYNTEHSAIWRVDVSTGVCEAKYHALCPFSKRTLQRVWVD 1380
1381 V DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1440 1381 V DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1440 1233 V DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1291 1233 – DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1291 1381 V DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1440 1237 V DNQ I HALFR HQLS EDY FGTLNY I LTAD SMNLVAMVGGPALLAKLHEEDKWTGEATLLLE 1440
1441 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGK DRY FYHQ FWI RMSDRTVIK 1500 1441 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDRY FYHQ FWI RMSDRTVIK 1500 1293 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDRY FYHQ FWI RMSDRTVIK 1351 1441 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDLY FYHQ FWI RMSDRTVIK 1500 1297 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDLY FYHQ FWI RMSDRTVIK 1500 1297 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDLY FYHQ FWI RMSDRTVIK 1500 1297 DYYTGP SELMLKLKP FEALAGAD I DAA I DPHL I FVLGPKGKDRY FYHQ FWI RMSDRTVIK 1500
1501 PHP DIHLNYLLVGTIAST DGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1560 1501 PHP DIHLNYLLVGTIAST DGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1560 1353 PHP DIHLNYLLVGTIAST DGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1411 1352 PHP DIHLNYLLVGTIASADGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1411 1501 PHP DIHLNYLLVGTIASADGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1560 1357 PHP DIHLNYLLVGTIAST DGSGELFCFFSYNQHKLVVQRGNGKQAEEPRPVTISPELGTI 1416
1561 SNFFCANNNLFSITNAGFILRLTSHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1620 1561 SNFFCANNNLFSITNAGFILRLTSHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1620 1413 SNFFCANNNLFSITNAGFILRLTSHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1472 1412 SNFFCTNNLFSITNAGFILRLTTHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1471 1561 SNFFCANNNLFSITNAGFILRLTSHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1620 1417 SNFFCANNNLFSITNAGFILRLTSHCTLALEAVNHQWLEHYEKDVDGGPWWTALPKLAKD 1476
1621 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1680 1621 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1680 1473 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1531 1472 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1680 1477 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1680 1477 HAAAIVSIVGLRDAEGSPVQAWLCSDRFVVAGPSLRGKPRHMAGLTEGGSKAWLWHIESE 1680

Epichloë_festucae_AR48	1681 D S G H V Y A O P T V R G K E L E T V F S P K A P F V K A E A V P D G R G V L V E H P F K T V A L T E S G L R Y T T K	E 1740
Epichloë festucae Fq1		E 1740
Epichloë festucae Fl1	1533 D S GHVY AOP T VR SK E L ET VF SP KAP F VK A EAVP D G R G V L V EHP F KT VA L T E S G L R Y T T K	
Epichloë festucae E2368	1532 DSGHVYAQPTVRSKELETVFSFKAPFVKAEAVPDGRGVLVEHPFKTVALTESGLRYTTK	
Epichloë festucae AR1	1681 DSGHVYAOPTVRGKELETVFSFKAPFVKAEAVPDGRGVLVEHPFKTVALTESGLRYTTK	
Epichloë festucae AR37	1537 DSGHVYAQPTVRGKELETVFSPKAPFVKAEAVPDGRGVLVEHPFKTVALTESGLRYTTK	
Epicnioe_restucae_AR37	1557 D'SGRUTAQFTURSKELETUFSFRAFFURALAUFDURGULVERFFRIVALLESGLRTTIK	L 1290
Epichloë festucae AR48	1741 GVVLILASER SMRLYGVDKVWOONRGDLSAELATLVNTWRHGETVVMLGSEPPOWYLMS	c 1000
	1741 GVVLILASER SMRLTGVDRVWQQNRGDLSAELATLVNTWRHGETVVMLGSEPPQWTLMS	
Epichloë_festucae_Fg1		
Epichloë_festucae_Fl1	1593 GVVLILASER SMRLYGVDKVWQQNRGDLSAELATLVNTWSHGETVVMLGSEPPQWYLTS	
Epichloë_festucae_E2368	1592 GVVLILASER SMRLYGVDKVWQQNRGDLSAELATLVNTWSHGETVVMLGSEPPQWYLTS	
Epichloë_festucae_AR1		- 1789
Epichloë_festucae_AR37	1597 GVVLILASER SMRLYGVDKVWQQNRGDLSAELATLVNTWSHGETVVMLGSEPPQWYLTS	S 1656
		-
Epichloë_festucae_AR48	1801 GK I LAAA EAT FTWL DAP TWL GADP S GAR GY AY VAAHR R I Y EL G E S S A E G K K S AA S Q E V A	
Epichloë_festucae_Fg1	1801 GK I LAAA EAT FTWLDAP TWLGADP S GAR GY AY VAAHRR I Y ELGES SAEGKK SAASQ EVA	
Epichloë_festucae_Fl1	1653 GKILAAAEAT FTWLDAP TWLGADP SGARGYAYVAAHRRIYELGES SAEEEK SAASQDVA	
Epichloë_festucae_E2368	1652 GKILAAAEAT FTWLDAP TWLGADP S GAR GYAY VAAHR RIYELGES S AEGKK S AASQEVA	
Epichloë_festucae_AR1	1790 DV F WK D S R G S R G D F Y L A R C P H V A W C R S Q R C K R I R	
Epichloë festucae AR37	1657 GKILAAAEAT FTWLDAP TWLGADP S GARGYAYVAAHRRIYELGES S AEGKK SAASQEVA	F 1716
Epichloë festucae AR48	1861 A SR FODVLAVKP SP GASF ROFALENVLYTILSOF EGDT SFMECVVP SSSWESLTGLVIE	W 1920
Epichloë festucae Fg1	1861 A S R FO D V L A V K P S P G A S F R O F A L E N V L Y T I L S O F E G D T S F M E C V V P S S S W E S L T G L V I E	W 1920
Epichloë festucae Fl1	1713 A S R FQ D V LAVK P S P G A S F R Q F A L E N V L Y T I L S Q F E G D T S F M E C V V P S S S W E S L T G L V I E	W 1772
Epichloë festucae E2368	1712 A S R F Q D V L A V K P S P G A S F R Q F A L E N V L Y T I L S Q F E G D T S F M E C V V P S S S W E S L T G L V I E	
Epichloë festucae AR1	1825 CRRPQKNLRIRREFCRGRKVGCVPGSRLCLTLPG	
Epichloë_festucae_AR37	1717 A S R F O D V L A V K P S P G A S F R O F A L E N V L Y T I L S O F E G D T S F M E C V V P S S S W E S L T G L V I E	
epienioe_restatute_ritor		
Epichloë festucae AR48	1921 K D O G O V O I E G S T R H P R P A D S F L G K R L D D D L I M M E I S T G R F L K I S R G L V M D P S C V I R F T D	K 1980
Epichloë festucae Fq1	1921 K DOGOVO I EGST R HP R P AD S F LGK R LDDDL I MM E I ST GR F LK I S R G LVM DP S C V I R FT D	
Epichloë festucae Fl1	1773 K DQ GQ V K I E G S T R H P R P A D S F L G K R L D D D L I MME I S T G R F L K I S R G L V M D P W C V T R F T D	
Epichloë festucae E2368	1772 K DQ GQ V K F E G ST R H P R P A D S F L G K R L D D D L I MME I ST G R F L K I S R G L VM D F W L V I R F T D	
Epichloë festucae AR1	1772 K D G G G V G T E G ST K H F K F A D S F E G K K E D D E T M M E T S T G K F E K T S K E E W D F S C V T K F D	
	1859SCLOAUP R	
Epichloë_festucae_AR37	1/// KDQGQVQTEQSTKHPKPADSFLGKKLDDDLTMMETSTGKFLKTSKGLVMDPSCVTKFTD	K 1830
Epichloë festucae AR48	1981 L L T V G N G S N E I O	1992
	1981 LLTVGNGSNEIQ	1992
Epichloë_festucae_Fg1		1992
Epichloë_festucae_Fl1	1833 L L T V G N G S A G N Q	
Epichloë_festucae_E2368	1832 L L T V G N G S N E I Q	1843
Epichloë_festucae_AR1		10.00
Epichloë_festucae_AR37	1837 LLTVGNGSNEIQ	1848

Appendix 19: Alignment of makes caterpillars floppy (Mcf) proteins from a range of *Epichloë festucae* strains using the alignment function of Geneious.

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_aotearoae\_MYA-1225 Epichloë\_brankovelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_Fg1 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_uncinata\_CBS\_102646\_

Epichioe\_uncinata\_CES\_102046\_ Epichioe\_amarillans\_E57\_ Epichioe\_amarillans\_E4668\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434\_ Epichioe\_fromicola\_AL04262\_ Epichioe\_festucae\_AR1 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_E368 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_gansuensis\_e7080\_ Epichioe\_mollis\_AL9924\_ Epichioe\_mollis\_AL9924\_ Epichioe\_typhina\_atCC\_200736\_ Epichioe\_typhina\_asubsp\_poae Epichioe\_molis\_AL9924\_ Epichioe\_typhina\_asubsp\_poae Epichloë\_typhina\_subsp.\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_uncinata\_CBS\_102646\_

Epichioe\_uncinata\_Cs5\_102046\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E4668\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brancheykri\_E4804\_ Epichioe\_branicola\_AL0434\_ Epichioe\_branicola\_AL04262\_\_ Epichioe\_festucae\_AR1 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F01 Epichioe\_festucae\_F01 Epichioe\_glyceriae\_ATCC\_200747\_ Epichioe\_molis\_AL9924\_ Epichioe\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp.\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_uncinata\_CBS\_102646

Epichloë\_amarillans\_E57 Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichnoe\_brachyenr\_24304\_ Epichnoe\_bromicola\_AL0434\_ Epichloe\_bromicola\_AL04262\_ Epichloe\_ehymi\_ATCC\_201551\_ Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloë\_festucae\_AR46 Epichloë\_festucae\_E2368 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fl1 Epichloë\_gansuensis\_e7080\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_uncinata\_CBS\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_aotearoae\_MYA-1229 Epichloë\_brankovelytri\_E4804\_\_ Epichloë\_brankovelytri\_E4804\_\_ Epichloë\_brankovel\_AL0434\_\_ Epichloë\_frankovelyte\_AL04262\_\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gausuensik e 7080 Epichloë\_restatue\_rts Epichloë\_gansuensis\_e7080\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924 Epichloë\_mollis\_AL9924 Epichloë\_typhina\_ATCC\_200736 Epichloë\_typhina\_subsp.\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_uncinata\_CBS\_102646\_

1 ATGGCTCATAAGTAAGAGCTTCCC       TCTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
1       ATGGCTCACAAGTAAGAGCTTCCCTCTGTCTCTCCCTCTCTTTTCTTTC
40       ATTCTTTTCTAAT
1       AC G CAT C 7         36       AT         1       GT C CAT G 51         1       AC G CAT C 7         61       CTT C T C T C T C T C T         61       CTT C T C T C T C T T         AC G CAT C 7         61       CTT C T C T C T C T T         AC G C AT C 7         61       CTT C T C T C T C T T         AC G C AT C 7         61       CTT C T C T C T C T T T         AC G C AT C 7         61       CTT C T C T C T C T T T T C T T T C T G T C T G C C AT G 103         1       AG G G C T C T A C G G AG T A G AG G G T         40       C G T T C         40       C G T T C         C G T T C       T C C C T G G G T G G G T G G G T G G G T G G G T G G G T G G G T G G G T G G G T G G G T G G C AT G 81         55       AAAAAAAAAAAAAA AAT C -       C C T C C C C G T C G T G T G T C C AT G 81         51       AAAAAAAAAAAAA AA T C -       C G T C C C G T C C G T C G T C G T C G T C C AT G 81         51       AAAAAAAAAAAAAA AA C A C C G T C C G T C C G T C C G T C G T C G T C C AT G 81         51       AAAAAAAAAAAAAA AA C A C G C C C C C C C
67 ATCG - GACTAACAT GTATCTTTG CAGCGCTGACGAAACGTCCATTGTCGAA 116 63 ATCG - GCTAACATGTATCTTTG CAGCGCTGACGAAACGTCCATTGTCGAA 112 83 GCCG - GACTAACATCCATCTTTG CAGCACTGACGAATCGTCCATTGTCGAA 132 76 GTCG - GACTAACATCCATCTTTG CAGCACTGACGAATCGTCCATTGTCGAA 105 56 ATTG - AACATACATCATCTTTG CAGCGCTGACGAATCGTCCATTGTCGAA 105 56 ATCGA - ACTAAAATCTATCATTT CAGCGCTGACGAATCGTCCATTGTCGAA 105 56 ATCGA - ACTAAAATCTATCATTGC AGCGCTGACGAATCGTCCATTGTCGAA 105 56 ATCGA - ACTAAAATCTATCATTGC AGCGCTGACGAATCGTCCATTGTCGAA 105 52 ATCG - GACTAACATCTATCATTGC CAGCGCTGACGAATCGTCCATTGTCGAA 105
52 ATCG - CACTAACATGTATCTTTG CAGCGTAACGAAACGTCCATTGTCGAA 101 8 GCCA - GTCCAACAGCCGAAGTGAGGGGTCAGAACAAAAAAAAGCGAAATGCAAAGCGAAGGGGTGAGGAGGGGTCAGAAGAAAAAAAA
117TTTTCGAAAGATCCCCTTGGTTTTATCACGAAGGG
67 CTCTTCCCCGCGCCGCGCCGCCGCCGCGGCGGCGGCGGCG
152 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACGTTGCTGGAAAA 197 148 - CGAAAAAGGCTATG CC GTC ATCAGCTTCGAC AACGTTGCTGGAAAA 193 168 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC AACATTCCTGGAGT 213 161 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 206 142 - GAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 206 142 - GAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 206 141 - CGAAGAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 186 141 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 186 137 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC - ACATTCCTGGAGGT 186 137 - CGAAAAAGGCTATG CC GTC ATCAGCTCCGAC - AACATTCCTGGAGGT 186
137       - CGAAAAAGCCTATG CC GTC ATCACCTCCGAC AACATTCCTGGACGA 182         127       CCCACGACGGCGCGG CC GGCCGCGCAACCCGGTCCAAC - AACATTCCTGGACGA 182         137       - CGAAAAAGCTATG CC GGCCGCGAACCCCGTCCAAC - AACATTCCTGCGACGA 182         126       CCCACGACGGCGGGG CC GGCCGCGAACCCCGGTCCAAC - AACATTCCTGCGACGA 182         137       - GGAACAAGCCTATG CC GGCCGCGAACCCGGTCCAAC - AACATTCCTGGACGA 182         126       CCCACGACGGCGGGG CC GGCCGCGAACCCGGTCCAAC - G - CTCTCCGAGGCCGA 182         138       TGCTAAGACCCTCGGGTACCTAGGTTCCCATAT - ATCTCT AGCCTCCTGAGGCCGA 171         141       - GGAACAAGCCTCGGGTACCTAGGTTCCCATAT - ATCTCT AGCCTCTCTAGGGGA 171         141       - AGAACAAGCCTATG CC GTC TTAGCTCCGAC - AACATTCCTGGACGT 234         167       - CGAAAGAGCTATG CC GTC TTAGCTCCGAC - AATATCACTGGACAT 186         167       - CGAAAGAGCTATG CC GTC TTAGCTCCGAC - AGAATTCCTGGACGT 212         167       - CGAAAAAGCTATG CC GTC TCAGCTCCCAAC - CACATTCCTGGACGT 212         163       - CGAAAAAGCGCTATG CC GTC ATCACCTCCGAC - AACATTCCTGGACGT 212         163       - CGAAAAAGCGCTATG CC GTC ATCACCTCCGAC - AACATTCCTGGACGT 212         163       - CGAAAAAGCGCTATG CC GTC ATCACCTCCGAC - AACATTCCTGGACGT 212         163       - CGAAAAAGCCTATG CC GTC ATCACCTCCGAC - AACATTCCTGGACGT 212         16

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_foaeF31

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_attearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_moblis\_AL9924\_ Epichloë\_moblis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_Pae\_Ps1 Epichloë\_typhina\_Subse\_Pae

Epichloë\_oncinata\_CoS\_100040\_ Epichloë\_amarillans\_E57 Epichloë\_aotearoae\_MYA-1229 Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_osis\_e7080\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_fromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F36 Epichloë\_festucae\_F36 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F36 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F36 Epichloë\_festucae\_F36 Epichloë\_molis\_AL3924\_ Epichloë\_typhina\_subsp\_poae\_F31 Epichloë\_typhina\_subsp\_poae\_F31 Epichloë\_uncinata\_C85\_102646\_

198       - ATCCCAAA       - AG       - TTTTTGCCGA       - CAAGACCAATTC       - 233         194       - ATGCCAAA       - AG       - TTTTTGCCGA       - CAAGACAATTC       - 233         194       - ATGCCAAA       - AG       - TTTTTGCGCG       - CAAGACAATTC       - 264         214       - CCAAG       - TA       - TTCTTATCG       - CAAGACAATTC       - CAACATTTC       - 246         207       - CCAAG       - TA       - TTCTTATCG       - CAAGACAATG       - GACATTTC       - 249         186       - TCC       - AG       - TTTTTTATAG       - GAGACGAAG       - GACATTTC       - 219         187       - CCAAG       - TG       - TTTTTATAG       - GAGACGAAG       - GACATTTC       - 219         183       CG       - TG       - TTTTTTATCG       - GAGACGAAG       - GACATTTC       - 219         183       CG       - TG       - TTTTTATCG       - GAGACGAAG       - GACATTTC       - 219
183 CG T C C A A A T G T T T T T T T T C G G A G A G G A A G G A C A T T C - 218 179 G G C T T - C G G A T T G T A G A G A G G C G G C C - C G A A C C G C T G G G G C C A T G C A 225 183 CG T C C A A A T G T T T T T T T C G G A G A C G A A G G A C A T T C - 218 178 G G C T T - C G G A T T G T A G A G A G C G G C C - C G A A C C G C T G T G G G G C C A T G C A 224
235
234       AGATTTTAACAGCG - TCGG AAATCTAGATGAACCGGTGT T - CG AAAT CAGG 281         230       AGATTTGACAGCG - TCGG AAATCTAGATGAACCAGTG CT - CG AAAT CAGG 281         247       AGATTTGACAGCG - TCGG AAATCTAGATGAACCAGTG CT - CG AAAT CAGG 287         240       AGATTTGAGAGCG - TCGG AAATCCAGATCAACCAGCGT - T - CG AAAT CAGG 287         220       AGATTTGAGAGCG - TCGG AAATCCAGATCAACCAGCGT - T - CG AAAT - CAGG 287         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 287         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         220       AGATTTGAGAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         220       AGATTTGACAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 267         219       AGATTTGACAGCG - TCGG AAATCTAGATCAACCAGCGT - T - CG AAAT - CAGG 266
219 AGATTTGACAGCG - TCGG AAATCTACATCAACCAGCGT - T - CG AAAT CAGG 266 226 GGA AATAGC - TGTG TACTCCGTACGAAGGACCGTGCT - CGGCAACCT - CCGC 275 219 AGATTTGACAGCG - TCGG AAATCTACATCAACCACCGT - T - CG AAAT CAGG 266 225 GGA AATAGCC - TGTG TACTCCGTACGAAGGACCGTGCT - CGGCAACCT - CCGC 274
268 A GATTTTAAGAGCG - T C G G A GATGCAGATCAACCACC GT - T - C CAAT - C AAG 315 225 A C G T C G A G T G C G A T C G G C G C G C G T G T A A G A C C G C A T G C C A C G C C C C A T A G A G G 282 220 A G A TTT G A G A G C - T C G G A A A T C T A A T C A C C A C C G C C T - T - C G A A A T - C A G C 267 246 A G A TTT G A G A G C - T C G G A A A T C T A A T C A C C A C C G C C T - T - C G A A A T - C A G C 283 247 A G A TTT G A G A G C - T C G G A A A T C T A G T C A A C C A C C G C T - T - C G A A A T - C A G C 293 248 A G A TTT G A G A G C - T C G G A A A T C T A G A T C A A C C A C C G T - T - C G A A A T - C A G C 293 249 A G A TTT G A G A G C G - T C G G A A A T C C A C C A C C A C C G C G T - T - C G A A A T - C A G C 289 249 A G A TTT G A G A G C G - T C G G A A A T C C A G C A T C A A C C A G C G T - T - C G A A A T - C A G C 289
282       TACCATGGACGCAACGCTGCGCCCGACACCGT       CTCGGCGTATCACTTG329         278       TACCATGGACCCAACGCTGCCCGACACCGT       CTCGGCGTATCACTTG329         295       TATTATGGACCCCACGCTGCCCCGACACCGT       CTCGGCGTATCACTTG329         288       TATTATGGACCCGACGCTGGCGCCGACACCGT       CTCGGCGTATCACTTG335         288       TATTATGGACCCGACGCTGCCCCGACACCGT       CTCGGCGTATCACTTG335         268       TACCATGGACCCCGACGCTGCCCCGACACCGT       CTCGGCGTATCACTTG335         268       TACCATGGACCCCGACGCTGCGCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCCGACGCTGGCGCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCCGACGCTGGCGCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCCGACGCTGGCCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCCGACGCTGGCCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGGCCCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGGCCGCGACACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGCCGACGCCGACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGCCGCGCGACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGCCGCGCGACCGT       CTCGGCGTATCACTTG315         268       TACCATGGACCCGACGCTGCCGCGCGCGCGCGCGCGCGCG
267 TACCAT GGAGCCCAC GCTGGCCGCCGACACCGT CT CGGCGTAT CACTTG 314 276 GCCCCTGACGCTAAT AATAT AGTTATTATAAT AATAAT AATAAT AAC CACGGCTAAT TAGTAA 335 267 TACCAT GGAGCCCACGCTGGCGCCGGCGCGCGCGCGC
316       TACCATGGACCCCGACGCTGCCGCCGACACCGT       TCGGCGTATCACTTG363         283       CAGCCAACATACG       CCCTGGCCCCGACACCGT       TCGGCGTATTTTTG329         284       TACTATGGACCCGAACACCGT       CCCGGCGTATTACTG337         294       TACTATGGACCCGAAGCTGTCGCCCGACACCGT       CTCGGCGTATCACCTT341         295       TACTATGGACCCGAAGCTGTGCCGCACACCGT       CTCGGCGTATTACTTG342         290       TACTATGGACCCCGAAGCTGTGCCGACACCGT       CTCGGCGTATTACTTG342         290       TACTATGGACCCCGAAGCTGTGCCGACACCGT       CTCGGCGTATCACCG337         297       TACTATGGACCCCGAAGCTGGCGCCGACACCGT       CTCGGCGTATCACCTG344
330       CGATACGACGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
315 GGATACAATGGAGGCGCGCAGACGTCTTGGACGCCCGCC - CAAATCGATATCCCCAA G 371 336 TACTATCATAAAGGCATGCACCCCCT - GCTGCCTACCGCACCTCG TCTCCAACGG 390 315 GGATACAATGGAGGCGCGCAGACGTCTTGGACGCCCGCC - CAAATCGATATCCCCAA G 371 335 TAGTATCATAAAGGCATGCACCCCCCT - GCTGCCTACCGCACCTCG TCTCCAACGG 389
364 GGATATAATGCAGGCGCGCAGACATCTTGGACGCCCGCC - CAAATCGATATCCCCAA - G 420 330 GGATACAATGGACGCGCGCAGAATCTTGGACGCCCCCC - CATATCGATATCCCCAT - C 386 316 GGATACAATGGAGGCGCGCAGAATCTTGGACGCCCGCC - CAAATCGATATACCCCAA - G 372 342 GGATACAATGGAGGCGCGCGAGAATCTTGGACGCCCGCC - CAAATCGATATCCCCAA - G 398 343 GGATACAATGGAGGCGCGCGAGACATCTTTGGACGCCCGCC
387       ATAAACCCGGGAA       CATAACTTGCTCTTC       ACAGGC       TCTCTCTCGGGT - TGC 434         383       ATAAACCCGGAA       CATAACTTGCTCTTC       ACAGGC       TCTCTCTCGGGT - TGC 434         400       ATAAACCCGGAA       CATAACTTGCTTTTC       ACAGGC       TCTCTCTCGGGT - TGC 434         933       ATAAACCCGGAA       CATAACTTGCTTTTC       ACAGGC       TCTCTCTCGGGT - TGC 440         373       ATAAACCCGGAA       CATAACTTGCTTTTC       ACAGGC
372       ATAAACCCCGGAA CATAACTTGCTTTTC ACAGGT TCTCTCTCGGGT - TGC 419         391       ACCTACATGTAATCCGTATCTACGCCTTTC TCTGCAGGTGAAAAGCCACGGTCTCGTGCGA450         372       ATAAACCCGGAA CATAACTTGCTTTTC ACAGGT TCTCTCTCGGGT - TGC 419         390       ACCTACATGTAATCCGTATCTACGCCTTTC TCTGCAGGTGAAAAGCCACGGTCTGCGGGCA449
421 ATAAACCCGCAA CACAACTTGCTCTTC ACAGGC TCTCTCTCGGGT -TGC 468 387 AAAAACCCGCAA CATGAGTTGCTTTTC ACAGGC ACTCTCTCGGGT -TGC 430 373 ATAAACCCGCAA CATAACTTGCTTTTC ACAGGC TCTCTCTCGGGT -TGC 420 399 ATAAACCCGGAA CATAACTTGCTTTTC ACAGGC TCTCTCTCGGGT -TGC 446 400 ATAAACCCGGAA CATAACTTGCTCTTTC ACAGGC TCTCTCTCGGGT -TGC 442 402 ACAAACCCGGAA CATAACTTGCTTTTC ACAGGC TCTCTCTCCGGT -TGC 442 402 ACAAACCCGGAA CATAACTTGCTTTTC ACAGGC TCTCTCTCCGGT -TGC 442 402 ACAAACCCGGAA CATAACTTGCTTTTC ACAGGC TCTCTCTCCGGT -TGC 449

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_unicinata\_Los\_102040\_ Epichioe\_amarillans\_E57 Epichioe\_antarillans\_E668 Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brankovelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_franticola\_AL04262\_ Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_gansuensis\_e7080\_ Epichioe\_anterias\_ATCC\_200747\_ Epichioe\_inebrans\_MYA-1228\_ Epichioe\_inebrans\_MYA-1228\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_inebrans\_M26430\_2026

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_moltis\_AL9924\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_s205\_Poae\_P31 Epichloë\_uncinata\_C85\_102646\_

435       T C C G T C A T A G T A A C A A C C T C A A C G A C A A C C A G T A C C G T G T C T A T C A C G A C T         431       T C C G T C A T A G T A A C A A A C C T C A A C G A C A A C C A G T A C C G T G T C T A T C A C G A C T         448       T C C G T C A T A G T A A C G A C A C T C A A C G A C A A C C A G T A C C G T G T C T A T C A C G A C T 492         441       T C C G T C A T A G T A A C G A A C C T C A A C G A C A A C C A G T A C C G T G T C T A T C A C G A T 492         421       T C C G T C A T A G T A A C G A A C C T C A A C G A C A C C A G T A C C G T G T C T A T C A C G A C T 472         421       T C C G T C A T A G T A A C G A A C C T C A A C G A C A C C A G T G T C T A T C A C G A C T 472         421       T C C G T C
420 TCCGTCATAGTAACGAACCTCAACGACAACCAGTACCGTGTCTATCACGACA471 451 TCGATCGCGCGCGCAGATAGTAACGAACCTCAACGACAACCAGTACCGTGTCTATCACGACA510 420 TCCGTC-ATAGTAACGAACCTCAACGACAACCAGTACCGTGTCTATCACGACA471 450 TCGATCGCGCGCAGATAGTAACGAACCTCAACGACAACCAGTACCGTGTCTATCACGACA509
469       TCCGTC ATAGTAACGAACCTCAACGACAGCCAGTACCGTGTCTATCACGACT 520         435       TCCGTC ATAGTTACGAACCTCAATGACAACCAGTACCGTGTCTATCACGACT 480         421       TCCGTC ATAGTTAACGAACCTCAATGACAACCAGTACCGTGTCTATCACGACT 482         421       TCCGTC ATAGTAAAGAACCTCAACGACACCAGTACCGTGTCTATCACGACT 472         447       TCCGTC
<ul> <li>487 CTCGACAGGATAGCTCGTTGTTGTACGAAAAAGTCGTCATGGCAGTTGACTACAGAGACT 542</li> <li>483 CTCGACAGGATAGCTCGTTGTTGTACAACGACGTCGTCATGGCAGTTGACTACAGAGACT 542</li> <li>500 CTCGACAGGATAGCTCGTTGTTGTACAACGACGTCGTCATGCCAGTTGACTACAGAGACT 559</li> <li>493 CTCGACAGGATAGCTCGTTGTTGTACAACGACGTCGTCATGGCAGTTGACTACAGAGACT 552</li> <li>473 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 532</li> <li>473 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 532</li> <li>473 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 532</li> <li>473 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 532</li> <li>473 CTCGACAGGATAGCTCGTTGTTGTACGACTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 531</li> <li>1 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTTGACTACAGAGACT 531</li> <li>11 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTTGACTACAGAGACT 531</li> <li>11 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTTGACTACAGAGACT 530</li> <li>12 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTTGACTACAGAGACT 530</li> <li>14 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTGACTACAGAGACT 530</li></ul>
510 CTCGACAGGATAGCTCGTTGTTGTACAATGACGTCGTCATGGCAGTTGACTACAGAGACT 569 521 CTCGACTGGATAGCTCGTTGTTTTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 580 487 CTCGAATGGATAGCTCGATGTTGTACGACAATGTCGTCATGACGACGTTGACTGGAGAGACT 546 473 CTCGACAGGATAGCTCGTTGTTGTACGACGACATGTCGTCATGGCAGTTGACTACAGAGACT 549 99 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 557 500 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 554 495 CTCGACAGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 554 502 CTCGACGGATAGCTCGTTGTTGTACGACGACGTCGTCATGGCAGTTGACTACAGAGACT 554
547 ATAAAC - ATCGCTATGAGAATACTGGGT ATGCCTGCGCATTCATGCGCTATCGCGAAGGT 605 543 ATAAAC - ATCGCTATGACAATACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 601 560 ATAAAC - ATCGCGATGCTAGTACTGGGT TGCCTGCGCATTCATGCACTATCGCGAAGGT 611 533 ATAAAC - ATCGCGATGCTAGTACTGGGT TGCCTGCGCATTCATGCACTATCGTGAAGGT 611 533 ATAAAC - ATCGCGGTGTTAGTACTGGTT TGCCTGCGCATTCATGCACTATCGTGAAGGT 591 533 ATAAAC - ATTGCGAACCTAGTACTGGGT TGCCTGCGCATTCATGCACTATCGCGAAGGT 591 532 ATAAAC - ATTGCGATGCTAGTACTGGGT TGCCTGCGCATTCATGCACTATCGCGAAGGT 591 532 ATAAAC - ATTGCGATGCTAGTACTGGGT TGCCTGCGCATTCATGCACTATCGCGAAGGT 591 532 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 593 532 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 93 532 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 93 532 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 590 571 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 590 572 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCGCATTCATGCACTATCGCGAAGGT 590 570 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCCGCATTCATGCACTATCGCGAAGGT 590 570 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCCGCATTCATGCACTATCGCGAAGGT 590 570 ATAAAC - ATCGCGATGCTAGTACTGGGT ATGCCTGCCGCATTCATGCACTATCGCGAAGGT 590
581       ATAAAC - ATCGCGATGCTACTACTGGGTTGGCCTGCGCATTCATGCACTATCGCGAAGGT       639         547       ATAAAC - TCCGCGAAGATGCTGGGAGGGTTGGCCTGCGCATTCATGCACTATCGCGAAGGT       605         533       ATAAAC - ATTTCGAGGCTTGGCTTGGCTTGCCCATTCATGCACTATCGCGAAGGT       591         558       - AAAC - ATCGCGATGCTAGTACTGGATTGCCCACCCATTCATGCACTATCGCGAAGGT       616         558       - AAAC - ATCGCGATGCTAGTACTGGGTTGCCTCGCCATTCATGCACTATCGCGAAGGT       618         550       ATAAAC - ATCGCGATGCTAGTACTGGGTTGCCTCGCCATTCATGCACTATCGCGAAGGT       618         555       ATAAAC - ATCGCGATGCTAGTACTGGGTTTGCCAGCACTTCATGCACTATCGCGAAGGT       613         552       ATAAAC - ATCGCGATGCTAGTACTGGGTTTGCCAGCACATTCATGCACTATCGCGAAGGT       620
606       CGCTGGTCTCTGTGCTTCCAGAGGC       AAACCCTACTTCAAAATGGAGCTGTTCGCCTG 662         602       CGCTGGTCTTGTGTGTTCCAGAGGC       AAACCCTACTTCAAAATGGAGCTGTTCGCCTG 653         619       CGCTGGTCATGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGGAGCTATTGTCCTG 675         612       CGCTGGTCATGTGTTTTCAGAGGC       AAACCATAGTTGCAAATGCGCGCTATTGTCCTG 675         612       CGCTGGTCATGTGTTTTCAGAGGC       AAACCATAGTTGCAAATGCGCGCTATTGTCCTG 675         612       CGCTGGTCTTGTGTTTTCCAGAGGC       AAACCCACGGCAATGCGCGCAATTGCACGTATTGTCCTG 648         592       CGCTGGAATTGCGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTATTGTCCTG 648         592       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 648         592       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 648         591       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         591       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         630       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         631       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         632       CGCTGGTCTCTGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         632       CGCTGGTCTCGTGTTTCCAGAGGC       AAACCCTACTTCAAAATGCACGTGTTGTCCTG 647         632       C
640 CCCT G G T C T C T G T A T T T C C A G A A G C - A A A C C T A G G T A C G G A T G G A C G T A T T G T C C T G 696 606 A T C T G G T C T T T G T A T T T C C A G A G A C A A A A T C A G A C A A A G A C G T G C T T T C C T A C C T C 655 592 C T C T G G T C T C T G T T T C C A G A G G C - A A A C C T A G C T G G A A T G G A C G T A T T G T C C T G 648 615 C G C T G G T C T T T G T T T C C A G A G G C - A A A C C A T A G T G C A A T G C A C G T G T T C C T C 675 619 C A T G T G T C T T T T G T T T C C A G A G G C - A A A C C T A G T G C A A T G C A G G G T G T C C T C 675 614 C C C T G G T C T T T T G T T T C C A G A G G C - A A A C C A T A G T T G C A A T C C A G G G T G T T C C - C C T C 677 621 C G C T G G T C A T T G T G T T T C C A G A G G C - A A C C A T A G T T G C A A T C C A C G T T T G C - C C T C 677
663 TACGATCCGCTGCCG AGAGATGGCCCATTCATAGTAGGGCAAGAGCCCTTGATTGA
697 TACAATCCCCTCCCCACAGATACCCCATCCATAATAGGGCCAGAGCCCTTCATTGAC753 666 TACAATGCCCGCCCCAGAGATCCCCTGATAATTGGTGCAGAGCCCTTCATTGAC753 649 TACAATCCCTGCCGAGAGATGCCCCGTACATAATAGGCCCAGAGCCCTTGATTGA

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gansuensis\_e7080\_ Epichloë\_enebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_gestucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E46804\_ Epichloë\_brancians\_AL04344\_ Epichloë\_brancians\_AL04262\_ Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_typhina\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1

Epichloë\_antarillans\_E57 Epichloë\_amarillans\_E57 Epichloë\_antarillans\_E57 Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

720 AT C C T G C C A C A AT C G T AT A A T C G G G A G C T A G T T C A AT C G C G T T T C G A C G A G A G T C G C C A A 779 716 AT C C T G C C A C A AT C G T AT A A T C G G G A G C T A G T T C A AT C G C G T T T C G A C G A G A G T C G C C G A 775 733 AT G G T G C C A G A AT C G T AT A A C C G G G A C T A G T T C A AT C G C G T T T C G A C G A G A G T C G C C G A 752 746 AT G G T G C C A G A AT C G T AT A A C C C G G A C C T A G T T C A AT C G C G T T T C G A C G A G A G C C C G A 753 709 AT G G T G C C A G A AT T G T A T A A C C C G G A C C T A G T T C A AT C G C G T T C G A C G A G A T C G C G A 753 706 C A A A AT T G T AT A C C C G G A C C T A G T T C A AT C G C G T T C G A C C A C A T C G C C A 759 706 AT G G T G C C A A A T T G T A T A A C C C G G A C T A G T T C A AT C G C G T T C G A C G A G A T C G C A 759 706 AT G G T G C C A A A AT C G T A T A A C C C G G A C T A G T T C A AT C G C G T T C G A C C A C A G T C C C G A 759 706 AT G G T G C C A A A T C G T A T A A C C C G G A C T A G T T C A A T C G C G T T C G A C C A G A G T C C C G A 754 708 AT G G T G C C A A A T C G T A T A A C C C G G A C C T A G T T C A A T C G C G T T C G A C C A G A G T C C C G A 754 708 AT G G T G C C A A A T C G T A T A A C C C G G A C C T A G T T C A A T C G C G T T C G A C C A G A G T C C C G A 754 708 AT G G T G C C A A A T C G T A T A A C C C G G A C C T A G T T C A A T C G C G T T C G A C C A G A G T C C C G A 754 705 AT G G T G C C A A A T C G T A T A A C C C G G A C C T A G T C C A T C G C T T C G A C C A G A G T C C C G A 754 705 AT G G T G C C A A A T C G T A T A A C C C G G A C C T A G T C A A T C G C G T T C G A C C A C A G A C C G C A 754 704 AT G G T G C C A A A T C G T A T A A C C C G G A C T A G T C C A T C G C T T C G A C C A C A G T C C C G A A 764 704 AT G G T G C C A A A A T C G T A T A A C C C G G A C T A G T C A A T C G C G T T C G A C C A C A G T C C C G A A 764 704 AT G G T G C C A G A T C G T A T A A C C C G G A C T A G T C A A T C G C T T T C G A C C A G A G T C C C G A A 764 70
754 CTGGTGCCAAATTCGTATAACCCGGACCTAGTTCAATCGCGTTTCGACGAGAGTCGCGAA 813 723 CTGGCTCCAAAATCGTATAACCCGAATCTAGTTCAATCGCGTTTCAACGAGAGTCGCAA 782 706 CTGGGCCCAAAATCGTATGACCCGGAGGAGTTCGAAGTCGTTTCGACGAGGTCGCAA 765 729 ATGGTGCCACGATCGTATAACCGGGAGCTAGTTCAATCGCGTTTCGACGAGGTCGCAA 765 733 ATGGTGCCACGATCGTATAACCGGGAGCTAGTTCAATCACCTTTCGACGACGAGTCGCAA 782 728 ATGGTGCCACGATCGTATAACCGGGAGCTAGTTCAATCGCGTTTCGACGACGAGGCCGCAA 787 735 ATGGTGCCACGATCGTATAACCGGGGAGCTAGTTCAATCGCGTTTCGACGACGAGGCCGGAA 787
780 AAGTGCCTGGAGCGCTTGCGGGACGCTCTTCACGAAGCTTTCTCACGATCCCTTTCATTAC 839 776 AAGTGCCTGGAGCGCTTGCGGGACGCTCGTCACGAAGCTTTCTACGATCCCTTTCATTAC 839 793 AAGTGCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCTTTCATGAC 852 786 AAGTGCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCTTTCATGGC 852 769 AAGTGCCTGGACCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCTTTCATGGC 845 760 AAGTACCGAGGACCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCCCCAGGGC 828 766 AAGTACCGAGGACGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCCCCAGGGC 828 766 AAGTACCGCGGACGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCCCCAGGGC 828 766 AAGTACCCGGGACGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCCCCAGGGC 828 765 AAGTGCCTGGAACGCCTTGCGGGACGCTCGCACGAAGCTTTCTAACGATCCCCCTCTCATTAC 824 268 AAGTGCCTGGAACGCCTTGCGGGACGCTCGCACGAAGCTTTCTAACGACCCCCTTTCATTAC 824 804 AAGTGCCCTGGAACGCCTTGCCGGACGCTCGCACGAAGCTTTCTAACGACCCCCTTTCATTAC 824 804 AAGTGCCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGACCCCCTTTCATTAC 824 804 AAGTGCCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGACCCCCTTTCATTAC 824 804 AAGTGCCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGACCCCTTTCATTAC 824 804 AAGTGCCCTGGAGCGCTTGCGGGACGCTCGCACGAAGCTTTCTAACGACCCCTTTCATTAC 824 804 AAGTGCCCTGGAGCGCTTGGCGGACGCTCGCACGAAGCTTTCTAACGACCCCTTTCATTAC 824 803 AAGTGCCTGGGACGCCTTGGCGGACGCTCGCACGAAGCTTTCTAACGACCCCTTTCATTAC 824 803 AAGTGCCTGGAGCGCTTGGCGGACGCTCGCACGAAGCTTTCTAACGACCCCTTTCATTAC 824
814 AAGAGCC GAGAGCGCTT GCAGGACGCT CACAAGGAGCTTT CT GACGAT CCCCCCAA GGGC 873 783 CAGAAC C GGACGCCT TACAGGATTT TC ACAT GACGT TG TT GCCAT CGCTACCAGGG 842 766 AAGT GCCCAGAGCGCT TG CACGACGCT CGCAGAAGCTT TC TAACGAT CCCT TAAT GGC 825 789 AAGT GCCT GGACGCCT TG CGACAGT TG GCACGAAGCT TT CT AACGAT CCCT TC CAT GG 848 793 AAGT GCCAGAGAGCGCT TG CGGACGCT GCACGAAGCT TT CT AACGAT CCCT TT CT CT CT CT CT CT CT CT CT TT CT ACGAT CCCT TT CT AACGAT CCCT TT CT ACGAT CCCT TT CT AACGAT CCCT TT CT AACGAT CCCT TT CT ACGAT CCCT TT CT A
840 GAGGATGGTGAATTTCAACCGTTCGAAGAAAACCGCATCAGTCTTCAAAATGAAGCCGTC 899 846 GAGGATGGTGAATTTCAACCATTCGAAGAAAACCGCATCAGTCTTAAAAATGAACCGCGTC 895 846 GAGGATGGTGAATTTCAACCGTTCGAAGAAAACCGCATCAGTCTTGACAATGAAGCCGTT 912 846 GAGGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 905 829 GTGGATGGTAATTTCAACCGTTCAAAGATAACTCCATTAAAATGACCACATGAAGCCGTT 808 820 ATGGATGGTGAATTTCAACCGTCAAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 888 826 AAGGATGGTGAATTTCAACCGTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGGATGGTGAATTTCAACCGTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTCGAAGAAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 884 826 AGAATGGTGAATTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 923 825 CAAGATGGTGAATTCAACCGTCGAAGAAACCACATCAGTCTGACAATGAAGCCGTT 884 826 AAGATGGTGAATTCAACCGTCGAAGAAACCACATCAGTCTTGACAATGAAGCCGTT 924
874 GT GGAT GGT AGATTT CAA CCGTT CGAAGAAAACT CCATTCATCTT GCAAT GAAGCCGT 933 843 GT GGAT GGT GATTT ACT CCGTT CGAGAAAACT CAAACTCAAACTCAAACCCAGCCGT 902 826 GAGGAT GGT GAATTT CAACCGTT CGAAGAAAACCACATCAGT CTT GACAAT GAAGCCGT 885 849 GAGGAT GGT GAATTT CAACCGTT CGAAGAAAACCACAT CAGT CTT GACAAT GAAGCCGT 908 853 AAGGAT GGT GAATTT CAACCGTT CGAAGAAAACCACAT AAGT CT GGACAAT GAAGCCGT 912 848 GAGGAT GGT GATTTT CAACCGTT CGAAGAAAACCACAT AAGT CT GGACAAT GAAGCCGT 907 855 GAGGAT GGT GAATTT CAACCGTT CGAAGAAAACCACAT CAGT CTT GGACAAT GAAGCCGT 907
900 C GCTATTCACAAGGACTTAGAACCGATCTTGAAGGCATTCTCAACAATAGGGGGCCT 956 896 C GCTATTCACAAGGACTTAGAACCGATCTTGGAGGCATTCTCAACAATAGGGGGCCT 952 913 C GCTATTCACAAGACTCAGAGCCGATCTTGATGGAGCATTCTCGAACAATAGGAGGCCT 959 906 C GCTATTCACAAAGACTCAGAGCGGATCTTGATGGCATTCTCGAAAATAGGAGGCCT 962 889 G GCTATTCACAAAGGCTTAGAGCGGATCTTGATGGCATCTCGAAAAATAGGAAGGCCT 962 889 C GCTATTCACAAGGACTCAGAAAGGATCTTGATGGCATCATCAAAAATAAGGAACGGACT 962 880 C GCTATTCACAAGGACTTAGAACGGATCTTGATGGCATCATCAAAAAATAAGGAACGGAATAGC 948 880 C GCTATTCACAAGGACTTAGAAACGATCTTGATGGCATCTCAACAAAATAAGGAACGGATAGC 948 880 C GCTATTCACAAGGACTTAGAACGGATCTTGATGGCATCTCAAGAAAATAGGAAGGGCCT 942 885 G GCTATTCACAAGGACTTAGAACCGAGTTGATGGCATTCTCAGAGATAGG CCT 938 388 G GCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAGATAGG CCT 938 924 G GCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAGATAGG CCT 938 924 G GCTATTCACAAGGACTTAGAACCGAGCTTGATGGCATTCTCAGAGATAGG CCT 938
934 G GCT ATT CAGAA GGA CT TAGAA A GGA T CT T CGT GGCA T CA T
957
949 CAGACGATA - CGGATAGAAAACCTA - CC

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_uncinata\_Cs5\_102040\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_fromicola\_AL04262\_ Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_festucae\_F11 Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_mobins\_AL9924 Epichioe\_typhina\_ATCC\_200747\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_moltis\_AL9924\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_s205\_Poae\_P31 Epichloë\_uncinata\_C85\_102646\_

993       GAGTTGGTGT       CACTCAGCGACCAGCTGT       1020         989       GAGTTGGTGT       CACTCAGCGACCAGCTGT       1016         1006       GCGTTGGTGT       CACTCAGCGACCAGCTGT       1013         990       GATTTGGTGT       CACTCAGCGACCAGCTGT       1013         991       GACTTGGTGT       CACTCAGCGACCAGCTGT       1013         991       GACTTGGTGT       CACTCAGCGACGACCAGCGGT       1017         991       GACTTGGTGT       CACTCAGCGACGACGACGGGGACGATATGTCTCTCAAACTGCCC1044         994       GACTTGGTGT       CACTCAGCGACGACCAGCTG	
1021	
1993 A T G G A T C C T T C T A G G A C G T A T G A T A T A A A A T A C C T T A A T C G A C A T G A C A T C T T 1147 1038 T T C A G G C A T T T A A G T A T T T T T T G A T C A G A T C A A G A A A T C T T 1080 1054 A T G G A T C C T T C T A G C A G T A T G A T T A T T G G A T A C T C A C T C A C C A T G C C A T C T 1071 1037 T C T A A C C G A A G G T T G T - T G T A G C T A C C T C A C C C G C C C T C T T 1074 1047 T T T A A C C G - A G A G T T G T T G A G C G A A C C A T G C C A T C T 1074 1042 C C A A C C A T C A - A A G G T T G T T G G C C G A C C C C C C C C C C C C T 1074 1046	
1060 CTCGGAATTATGACTGTACATTCCTCTGGCTGCAGCAAAAGATTAGAAAGGGATTTGAAG 1119 1056 CTCGGAATTATGACTATACATTCCTCTGGCTGCACCAAAAGATTAGAAAGGGGATTTGAAG 1119 1073 CTCGGAATTATGACTTACATACCTCTGGCTGAACCAAAAGGAGGAGAAAGGGGGTTTGCAG 1132 1057 CTCGGAATTATGACTTACATACCTCTGGCTGAACCAAAAGGAGGAAAAGGGGGTTTGCAG 1130 1100 CTGAGAAGTATGACTATACATACCTCTGGCTGAACCAAAAGGAGGAAAGGGGGTTGCAG 116 1100 CTGAGAAGTATGACTATACATACCTCTGGCTGAACCAAAAGGAGGCAAAGGGGGTTGCAG 116 1040 CTGAGAAGTATGACTATACATACCTCTGGCTGAACCAAAAGGAGGCGAAAGGGGGTTGCAG 116 1042 CTCGGAATTATGACTTACATACCTCTGGCTGAACCAAAAGGAGGAGAAGGGGGTTGCAG 1101 545 CTCGGAATTACGACTATACATACCTCTGGCTGAACCAAAAGGAGGAGAAAGGGGGTTTGCAG 1101 1042 CTCGGAATTACGACTATACATACCTCTGGCTGAACCAAAAGGAGGAAAGGGGGTTTGCAG 1140 1042 CTCGGAATTACGACTATACATACCTCTGGCTGAACCAAAAGGAGGAAAGGGGGTTTGCAG 1140 1042 CTCGGAATTACGACTTTACATACCTCTGGCTGAACCAAAAGGAGGAAAGGGGGTTTGCAG 1140 1042 CTCGGAATTACGACTTTACATACCTCTGGCTGAACCAAAAGGAGGAAAAGGGGGTTTGCAG 1140 1042 CTCGGAATTACGACTTTACATACCTCTGGCTGAACCAAAAGGAGGAAAGGGGGTTTGCAG 1140	
1148 CTGAGAAAGTATGACTATACATACCTCTGGCTGAAGCAAAAGGAGGCAAAGGGGTTTGCCG 1207 1081 CTGAGAAGTATGACTATACATACCTCTGGCTGAAGCAAAAGGAGGGGGGCAGGGGGTTTGCCG 1207 109 CTGAGAGCATACATCACATTCCTCTGCGCTGAACCAAAAGGAGGAGGAGAGGAGGAGGAGAGGGGTTTGCAG 1168 1075 CTCGGAATTATGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGGGAAAGGGGGTTTGCAG 1134 1085 CTGAGAGTATGACTTTACATACCTCTGGGTGAACCAAAAGGAGGAGAAAGGGGGTTTGCAG 1139 1084 CTCAGGATATGACTTTACATACCTCTGGCTGAAGCAAAAGGAGGAGAAAGGGGGTTTGCAG 1143	
1120 CAGT CGT GAAACAAGGC - AT CAGT CT GCAGGCACCGCT AGGAGGCACAGCCGGC GAACGT 1178 1116 CAGT TGT GAAACAAGGC - AT CAGT CT GCAGGCACCGCT AGGAGGCACAGCCGGC GAACGT 1171 1133 CAGT CGT GAGAGAGGCGAACA - TCGGCAACACCCGCT AGGAGGCACAGCCGGCCAACGT 1191 1117 CAGT CGT GAGAGAGGCGAGCA - TCGGCAAACACCGCT AGGAGGCACAGCCGGCCAACGT 1191 1117 CAGT CGT GAGAGAGGCGAGCA - TCGGCAAGCCGCT AGGAGGCACAGCCGGCCAACGT 1212 1106 CAGT CGT GAGAGAGGCGACCA - TCGGCAAGCCGCT AGGAGGCACAGCCGGCGACGT 1212 1106 CAGT CGT GAGAGAGGCGACCA - TCGGCAAGCACCGCT AGGAGGCACAGCCGGCGAACGT 1212 1106 CAGT CGT GAGAGGCGAGCA - TCGGCAAGCACCC T TAGGAGGCACAGCCGGCGAACGT 1160 1102 CAGT CGT GAGAGAGGCGACCA - TCGGCAACACCT CTAGGAGGCACAGCCGGCCAACGT 1160 1102 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT TAGGAGGCACAGCCGGCCAACGT 1160 1102 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT TAGGAGGCACAGCCGGCCAACGT 1160 1102 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT TAGGAGGCACAGCCGGCCAACGT 1199 1102 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT CTAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT T TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT T TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCT T TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAACCACCT T TAGGAGGCACAGCCGGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAACCCT T TAGGAGGCCACGCCGCCCACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAACCCT T TAGGAGGCCACGCCGCCCACGC 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGCCAACCT T TAGGAGGCCACGCCGCCCACGCCGCCAACGT 1160 1140 CAGT CGT GAGAGAAGGCGACCA - TCGCCAACCCT T TAGGAGG	
1208 CAGT CGT GAGAGAAGGCGACCA - TCGCCAGGCACCGCT AGGGGGCACAGCT GGCGAACGT 1266 1141 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAGCACCACT AGGAGGCACAGCCGGCGAACGT 1299 1169 CAGT CGT GAGACAAGGCGACCA - TCGGCAAGCCGCCACGCT AGGAGGCACAGCCGGCGACGT 1227 1135 CAGT TGT GAGAGAAGGCGACCA - TCGGCAAACACCGCT AGGAGGCACAGCCGGCCAACGT 1233 1140 CAGT CGT GAGAGAAGGCGACCA - TCGGCAAACACCCGT AGGAGGCACAGCCGGCCAACGT 1233 1140 CAGT CGT GAGAGAGGGGACCA - TCGGCAAACACCGCT AGGAGGCACAGCCGGCCACGT 1233 1144 CAGT CGT GAGAGAGGCGAGCA - TCGT CAACACCGCT AGGAGGCACAGCCGGCCAACGT 1202	
1179 CTCTCCGAACAGAGGTTCAGT GAGCTTTTAGTCGGCGATAACGAGTTTTCAACGGGATAC 1238 1175 CTCTCCGAACAGAGGTTCAGTGAGCTTTTAGCGGCGGCATAACGAGTTTTCAACGGGATAC 1234 1192 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAACGGGATAC 1235 1219 CTCTCCGAACAGGGTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTGCAACGGGATAC 1235 1229 CTCTCCGAACAGGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTGCAACGGGATAC 1238 1222 CTCTCCGAACAGGAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTCGAACGGGATAC 1238 1235 CTCTCCGAACAGGGTCAGGGGGCTTTTAGCGGGCGATAACGAGTTTCAACGGGATAC 1241 1165 CTCTCCGAACAGGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTCAACGGGATAC 1224 1161 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGCATAACGAGTTTTCAACGGGATAC 1220 1200 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAAGGGGATAC 1220 1200 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAACGAGTTTTCAGGGGGATACCGAGTTTTCAGGGGGATACCGAGTTTTAGCGGGCGATACCG	
1267 CTCTCCGAACAGAGGTTCAGGAGGCTTTTAGCGGGGCGATAACGAGTTTTCAACGGGATAC 1326 1200 CTCTCCGAACAGCGGTCAGGGCGTCTTTGGCGGGGGGATAACGAGTTTTCAACGGGATAC 1259 1228 CTCTCCGAACAGGGGCGGTCGTTGGGCCTTTTAGCCGCGCGATAACGAGTTTTCAACGGGATAC 1287 1394 CTCTCCGAACAAAGGTTCAGGGGGCTTTTAGCGGGCGATAATGATTTTTCAACGGGATAC 1253 1204 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAATGATTTTTCAAGCGGATAC 1253 1399 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAATGATTTTTCAAGCGGATAC 1258 1203 CTCTCCGAACAAAGGTTCAGGGAGCTTTTAGCGGGCGATAATGATTTTTCAAGCGGATAC 1258	

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Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL0434. Epichloë\_bromicola\_AL0434\_ Epichloë\_elymi\_ATCC\_201551\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gansuensis\_e7080\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924 Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

1239 AA G G A T T A T G G A T C A G T C G A G A T T C G G G A T A C G A G C A T G A C A T T A T G G A T C A G T C G A G A T T C C G G G A T A C G A G C A T G A C A T T C C G A A A T C 1294 1235 AA G G A T T A T G G A T C A G T C G A G A T T C C G G G A T A C G A G C A T G A C A T C A C A C T A T C G A A T C 1294 1252 AA T G C A T A T G G A T C A G T C G A G A T T C C G G G A T T C G A G C A T G A C A T T C C G A A A T C 1295 1279 AA G C A T A T G G A T C A G T C G A G A T T C G G G A T A C G A C C T G A C A T T C C G A A A T C 1295 1279 AA G C A T A T G C A T C A G T C G A G A T T T C G G G A T A C G A C C T G A C A T T C C C A A A T C 1338 1282 AA G A C A T A C G C A T C C G A G A T T T C G G G A T A C G A G C A T G A C A T T A C C C A A A T C 1318 1225 AA G A C A T A C G A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A A T C 1318 1226 AA G A C A T T G A A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 724 AA T A C A T T G A A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 724 AA T A C A T T G A A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 724 AA T A C A T T A G A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 1221 AA T A C A T T A G A T C A G T C G A G A T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 1221 AA T A C A T T A G A T C A G T C G A G A T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1280 1221 AA T A C A T T A G A T C A G T C G A G A T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1319 1221 AA T A C A T T A G A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1319 1221 AA T A C A T A T G A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1319 1221 AA T A C A T A T G A T C A G T C G A G A T T T C G G G A T A C G A G C A T G A C A T T C C G A A T C 1319 1231 A T A C A T A T A G A T C A G T C G A G A T T T C
1327 AAGACATATGCATCAGTCGAGATTTCGGGGATACGAGCATGACATGACACTATCCGAAATG 1360 AAGACATATGGATCAGTCGAAATTTCGGGGATACGAGCATGACATGACACTATCCGAAATG 1388 GAGAAGTATGGATCAGTCGAAATTTCGGGATACGAGCATGACATGACACTATCCGAAATG 1347 1254 AATGCATATGAATCAGTCGAGATTCCGGGATACGAGCATGACATGACACTATCCGAAATA 1313 1264 AATGCATATGAATCAGTCGAGATTCCGGGATACGAGCATGACATGACACTATCCGAAATA 1328 1259 AATGCATATGAATCAGTCGAGATTCCGGGATACGAGCATGACATGACACTATCCGAAATA 1328 1263 AATGCATATGAATCAGTCGAGATTCCGGGATACGAGCATGACATGACACTATCCGAAATA
1299 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAATCCAACAGGACGATTGCTT 1358 1295 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAAGCCCAACAGGACGATTGCTT 1354 1312 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAGGCGCTGCTT 1371 1326 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAGGCGCGTTGCTT 1355 1339 ATACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGCGCGTTGCTT 1381 1342 GTACAACTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGACGCGTTGCTT 1401 1285 GTACGGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGTTGCTT 1401 1285 GTACGGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGTTGCTT 1401 1281 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGCTTGCTT
1387 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGTTGCTT 1446 1320 ATAAAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGTTGCTT 1379 1348 GTACAGTTGTTCGACCGATCGTCGACAAGCTTGACGCAAACCGAACAAGGAGCGTTGCTT 1407 1314 GTACAGTTGTTCGACCGATCGTCGACAAGTTTGACGCAAACCGAACAAGGCGCGTGCTT 1378 1324 GTACAGTTGTTCGACCGATCGTCGACAAGTTTGACGCAAACCGAACAAGGCGCGTTGCTT 1388 1319 GTACAGTTGTTCGACCGATCGTCGACAAGTTTGACGCAAACCGAACAAGGCGCGTTGCTT 1378 1323 GTACAGTTGTTCGACCGATCGTCGACAA
1359 CGTCGCATTGAACTGGCCAGAAAGGAGTTGTTCAATGAGTCTGTCGGCAGAAAACCAAC 1418 1355 CGTCGCATTGAACTGGCCAGAAAGGAGTTGTTCAATGAGTCTGTCGGCAGAAAACCAAC 1418 1352 CGTCGCATTGACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1431 1356 CGTCGCATTGACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1458 1402 CGTCGCATTGAACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1451 1345 CGTCGCATTGAACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1451 1345 CGTCGCATTGAACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1451 1345 CGTCGCATTGAACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1451 1345 CGTCGCATTGAACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1461 1345 CGTCGCATTGACTGGCCAGAAAGGAGGTGTTCAATGAGTCTATCTGGCAGAAAACCAAC 1400 1341 CGTCGCATTGACTTGGCCAGAAAGGAGTTGTTCAATGAGTCTGTGGCGCAGAAAACCAAC 1400 1380 CGTCGCATTGACTTGGCCAGAAAGGAGTTGTTCAATGAGTCTGTGGCGCAGAAAACCAAC 1400 1380 CGTCGCATTGACTTGGCCAGAAAGGAGGTTGTTCAATGAGTCTGTGGCGCAGAAAACCAAC 1400 1380 CGTCGCATTGACTTGGCCAGAAAGGAGGTTGTTCAATGAGTCTGTGGCGCAGAAAACCAAC 1400
1447 CGTCGCATTGACTTGGCCAGAAAGGAGTTGTTCAATGAGTCCATCTGGCAGAAAACCAAC 1506 1380 CGTCGCATTGAACTGGCCAGAAAGGACTTGTTCAATGAGTCTGTCT
1419 GACGTCATTGCTATGTTCCAAGATATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1478 1415 GACGTCATTGCTATGTTCCAAGAGATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1474 1432 GACGTCATTGCTATGTTCCAAGAGATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1474 1416 GACGTCATTGCTATGTTCCAAGAGATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1518 1462 GACGTCATTGCTATGTTCCAAGATATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1518 1462 GACGTCATTGCTATGTTCCAAGATATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1518 1463 GACGTCATTGCTATGTTCCAAGATATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1518 1464 GACGTCATTGCTATGTTCCAAGATATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1518 1469 GACGTCATTGCTATGTTCCAAGATATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1548 1401 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1464 1401 GACGTCATTGCTATGTTCCAAGAGATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1404 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1404 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1400 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1400 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1400 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1400 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460 1439 GACGTCATTGCTATGTTCCAAGACATGGGGGGTTACACGAAACCTATGCCACAAGACATT 1460
1507 GACGTCATTGCCATGTTCCAAGACATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1566 1440 GACGTCATTGCCATGTTCCAAGACATGGGGGGGTTCTACGAAACCTATGCCACAAGACATT 1468 GACGTCATTGCTATGTTCCAAGACATGGGGGGGTTATACGAAACCTATGCCACAAGACATT 1434 GACGTCGTTGCTATGTTCCAAGACATGGGGGGGTCATACGAAACCTATGCCACAAGACATT 1444 GACGTCATTGCTATGTTCCAAGACATGGGGGGTCATACGAAACCTATGCCACAAGACATT 1439 GACGTCGTTGCTATGTTCCAAGACATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1444 GACGTCGTTGCTATGTTCCAAGACATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1448 GACGTCGTTGCTATGTTCCAAGACATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1448 GACGTCGTTGCTATGTTCCAAGAGATGGGGGGTTATACGAAACCTATGCCACAAGACATT 1502
1479 CTTCTCCATGCAATTCCCGATGAGTTAGGCGGCGGCGGTGCTATCCTCTGGTCTACGCC 1538 1475 CTTCTCCATGCAATTCCCGATGAGTTAGGCGGCGGCGGTGGTATCCTCTGGTCTATGCC 1534 1492 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1535 1519 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1538 1522 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTACGCC 1578 1465 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTACGCC 1524 1461 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTACGCC 1524 1461 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTACGCC 1524 1461 CTTCTCCATGCAATTCCCGATGAGCATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1520 1461 CTTCTCCATGCAATTCCCGATGAGCTATGGCGGCGGCGGTTGCTATCCTCTGGTCTATGCC 1520 1500 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGGTTGCTATCCTCTGGTCTATGCC 1520 1500 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1520 1500 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1520 1461 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGCTTGCTATCCTCTGGTCTATGCC 1520 1462 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGCTTGCTATCCTCTGGTCTATGCC 1520 1500 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGTTGCTATCCTCTGGTCTATGCC 1520 1461 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGCTTGCTATCCTCTGGTCTATGCC 1520 1469 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGCTTGCTATCCTCTGGTCTATGCC 1520
1567 CTCCTCCATGCAGTTCCCGATGAGTATGGCGGCGGCGGCGTTGCTATCCTCTGGTCTACGCC 1626 1500 CTTCTTCGTGCAGTTCCCGATGAGCATGGTGGCGGCGCCGTTGCTATCCTTTGGTCTACGCC 1559 1528 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGCTTGCTATCCTCTGGTCTATGCC 1587 1494 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCC 1583 1504 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCC 1563 1499 CTTCTCAATGCAATCCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCC 1558 1503 CTTCTCCATGCAATTCCCGATGAGTATGGCGGCGGCGGTGCTATCCTCTGGTCTATGCC 1562

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Epichioe\_uncinata\_CBS\_102040\_ Epichioe\_amarillans\_ES7\_ Epichioe\_amarillans\_E4668\_ Epichioe\_brachvelytri\_E4804\_ Epichioe\_brachvelytri\_E4804\_ Epichioe\_bromicola\_AL0434\_ Epichioe\_formicola\_AL04262\_ Epichioe\_festucae\_AR1 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_F01 Epichioe\_festucae\_F01 Epichioe\_festucae\_F11 Epichioe\_glyceriae\_ATCC\_200747\_ Epichioe\_moltis\_AL9924\_ Epichioe\_typhina\_subsp\_Poae Epichioe\_typhina\_subsp\_Poae Epichioe\_typhina\_CBS\_102646\_ Epichioe\_uncinata\_CBS\_102646\_

Epichioe\_unicinata\_Less\_1020+0-\_\_\_\_ Epichioe\_amarillans\_E57 Epichioe\_antarillans\_E668\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brankovelytri\_E4804 Epichioe\_bromicola\_AL0434 Epichioe\_frankovelytri\_E4804 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_R488 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_gansuensis\_e7080 Epichioe\_gansuensis\_e7080 Epichioe\_mobina\_ATCC\_200747\_ Epichioe\_mobina\_ATCC\_200736\_ Epichioe\_typhina\_subsp\_Poae Epichioe\_typhina\_subsp\_Poae Epichioe\_topaina\_b10264

Epichloë\_antarillans\_E57 Epichloë\_amarillans\_E57 Epichloë\_antarillans\_E57 Epichloë\_aotearoae\_MYA-1229 Epichloë\_brachyelytri\_E4804 Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F11 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_moblis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F11 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

1539       ATGTCAGTGGCACTGGCATCGTCGGAATTCGC
1627 ATGTCAGTGGCACTGGCGTCGTCGGCATTTCGCCATTGATCAACTCTGC1674 1560 ATGTCAGTGGCATTGGCATCGTCGGATTTCGCCATTGATTTCGTCATTGACCAACTTTGT1619 1588 ATGTCAGTGGCACTGGCATCGTCGGAATTCGCCATTGACCAACTCTGT1635 1554 ATGTCAGTGGCACTGGCATCGTCGAATTTCGCCATTGACCAACTCTGC1601 1564 ATGTCAGTGGCACTGGCATCGTCGAATTTCGCCATTGACCAACTCTGC1611 1559 ATGTCAGTGGCACTGGCATCGTCGAATTTCGC
1587 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCTC1646 1583 GCCAAGCTGGTCGGCCTCTCCCCCAAACAATGAATCCCGACATGAAGAATGCGAAGTTGCTC1649 1584 GCCAAGCTGGTCGGCCTCTCCCCTAACAGTGCAGCCGACATGAAGAACGCGGAAGTTGCTC1659 1584 GCCAAGCTGGTCGGCCTCTCCCCTAACAGTGCAGCCGACATGAAGAATGCGAGCTGCTC1689 1570 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCT1689 1573 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCT1689 1573 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCT1689 1573 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCT1689 1573 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCCGACATGAAGAATGCGAAGTTGCT1689 1578 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAATCAAGCGCGACATGAAGAACGCGGAGTTGCT1628 1072 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAAGCACACTGAAGAACGCGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAACGCGGACATGAAGAACGCGGAGTTGCT1627 1569 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAACGCGCACATGAAGAACGCGGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAAGCACACGCGACATGAAGAACGCGGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAAGCACACGCGACATGAAGAACGCGGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAAGCACACGCGACATGAAGAACGCGGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAACCGCGCACATGAAGAACGCGGGAGTTGCT1628 1608 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAACCAGCCGCACATGAAGAACGCGGGAGTTGCTC1628 1607 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAACCAGCCGCACATGAAGAACGCGGGAGTTGCTC1628
1675 GCCAAGCTGGTCGGCCTCTCCCCTAACAATGAAGCCGACATGAGGAATGCGCAGTTGTTC 1734 1620 GCCAAGCTAGTCGCCCTCTCCCCGAATAATAAATCCGACATGATGATGCGCAGTTGTTG 1679 1636 GCCAAGCTGGTCGGCCTCTCCCCTAACAATCAAGCCCACATGAAGAACGCGAGTTGCTC 1695 1602 GCCAAGCTGGTTGGCCTCTCCCCTGACAATGCAGCCGACATGAAGAACGCAGAGTTGCTC 1661 1612 GCCAAGCTGGTTGGCCTCTCCCCTAACAATCAAGCCGACATGGAGAACGCAGAGTTGTC 1671 1607 GCCAAGCTGGTTGGCCTCTCCCCTAACAATGCAGCCGACATGAAGAACGCAGAGTTGTC 1666 1611 GCCAAGCTGGTTGGCCTCTCCCCTAACAATGCAGCCGACATGAAGAACGCAGAGTTGCTC 1670
1647 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCAAGCAGCAGCAGGGCTTCAAGACCCATC 1706 1643 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCAAGCAGCAGCAGGGCTTCAAGACCCATC 1709 1660 AAGCGATGCTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGGCTTCAAGACCCATC 1719 1644 AAGCGATGCTTGGAAGATCTTCACACCAGCTACCCGGCAGCAGGGGCTTCAAGACCCATC 1749 1637 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGGCTTCAAGACCCATC 1749 1639 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGGCTTCAAGACCCATC 1749 1639 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGGCTTCAAGACCCATC 1749 1639 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGCAGCACAGAGCCTTCAAGACCCATC 1749 1639 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGCTTCAAGACCCATC 1688 1132 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGAGGCTTCAAGGCCCATC 181 1629 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGAGGCTTCAAGGCCCATC 182 1628 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGCAGGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACCACCAGCTACCCAGCAGCAGAGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACCACCAGCTACCCAGCAGCAGAGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACCCAGCAACCCAGCAGCAGAGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGAGGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGAGGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGAGGGCTTCAAGGCCCATC 182 1668 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGGGCTTCAAGGCCCATC 182
1735 AAGCGATGTTTGGAAGATCTTCACACCAGCTACCCAGCAGCAGGGGCTTCAAGACCCATC 1794 1880 AAGCGATGTTTGGAAGATCTTCACACCAGTACCCAGGCAGG
1707 GGCAATATGACGCTCGAGGAGGCCGTCG GCATGCTCGAGAAAAGCACCCGAAACCACCACTA 1766 1703 GGCAATATGACGCTTGAGGAGGCCGTCGGCATGCTCGAGAAAGCACCCGAAACCACCACA 1769 1704 GGCAATATGACGCTTGAGGAGGCCGTCAGCGTGGTGGAGAAAGCACCGAAACCACCACA 1779 1704 GGCAAGATGACGCTCGAGGAGGCCGTCAGCGTGGTGGAGAAAGCACCGAAACCACCACA 1763 1747 GGCAAGATGACGCTCGAGGAGGCCGTCAGCGTGGCGGAGAAAGCACCGAAACCACCACA 1809 1893 GGCAATACGACGCTCGAGGAGGCCGTCGGCATGCTCGAGAAAAGCACCGAAACCACCACA 1809 1893 GGCAATACGACGCTCGAGGAGGCCGTCGGCATGCTCGAGAAAAGCACCGAAACCACCACA 1752 1889 GGCAATATGACGCTTGAGGAGGCCGTCGGCATGCTCGAGAAAGCACCGAAACCACCACA 1752 1889 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAAAGCACCGAAACCACCACA 1748 192 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAGGACCCGAAACCACCACA 1748 1728 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAGGACCCGAAACCACCACA 1748 1728 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAGGACCCGAAACCACCACCAC 1748 1728 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAGGACCCGAAACCACCACCAC 1748 1727 GGCAATATGACGCTTGAGGAGGCCGTCAGCATGCTCGAGAGGACCCGAAACCACCACCAC 1748
1795 GGCAATATGACGCTTGAGGAGGCCGTCAGCGTGCTCGAGAAAAGCACCGAAACCACCACA 1854 1740 GGCAACATGACGCTTGAGGAGGCCGTCAGCATACTCGAGAAAAGCACCCGAGACCACCACA 1799 1756 GGCAATATGACGCTTGAGGAGGCTGTCAGCATGCTCGAGAAAAGCACCCGAAACCACCACA 1815 1722 GGCAATATGACGCTTGAGGAGGCTGTCAGCGTGCTCGAGAAAAGCACCCGAAACCACCACA 1781 1732 GGCAATATGACGCTCGAGGAGCCATCACGGTGCTCGAGAAAAGCACCCGAAACCACCACA 1781 1727 GGCAATATGACGCTCGGGGAGCCGTCAGCGTGCTCGAGAAAAGCACCCGAAACCACCACA 1780 1731 GGCAATATGACGCTTGGGGAGGCCGTCAGCGTGCTCGAGAAAAGCACCCGAAACCACCACA 1790
1767 TTAGCAATGCACACAGAAGTACATTCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1826 1763 TTAGCAATGCACACAGAAGTACATTCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1822 1780 TTAGCAATGCACACAGAAGTACATCAATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1823 1807 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1823 1807 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1869 1753 TTAGCAATGCACACAGAAGTACATGCAATGTTTCTGGGCCTTACAAAGAATGGCAATTCA 1869 1253 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1869 1254 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1869 1254 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1808 1252 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1808 1258 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1808 1749 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1808 1788 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCTTACAAAGAATGGCAATTCA 1808
1855 TTACCAATGCACACAGAAGTACATGCAATGTTGCTGGGCGTTACAAAGAATGGCAATTCA 1914 1800 TTAGCAATGCACACAGAAGTACATGCAATGTTGCTGGGCGTTACGAGGACTGGCAATTCG 1859 1816 TTACCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCGTTACAAAGAATGGCAATTCG 1851 1782 TTACCAATGCACACAGAAGTACATGCAATGTTGCTGGGCCGTTAGAAAGAA

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR31 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_mollis\_MYA-1228\_ Epichloë\_mollis\_MYA-1228\_ Epichloë\_mollis\_MSA-1228\_ Epichloë\_mollis\_MSA-200747\_ Epichloë\_mollis\_MSA-200748\_ Epichloë\_mollis\_MSA-200748\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL0434. Epichloë\_bromicola\_AL0434\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_molis\_AL9324\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_Poae\_F31 Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL0434. Epichloë\_bromicola\_AL0434\_ Epichloë\_elymi\_ATCC\_201551\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gansuensis\_e7080\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924 Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

1827 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1886 1823 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1889 1840 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1889 1844 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1920 1870 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1920 1870 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1920 1813 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1868 1312 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1868 1312 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAGCG 1871 1809 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1868 1848 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1868 1849 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1868 1849 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1868 1847 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1868
1915 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGAAGCG 1974 1860 ACTTTGTGGCATTTCTATGATCCGAAGTTCGCCATTGCCACGTTCAACTCAGGGGAAGCG 1935 1876 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCAGGGGGAGCG 1935 1842 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCGGGGGAGCG 1910 1852 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCGGGGGAGCG 1910 1847 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCAAGTCGGGGGAGCG 1910 1851 ACCTCGTGGCATTTCTATGATCCGAACTTCGCCATTGCCACGTTCGAGTCGAGGGGAGCG 1910
1887 CTACTTGAAGCCACCACCAAGTT TTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1946 1883 CTACTTGAAGCCACCACCAAGTTTTTCGAAGAGTCGGGATTCGCCAAGGTCTACGAAGCC 1959 1900 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1959 1884 CTACTTGAAGCCACCACCAAGTTCTTCGGACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1958 1930 CTACTTGAAGCCACCACCAAGTTTTTCGGACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1988 1873 CTACTTGAAGCCACCACCAAGTTTTTCGGACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1873 CTACTTGAAGCCACCACCAAGTTTTTCGGACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1873 CTACTTGAAGCCACCACCAAGTTTTTCGGACGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1873 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1874 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1875 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1876 CTACTTGAAGCCACCACCAAGTTCTTCGAACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1879 CTACTTGAAGCCACCACCAAGTTCTTCGAACAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1908 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1908 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938 1909 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1938
1975 CTACTTGAAGCCACCACCAAGTTTTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 2034 1920 CTGCTTGAAGCCACCACCAAGTTTTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1979 1936 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1961 1912 CTACTTGAAGCCACCACCAAGTTCTTCGAGGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1971 1907 CTACTTGAAGCCACCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1971 1910 CTACTTGAAGCCGCCACCAAGTTCTTCGAAGAGTCGGGATTCGCCAAAGTCTACGAAGCC 1970
1947 CAGGGTCAGACACCTAGTTCGACTTTTTCGATTTTCCATATTGATGCGGAAAGGGTGTCTCGCATC2006 1943 CAGGGTCAGACACCTAGTTTCGACTTTTTCCATATTGATGCGGAAAGGGTGTCTCGCATC2001 1940 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGATTGATGCCGAAAGGGTGTCTCGCATC2011 1944 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGTCTCGCATC2004 1987 CAGGGTCAGACACCCAGTTTCGACTTTTTCCAGATTGATGCGGAAAGGGTGCTCGCATC2004 1990 CAGGGTCAGACACCCAGTTTGGACTTTTTCCAGATTGATGCGGAAAGGGTGCTCGCATC2004 1992 CAGGGTCAGACACCCAGTTTGGACTTTTTCCAGATTGATGCGGAAAGGGTGCTCGCATC2004 1932 AAGGGTCAGACACCCAGTTTGGACTTTTTCCAGATTGATGCGGAAAGGGTGCTCGCATC2004 1932 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC2004 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGCACCCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGGATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTTCGACTTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTCGACTTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTCGACTTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTCGACTTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC499 1929 CAGGGTCAGACACCCAGTTCGACTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC9027 1929 CAGGGTCAGACACCCAGTTCGACTTTTCCGCCGATGATGCGGAAAGGGTGCTCGCATC9027 1929 CAGGGTCAGACACCCAGTTCGACTTTTCCGCCGATGATGCGGAAAGGGTGCTCGCATC9027 1929 CAGGGTCAGACACCCAGTTCGACTTTTCCGCCTGCATC3027 1929 CAGGGTCAGACACCCAGTTCGACTTCGACTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC9027 1929 CAGGGTCAGACACCCAGTTCGACTTCGACTTTTCCGCATTGATGCGGAAAGGCGGTGCTCGCATC9027 1920 CAGGGTCAGACACCCAGTTCGACTTCGACTTTTTCCGCATTGATGCGGAAAGGGTGCTCGCATC9027 1920 CAGGGTCAGACACCCAGTTCGACTTCGA
2035 CAGGGTCGGACACCCAATTTCGATTTTTCCACATTGATGCGGAAAGGGTGTCTCGCATC 2094 1980 CAGGGTCAGACACCCAGTTTCCATATTTTCCACATTGACGCGGAAAGGGTTTCTGGCATC 2039 1996 CAGGGTCAGACACCCAGTTTCGGATTTTTCCGCATTGATACGGAAAAGGTTCTGCATC 2052 1922 CAGGGTCAGACACCCAGTTTCGAATTTTTCCAGATTGATGCCGAAAGGGTGTCTCGCATC 2021 1922 CAGGGTCAGACACCCAGTTTCGATTTTTTCCAGATTGATGCCGAAAGGGTGTCTCGCATC 2021 1972 CAGGGTCAGACACCTAGTTCCGACTTTTTCCAGATTGATGCCGAAAGGGTGTCTCGCATC 2026 1971 CAGGGTCAGACACCAAGTTTCGACTTTTTCCG
2007 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGAGACCCTGCTCGAGACC 2066 2003 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGAGACCCGGCTCGAGACC 2067 2020 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2067 2044 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2068 2047 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2069 2050 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGAGACCCTGCTCGAGACC 2069 2050 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGGAGACCCTGCTCGAGACC 2069 2050 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGGAGACCCTGCTCGAGACC 2069 2050 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTCAGCCTGGAGACCCTGCTCGAGACC 2049 2050 GGATTTGACCACACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2058 GGATTTGACACGCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGCCCACGACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACACGACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACACGACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACACGACTTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACACGACTGACCGTCGCCGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACGCCCGCGCCGCGGCCGGTCGGCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACGCCGACGTGGCCGCGCCGGTCGGGCGGAGACCCTGCTCGAGACC 2048 2028 GGATTTGACGCCGCGCGCGGACCTGGTTCAGCCGGAGACCCTGCTCGAGACC 2048 2027 GGATTTGGCCCACGACTGGCCGCGCCGGCTGGTTCAGCCGGGGGGAGCCCTGCTCGAGACC 2048 2027 GGATTTGGCCACGACTGGCCGCGCCGGCTGGTTCAGCCGGGGGGGG
2095 GGATTCGACCACAACTTGACCGTCGCCGACCTGGTCCAGCCTGAGACCCTGCTCGAGACC2154 2040 ATATTGGACTACAACTTGACCGTCGCCGACCTGGTTCGGCCTGAGACCCTGCTCGAGACC2099 2056 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTGAGCCGGACACCTGCTCGAGACC2115 2022 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTGAGCCGGACACCTGCTCGAGACC2081 2032 GGATTTGGCCACAACTTGACCGTCGCCGACCTGGTTGAGCCGGACACCTGCTCGAGACC2086 2030 GGATTTGACCACAACTTGACCGTCGCGACCTGGTTGAGCCGGACACCTGCTCGAGACC2088 2030 GGATTTGACCACAACTTGACCGTCGCCGACCTGGTTGAGCCGGACACCTGCTCGAGACC2088
2067 ATAGCCTCCGAGCATAATACGGCGAATTTCATACAGGATCCTGGCGAGTTGACGTCTTCC 2126 2063 ATAGCCTCCGAGCATAATACGGCGAATTTCATACAGGATCCTGGCGAGTTGACGTCTTCC 2122 2080 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGATCCTGGCGAGTGACGTCTTCC 2123 2064 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGATCCTGGCGAGTGACGTCTTCC 2123 2107 ATAGCCTCCGAGCATAAGACGGCGACTTTCCTCAGGATCCTGCGCGAGTGACGTCTCC 2123 2053 ATAGCCTCCGAGCATAAGACGGCGACTTTCCTCCAGGATCCTGCGCGAGTGACGTCTCC 2123 2053 ATAGCCTCCGAGCATAAGACGGCGACTTTCCTCCAGGATCCTGCGCGAGTGACGTCTTCC 2123 2054 ATAGCCTCCGAGCATAAGACGGCGACTTTCCTCCAGGATCCTGCGCGAGTGACGTCTCC 2123 2053 ATAGCCTCCGAGCATAAGACGGCGACTTTCCTCCAGGATCCTGCGCGAGTGACGTCTCC 2123 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCGAGTGACGTCTCC 2103 2053 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCGAGTGACGTCTCC 2103 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCGAGTGACGTCTCC 2103 2048 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCGAGTGACGTCTCC 2108 2048 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCCAGTTGACGTCTCC 2108 2048 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCGCCAGTTGACGTCTCC 2108 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCTGCCCGCGAGTGACGTCTCC 2108 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCCTGCGCCAGTTGACGTCTCC 2108 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCCTGCGCCAGTTGACGTCTCC 2108 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGACCCCTGCGCCAGTTGACGTCTCC 2108 2049 ATAGCCTCCGAGCATAAGACGGCGACTTTCATACAGGATCCTGCCCAGTGACGTCTCC 2108
2155 AT AGCCTCCGAGCATAAGACGGCGACTTTCATCCAGGATCCTGCGCAGTTGACGGCTTCC 2214 2100 GT AGCCTCTCAGCAGCGCGACGCGCGACTTTCCTCCAAGATCCTGCGCAGTTGACGGCTTCC 2159 2116 AT AGCCTCCGAGCATAAGAGGGGCGACTTTCGTACAGGATCCTGCGCGAGTTGACGTCTCC 2175 2082 AT AGCCGCTGATCGTAAGACGGCGACTCTCTATCATGATCCTGCGCGAGTGACATCTTCC 2141 2092 AT AGTCTCCGAGCATAATAGGGGCGACTCTCTATCATGACTCTGGCCAGTTGACATCTTCC 2140 2087 AT AGCCGCTGATCGTAAGACGGGGCGCTCTCTATCATGATCCTGGCCAGTTGACATCTTCC 2146 2090 AT AGCCTCTGATCGTAAGACGGTGACTCTCTTACATGATCCTGGCCAGTTGACATCTTCC 2149

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_uncinata\_Cs5\_102040\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_fromicola\_AL04262\_ Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_festucae\_F11 Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_mobins\_AL9924 Epichioe\_typhina\_ATCC\_200747\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

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2127 CACAACTTTAGCGCCCAAACCGAGCTTTCGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2186 2123 CACAACTTTAGCGCCCAAACCGAGCTTTCGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2182 2140 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2199 2124 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2226 2170 CGCACTTTCGGCCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2229 2113 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2229 2113 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2188 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2168 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2168 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2168 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2168 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2168 1612 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2168 2148 CGCACTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGCCAGCTTGG 2207 2109 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGAGCCAGCTTGG 2207 2109 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGAGCCAGCTTGG 2207 2109 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGAGCCAGCTTGG 2206 2215 CGCGCTTTCGGCCCCAAACCGGCGCGCGCGCTTTGGGAGCTTGGGGGAGCCAGCTTGG 2206	
2160 GACGCTTTTAGCGCCCAAACCGAGCTTTTGGAAACCTTGGGCTTGGGGGAGGCAGCTTGG 2219 2176 CGCAATTTTAGCGCCCAAACCGAGCTTTTGGAAATCTTGGGCTTGGGGGAGGCAGCTTGG 2212 2122 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAACCTTGCGCTTGGGGGAGGCAGCTTGG 2201 2152 CACAGTTTTAGCGCCCAAACCGAGCTTTTGGAAATATTGGACTTGGGGGAGGCAGCTTGG 2211 2147 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAACCTTGCGCTTGGGGGAGGCAGCTTGG 2206 2150 CGCACTTTTAGCGCCCAAACCGAGCTTTTGGAAACCTTGGGCTTGGGGGAGGCAGCTTGG 2209	
2187 C GCGAT GCCACAGCCAGGCT GGAAGAAAGCACT GGTAT G GGTGAGCACT GGAT GCCT AT C 2246 2183 CGCGAT GCCACAGCCAGGCT GGAAGAAAGCACT GGTAT GGGTGAGCACT GGAT GCCT AT C 2242 2200 CGCGAT GCTACAGCCAGGCT GGAAGAAAGC CT GGTAT C GGTGAGCACT GGAT GCCCAT C 2259 2184 CGCGAT GCTACAGCCAGGCT GGAAGAAAGC CT TGGTAT GGGTGAGCACT GGAT GCCCAT C 2238 2227 CGCGAT GCTACAGCCAGGCT GGAAGAAAGCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2239 2173 CGCGAT GCTACAGCCAGGCT GGAAGAAAGCACT GGTT GGGTGAGCACT GGAT GCCCAT C 2239 2173 CGCGAT GCTACAGCCAGGCT GGAAGAAAGCACT GGTT GGGTGAGCACT GGAT GCCCAT C 2239 2173 CGCGAT GCTACAGCCAGGCT GGAAGAAAGCACT GGTT GGGTGAGCACT GGAT GCCCAT C 2239 2169 CACGAT GCCACAGCCAGGCT GGAAGAAAGCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2238 2174 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2238 2189 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2238 2280 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2238 2208 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2208 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2208 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2207 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2207 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2207 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2207 CACGAT GCCACAGCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248 2207 CACGAT GCCACACCCAGGCT GGAAGAAACCACT GGTAT GGGTGAGCACT GGAT GCCCAT C 2248	
2275 CGCGATGCTACAGCCAGGCTGGAAGAAAGCACTGGCGTGGGGTGAGCACTGGATGCCTATC 2334 2220 CGCGATGCGACACCCAGGCTGGAAGAAACCACTGGTATGGGTGAGCACTGGATGCCTATC 2279 2236 CGCGATGCCACCCAGGCTGGAAGAAACCACTGGTATGGGTGAGCACTGGATGCCCATC 2295 2202 CGCGATGCTACAGCCAGGCTGGAAGAAAGCCTCGGTTGGGTGAGCACTGGATGCCCATC 2271 2202 CGCGATGCTACAGCCAGGCTGGAAGAAAGCCTCGGTTGGGTGAGCACTGGATGCCCATC 2272 2202 CGCGATGCTACAGCCAGGCTGGAAGAAAGCCTCGGTTGGGTGAGCACTGGATGCCCATC 2272 2202 CGCGATGCTACAGCCAGGCTGGAAGAAAGCCCTCGGTTCGGGTGAGCACTGGATGCCCATC 2272 2202 CGCGATGCTACAGCCAGGCTGGAAGAAAGCCCTGGATCGCCTGGATGCCCATC 2269	
2247 CTTGAAACGATGAAGGAGGCCGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTT 2306 2243 CTTGAAACGATGAAGGAGGCCGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTT 2302 2240 CTTGAAACGATGAAGGAGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCTC 2319 2244 CTTGAAACGATGAAGGAGGGCCGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTC 2330 2287 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTC 2349 2233 CTTGAAACGATGAAGGAGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTC 2349 2233 CTTGAAACGATGAAGGAGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTC 2349 2233 CTTGAAACGATGAAGGAGGGCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTC 2349 2232 CTTGAAACGATGAAGGAGGGCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTT 2282 2239 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCTT 2282 2239 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCATCATCT 2282 CTTGAAACGATGAAGGAGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2282 2282 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2282 2292 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2282 2292 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2282 2292 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2282 2292 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2288 2266 CTTGAAACGATGAAGGAGGGCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2288 2267 CTTGAAACGATGAAGGAGGGCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCAATCTT 2288	
2335 CTTGAAACGATGAAGGAGGGCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATCTC 2394 2880 CTTGAAACGATGAGGGGGGGGGGGGAGAAAGGGTCCTATGAGGTGCAATTCATCATCTC 2329 2296 CTTGAAACGATGAAGGAGGGCCGGTGAGAAAGGGTCCTATGAGGTGCAATTCATCATATT 2262 CTTGAAACGCTGAAGGAGGGCGGTGAGGAAGGGTCCTATGAGGTGCAATTCATCATCATC 2272 CTTGAAACGCTGAAGGAGGGCGGTGGGGAGGGCCCTATGAGGTGCAATTCATCATCATC 2267 CTTGAAACGGTGAAGGAGGGCCGGTGAGGAGGGTCCTACGAGGTGCAATTCATCATCATC 2270 CTTGAAACGCTGAAGGAGGGCGGTGAGGAAAGGGTCCTACGAGGTGCAATTCATCAATCT 2329	
2307 AACAACAAGAAT G G GACCAAGT G GATT A GT A C GG AAAGT C C C G C C AT CAAAGAT T T C AA G 2366 2303 AACAACAAGAAT G G G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T T C AA C 2362 2304 AAGAACAAGAAT G A G C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T T C AA C 2379 2304 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T T C AA C 2373 2307 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T T C AA C 2409 2309 AAGAACAAGAAT G A G C C AAGT G G AT T A GT A C G G AAAGT C C AC G C C AT C AAAGAT T T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C AT C AAAGAT T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AAAGT C C C G C C C T C AAAGAT T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AA G T C C C G C C AT C AAAGAT T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A GT A C G G AA G T C C C G C C AT C AAAGAT T C AA C 2409 2293 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A C G G AAGT C C C G C C AT C AAAGAT T C AA C 2409 2289 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A C G G AAGT C C C G C C C AT C AAAGAT T C AA C 2348 2328 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A C G G AAGT C C C G C C AT C AAAGAT T C AA C 2348 2327 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A C G G AAGT C C C G C C AT C AAAGAT T C AAC 2348 2327 AAGAACAAGAAT G A G A C C AAGT G G A T C G G A T A G T A G G A C T C AAAGAT T C AAC C 2348 2327 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A G G AAGT C C C G C C AT C AAAGAT T C AAC 2348 2327 AAGAACAAGAAT G A G A C C AAGT G G AT T A G T A G G AAGT C C C G C C AT C AAAGAT T C AAC 2348 2327 AAGAACAAGAAT G A G A C C AAGT G G A T T G T A G G G A T T G G A A G A C C AAGAT T C AAC C 347 C C G A C A AGAAT G A G A C C AAGT G G A T C G G A AGT C C G G A AGT C C AAGAT T C AAC C 2348 2327 AAGAACAAGAAAT G A G A C C AAGT G G A	
2395 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCGGCATCAAAGATTTCAAG 2454 2340 AAGAACAAGAATGAGACCAAGTGGCTCAGCACGAAAGTCCCGGCCATCAAAGATTTCAAG 2356 GAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCGGCCATCAAAGATTTCAAG 2322 AAGAACATGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCGCCATCAAAGATTTCAAC 2332 GAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCGCCATCAAAGATTTCAAC 2332 AAGAACAATGAGACCAAGTGGATTAGTACGGAAAGTTCCCCGCATCAAAGATTTCAAC 2330 AAGAACAATGAGACCAAGTGGATTAGTACGGAAAGTTCCCCCATCAAAGATTTCAAC 2380 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTTCCCCCATCAAAGATTTCAAC 2380 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCCGCCATCAAAGATTTCAAC 2380 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCCGCCATCAAAGATTTCAAC 2380 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCCGCCATCAAAGATTTCAAC 2380 AAGAACAAGAATGAGACCAAGTGGATTAGTACGGAAAGTCCCGCCATCAAAGATTTCAAC 2380	
2367 GCGCGTCTTGATGAGCACCTCAAGACTCTAAGCAAAACATACGAGTTTGAGTCAGGGTCG 2426 2363 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTTGAGTCAGGGTCG 2423 2380 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTGAGTCAGGGTCG 2423 2364 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTTGAGTCAGGGTCG 2423 2407 GCGCGTCTTGATGAGCACCTCAAGCACAGCATAGCAAAACATACGAGTTTGAGTCAGGGTCG 2423 2408 GCGCGTCTTGATGAGCACCTCAAGCACACATACGAAACATACGAGTTGAGTCAGGGTCG 2423 2409 GCGCGTCTTGATGAGCACCTCAAGCACAACATACGAGTTGAGTCAGGGTCG 2459 2353 GCGCGTCTTGATGAGCACCTCAAGCACAAACATACGAGTTGAGTCAGGGTCG 2459 2353 GCGCGTCTTGATGAGCACCTCAAGCACAAACATACGAGTTGGAGTCAGGGTCG 2412 2349 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTCG 2412 2349 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTC 12408 2388 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCGAGGGTC 12408 2388 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTC 12408 2388 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTC 12408 2387 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTC 12408	
2455 GCGCGTCTTGATGAGCACCTCGGGACTCTAAGCAAAACATACGAGTTTGAGTCAGGGTCG 2514 2400 GCGCGTCTTGATGAGCACCTCGAGAGCTCTAAGCGAAACATATGGAGTTCGAGTCAGGGTCG 2459 2416 GCGCGTCTTGATGAGCACCTCGAGACTCTAAGCAAAACATACGAGTTCGAGTCAGGGTCG 2475 2382 GCGCGTCTAGATGAGCACCTCGAGACTCTAAGCAAAACACACAC	

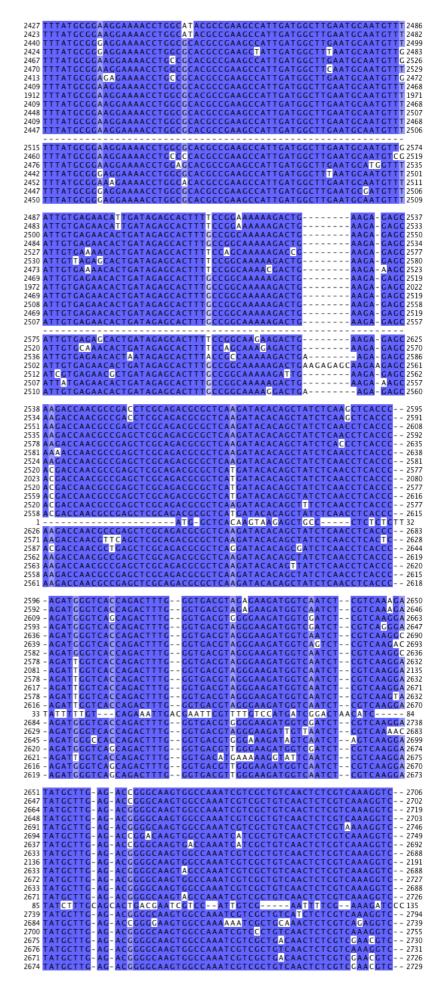
Epichloë\_amarillans\_ES7\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloē\_amarillans\_ES7\_ Epichloā\_amarillans\_E4668\_ Epichloā\_actearoae\_MYA-1229\_ Epichloā\_brachyelytri\_E4804\_ Epichloā\_bromicola\_AL0434\_ Epichloā\_bromicola\_AL04262\_ Epichloā\_festucae\_AR1 Epichloā\_festucae\_AR37 Epichloā\_festucae\_AR37 Epichloā\_festucae\_AR48 Epichloā\_festucae\_F31 Epichloā\_festucae\_F31 Epichloā\_festucae\_F31 Epichloā\_glyceriae\_ATCC\_200747\_ Epichloā\_glyceriae\_ATCC\_200747\_ Epichloā\_moltis\_AL9924\_ Epichloā\_typhina\_subsp\_Poae Epichloā\_typhina\_subsp\_Poae Epichloā\_typhina\_S05\_Poae\_P51 Epichloā\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gestucae\_F11 Epichloë\_gansuensis\_e7080\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924 Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gasvensis\_e7080\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_moltis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_uncinata\_CBS\_102646\_

Epichloē\_amarillans\_E57\_ Epichloē\_amarillans\_E4668\_ Epichloē\_aotearoae\_MYA-1229\_ Epichloē\_brachyelytri\_E4804\_ Epichloē\_bromicola\_AL0434\_ Epichloē\_bromicola\_AL04262\_ Epichloē\_festucae\_AR1 Epichloē\_festucae\_AR37 Epichloē\_festucae\_AR48 Epichloē\_festucae\_F31 Epichloē\_festucae\_F31 Epichloē\_festucae\_F31 Epichloē\_galveriae\_ATCC\_200747\_ Epichloē\_galveriae\_ATCC\_200747\_ Epichloē\_inebrians\_MYA-1228\_ Epichloē\_typhina\_subsp\_poae Epichloē\_typhina\_subsp\_poae Epichloē\_typhina\_subsp\_poae Epichloē\_paceiae\_ATCC\_200746\_ Epichloē\_typhina\_subsp\_poae Epichloē\_typhina\_subsp\_poae Epichloē\_tucata\_C85\_102646\_\_



Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_unicinata\_Cs5\_1020+0\_\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_antarillans\_E4668\_ Epichioe\_branicola\_AL0434\_ Epichioe\_branicola\_AL0434\_ Epichioe\_festucae\_A817 Epichioe\_festucae\_A817 Epichioe\_festucae\_A837 Epichioe\_festucae\_A837 Epichioe\_festucae\_F11 Epichioe\_festucae\_F11 Epichioe\_gasuensis\_e7080\_ Epichioe\_alyceriae\_ATCC\_200747\_ Epichioe\_inebrians\_MYA-1228\_ Epichioe\_inebrians\_MYA-1228\_ Epichioe\_ityphina\_subsp\_poae Epichioe\_typhina\_subsp\_poae Epichioe\_typhina\_sch5\_102646\_ Epichioe\_uncinata\_C85\_102646\_

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

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2746       CAACGTTGTTCTCGACGCCTA         2747       CAACGTTATCCTCGACGCCTA         2749       CAACGTTATCCTCGACGCCTA         2790       CAACGTTATCCTCGACGCCTA         2790       CAACGTTATCCTCGACGCCTA         2790       CAACGTTGTTCTCGACGCCTA         2736       CAACGTTATCCTCGACGCCTA         2732       CAACGTTATCCTCGACGCCTA         2747       CAACGTTATCCTCGACGCCTA         2770       CAACGTTATCCTCGACGCCTA         2783       AGTGTTATCCTCGACGCCTA         2779       CAACGTTATCCTCGACGCCTA         2770       CAACGTTATCCTCGACGCCT	CGAGCTTGCCCAC - AGGGA CGAGCTTGCCCAC - ACGGA CGAGCTTGCCCAC - ACGGA	A A T G A T G C A C A G A G A G A C C G C 2804 A A T G A T G A T G C A C A G A A A G C C C 2821 A T G A T G A T G C A C A A G A G A C C C 2805 A A T G A T G T G C A C A A G A G A C C C 2805 A A T G A T G T G C C A G A G A G C C C 2848 A A T G A T G C C C A G A G A G C C C 2794 A A T G A T G C C C A G A G A G C C C 2793 A A T G A T G C C C A G A G A G C C C 2793 A A T G A T G C C C A G A G A G C C C 2793 A A T G A T G C C C A G A G A G C C C 2790 A A T G A T G C C C A G A G A G C C C 2790 A A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C C A G A G A G C C C 2828 A T G A T G C C A C A G A G A G C C C 2832 A T G A T G A C G A C A G A G A G C C C 2831 A A C G A T G C A C A G A G A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C 2833 A A C G A T G C A C A G A A A G C C C C 2833 A A C G A T G C A C A G A A A G C C C C 2833 A A C G A T G C A C A
2809 T C T T T G G C A C A A C A G T T G G C T T         2805 T C T T T G G C A C A A C A G T T G G C T T         2822 T C T T T G G C A C A A C A G T T G G C T T         2849 T C T T T G G C A C A A C A G T T G G C T T         2852 T C T T T G G C A C A A C A G T T G G C T T         2795 T C T T T G G C A C A A C A G T T G G C T T         2791 T C T T T G G C A C A A C A G T T A G C T T         2791 T C T T T G G C A C A A C A G T T G G C T T         2791 T C T T T G G C A C A C A G T T G G C T T         2791 T C T T T G G C A C A C A G T T G G C T T         2791 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T C T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T         2807 T T T T G G C A C A C A G T T G G C T T      <	TTGATTCGG - TCACGTTCC TTGATTCGG - CCACGTTCC TTGATTCGG - CCACGTTCC TGATTCGG - CCACGTTCC TGATTCGG - CCACGTTCC TGATTCGG - CCACGTTCC TGATTCGG - CCACGTTCC TGATTCGG - CCACGTTCC	TACC
2863       CTATTGGTGCCGGATTCATAG         2859       CTATTGGTGCCGGATTCATAG         2860       CTATTGGTGCCGGATTCATAG         2905       CTATTGGTGCCGGATTCATAG         2906       CTATTGGTGCCGGATTCATAG         2849       CTATTGGTGCCGGATTCATAG         2849       CTATTGGTGCCGGATTCATAG         2849       CTATTGGTGCCGGATTCATAG         2849       CGTTGGTGCCGGATTCATAG         2848       CGTTGGTGCCGGACTCTAG         2845       CGTTGGTGCCGGACTCTAG         2845       CGTGGTGCCGGACTCTAG         2845       CGTTGGTGCCGGACTCTAG         2845       CGTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2845       CGTTGGTGCCGGACTCATAG         2850       CATTGGTGCCGGACTCATAG         2860       CTATTGGTGCCGGACTCATAG         2877       TATTGGTGCCGGACTCATAG         2888       CCTTGGTGCCGGACTCATAG         2888       CCTTGGTGCCGGACTCATAG         2888       CCTTGGTGCCGGACTCATAG         2888       CTTGGTGCCGGACTCATAG <tr< td=""><td>GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC</td><td>C T C C T G G G A G G C T C 2914         C T C A T G G G A G C T G T C 2931         C T C A T G G G A G C C T C 2915         C T C A T G G G A G C C T C 2915         C T C A T G G G A G C C T C 2958         G T C T T G G G A G C C T C 2904         C T C G T G G G A G C C T C 2904         C T C G T G G G A G C T C C 2903         G T C G T G G G A G C T T C 2900         C T C G T G G G A G C T T C 2900         C T C G T G G G A G C T G T C 2939         C T C G T G G G A G C T G T C 2900         C T C G T G G G A G G T G T C 2900         C T C G T G G G A G G T G T C 2933         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 29567         G T C T T G G G A G G T G T C 29567         G T C T T G G G A G G T G T C 29567         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2938    </td></tr<>	GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGTCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC GAGCCACTACCGCCGTCGC	C T C C T G G G A G G C T C 2914         C T C A T G G G A G C T G T C 2931         C T C A T G G G A G C C T C 2915         C T C A T G G G A G C C T C 2915         C T C A T G G G A G C C T C 2958         G T C T T G G G A G C C T C 2904         C T C G T G G G A G C C T C 2904         C T C G T G G G A G C T C C 2903         G T C G T G G G A G C T T C 2900         C T C G T G G G A G C T T C 2900         C T C G T G G G A G C T G T C 2939         C T C G T G G G A G C T G T C 2900         C T C G T G G G A G G T G T C 2900         C T C G T G G G A G G T G T C 2933         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 2938         G T C G T G G G A G G T G T C 29567         G T C T T G G G A G G T G T C 29567         G T C T T G G G A G G T G T C 29567         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2942         G T C A T G G G A G G T G T C 2938
2919 AGT GT TATTCT C GGAGGACT G 2913 AGT GT CATTCT C GGAGGACT G 2923 AGT GT CATTCT C GGAGGACT G 2959 AGT GT CATTCT C GGAGGACT T 2959 AGT GT CATTCT C GGAGGACT T 2959 AGT GT CATTCT C GGAGGACT T 2905 AGT GT CATTCT C GGAGGACT T 2901 AGT GT CATTCT C GGAGGACT T 2902 AGT GT CATTCT C GGAGGACT T 2903 AGT GT CATTCT C GGAGGACT T 2924 AGT GT CATTCT C GGAGGACT C 2952 AGT GT CATTCT C GGAGGACT C 2954 AGT GT CATTCT C GGAGGACT C 2953 AGT GT CATTCT C GGAGGACT C 2954 AGT GT CATTCT C GGAGGACT C 2953 AGT GT CATTCT C GGAGGACT C 2954 AGT GT CATTCT C GGAGGACT C 2953 AGT GT CATTCT C GGAGGACT C 2954 AGT GT CATTCT C GGAGGACT C 2953 AGT GT CATTCT C GGAGGACT C 2954 AGT GT CATTCT C GGAGGACT C	GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTTGGCGCC-TG GCCTTTGGCGTGGCG	

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gansuensis\_e7080\_ Epichloë\_enebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_gestucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E46804\_ Epichloë\_brancians\_AL04344\_ Epichloë\_brancians\_AL04262\_ Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_typhina\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1

Epichloë\_uncintar\_CD\_105040\_ Epichloë\_amarillans\_E57\_ Epichloë\_antarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_branicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_gasuensis\_e7080\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobilis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_uncintata\_CB5\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

2977 AAATTGCTCCAAAAGCAGAGGCTGT GGGTCGCTATTTC - GGTGACGCCGACGGGGCC 2973 AAATTGCTCCAAAAGCAGAGGCGGTGT GGGTCGCTATTTC - GGTGACGCCGACGAGGGC 2974 AAATTGCCGAAAAAGCAAAGCCGTGT GGGTCGCTATTTC - GGTGACGCCGACGAGGGC 2974 AAATTGCCGAAAAAGCAAAGCCGTGT GGGTGGCTATTTC - GGTGACGCCGACGACGACGAC 3020 AAATTGTCGAAAAAGCAAAGCACGCGTGT GGGTGGCTATTTC - GGTGACGCCGACGACGACGA 2963 AAATTGCCGAAAAAGCACGCGGTGT GGGTGGCTATTTC - GGTGACGCCGACGACGACGA 2959 AAATTGCCGAAAAAGCACGGCGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGA 2959 AAATTGCCCCAAAGCAGGCGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGAC 2959 AAATTGCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGAC 2959 AAATTGCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGAC 2959 AAATTGCCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCCAAAGCAGGCGGGTGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCCAAAGCAGGCGCGGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCCAAAGCAGGCGCGGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2959 AAATTGCCCCCAAAGCAGGCGCGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2950 AAATTGCCCCCAAAGCAGGCGCGGT GGGTCGCTATTTC - GGTGACGCCGACGACGACGC 2950 AAATTGCCCCCAAAGCAAAGCAAAGCCGCT GGGTCGCTATTTC - GGTGACGCCGACGACGACGCC 2950 AAATTGCCCGCAAAAGCAAAGCAAAGCCGCGT GGGTCGCTATTTC - GGTGACGCCGACCACGCC 2010 GAATTGCCCGAAAAGCAAAGCAAAGCAAAGCTGT GGGTCGCTATTTC - GGTGACGCCGACCAACGCC 2010 AAATTGCCCGAAAAAGCAAAGCAAAGCTGT GGGTCGCTATTTC - GGTGACGCCGACCACGCC 2010 AAATTGCCCGAAAAAGCAAAGCAAAGCAAAGCTGT GGGTCGCTATTTC - GGTGACGCCGACCAACGCC 2020 AAATTGCCCGAAAAAGCAAAGCAAAGCAAAGCAAGCGCGT GGGTCGCTATTTC - GGTGACGCCGACGACGCC 2020 AAATTGCCCGAAAAAGCAAAGCAAAGCAAAGCTGT	3028 3045 3072 3072 3078 3014 2517 3014 3054 3014 3054 3014 3052 7446 3052 7446 3120 3081 3055 3081 3057 3057
3033       TACAAAGCTGGAGGAT       TCAAGTACGATGAGAAGGATAAGATTCT	3081 3098 3098 3125 3125 3071 3067 3067 3067 3067 3105 504 3173 3118 3134 3134 3109 3110
3086       GTTTGGTGC TGTCATCGGCGAAATCGA C - GCGGCCGGTAACGT - C - A	- 3125 - 3142 - 3126 - 3169 - 3172 - 3115 - 3111 - 2614 - 3111 - 3110 - 3111 - 3149 G 564 - 3217 - 3162 - 3178 - 3153 - 3153 - 3155
3130       A G T T C G A C A G C C A A T A C A T C T A T C G C A - C C A G G C A C G G A T C C A C A G G         3126       A G T T C G A C A G C C A A T A C A T C T A T C G C A - C A G G C A C G G A T C C A C A G G         3127       A T T C G A C A G C C A A C A C A T C T A T C G C A - C T G G C A C G G A T C C A C A G G         3170       A T T C G A C A G C C A A C A C T C T A T C C C A - C T G G C A C G A T C C A C A G G         3170       A T T C G A C A G C C A A T A C A T C T A T C G C A - C C T G G C A C G A T C C A A G G         3170       A T T C G A C A G C C A A T A C A T C T A T C G C A - C C T G G C A C G A T C C A A G G         3116       A T T C G A C A G C C A A T A C A T C T A T C G C A - C C T G G C A C G A T C C A A G G         3112       A T T C G A C A G C C A A T A C A T C T A T C G C A - C C T G G C A C G A T C C A A G G         3112       A T T C G A C A G C C A A T A C A T C T A T C G C A - C T G G C A C G T G C C A C A G G         3112       A T T C G A C A G C C A A T A C A T C T A T C G C A - C T G G C A C G T G C C A C A G G         3112       A T T C G A C A G C C A A T A C A T C T A T C G C A - C T G G C A C G T G C C A C A G G         3112       A T T C G A C A G C C A A T A C A T C T A T C G C A - C T G G C A C G T G C C A C A G G         312       A T T C G A C A G C C A A T A C A T C T A T C C C A	- 3171 - 3188 - 3172 - 3209 G 3219 G 3162 G 3158 G 3158 G 3158 G 3158 G 3158 G 3157 - 3195 - 612 - 3263 G 3211 A 3224 G 3200 G 3209 G 3158 G 3158 G 3158 G 3158 G 3157 - 3195 - 3195 - 3263 G 3211 - 3263 G 3211 - 3263 G 3211 - 3263 G 3209 G 3209 - 3263 G 3211 - 3263 G 3209 G 3209 - 3263 G 3209 - 3263 - 3263 G 3209 - 3263 G 3209 - 3263 G 3209 - 3263 G 3209 - 3263 G 3209 - 3263 - 3265 - 3265 - 3265 - 3265 - 3265 - 3265 - 3265 - 3265 - 3265
3176       GTCAGGCAG       AATCA       ACTACTTCTTCTGGGCCGGCCATTTTCCCAGAATC       ATCACGC         3172       GTCAGGCAG       AATCA       ACTACTTCTTCTGGGCCGGCCATTTTCCCAGAATC       ATCCACGC         3189       GTCAGGCAG       AATCA       ACTACTTCTTCTGGGCCGCCATAAGCCCAGAATG       ATCCAAGC         3173       GTCAGGCAG       AATCA       ACTACTTCTTCTGGGCCGGCCATAAGCCCAGAATG       ATCCAAGC         3173       GTCAGGCAG       AATCA       ACTACTTCTTCTGGGCCGGCCATAAGCCCAGAATG       ATCCAAGC         3210       TTAGGCATC       ACTACTTCTTCTGGGCCGGCCATAAGCCCAGAATG       ATCCAAGC         3210       TTAGGCATC       ACTACTTCTTCTGGGCCGGCCATAAGCCCAGAATG       ATCCAACG         3210       TTAGGCATC       ACTACTTCTTCTGGGCCGGCGACATTTTCCCAGAATG       ATCCACG         3163       TCAGGCACA       ACTACTTCTTCTGGGCCGGCGACAAGCCCAGAATG       ATCCACG         3159       TCGGGCACA       AATCA       ACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATG       ATCCACG         3159       TCGGGCACA       AATCA       ACTACTTCTTCTGGGTCGGCGACAAGCCCAGAATG       ATCCACG         3159       TCGGGCACA       AATCA       ACTACTTCTTCTGGGTCGGCGACAAAGCCCAGAATG       ATCCACG         3159       TCGGGCACA       AATCA       ACTACTTCTTCTGGGTCGGCGACAAAGCCCAGAATG       ATCCACG         3159       TCG	A 3228 A 3245 A 3229 G 3250 A 3275 A 3218 A 3214 A 3214 A 3214 A 3253 A 3214 A 3252 A 646 A 3320

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atoeraoa\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_R81 Epichloë\_festucae\_R81 Epichloë\_festucae\_A848 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F11 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_SL05\_Poae\_F31 Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atoeraoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gansuensis\_e7080\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_SL55\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_A817 Epichloë\_festucae\_A837 Epichloë\_festucae\_A837 Epichloë\_festucae\_A848 Epichloë\_festucae\_F91 Epichloë\_festucae\_F91 Epichloë\_festucae\_F11 Epichloë\_ansuensis\_e7080\_ Epichloë\_molins\_AL9924\_ Epichloë\_molins\_AL9924\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_C85\_102666\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E3368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveriae\_ATCC\_200747\_ Epichloë\_molrians\_MYA-1228\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_PaeaeP31 Epichloë\_typhina\_Sat05\_PaeaP51 Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

3233 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCAGC - GGAA 3229 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCAGC - GGAA 3240 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCAGC - GGAA 3251 TTACCGTGGGTTATAGTACTTAGGACTTAGGACAT - AGACCCTTGTGCGCCAGC - GGAA 3251 TTACCGTGGGTTAT - TAATGTGCGA - GAAGGCATTGGAGCTCCGGCCAGC - GGAA 3251 TTACCGTGGGTAT - TCATGTGCGA - GAAGGCATTGGAGCTCCGGCCAGC - GGAA 3219 TAAGTCCCAGGCTAT - TCATGTGCGA - GAAGGCATTGGAGCTCCGGCCAGC - GGAA 3215 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCTCCGGCCAGC - GGAA 3215 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCAGC - GGAA 3215 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTCGAGCCCCGGCCAGC - GGAA 3215 TAAGTCCCAGGCTAT - TAATGTGCGA - GAAGGCATTCGAGCCCCGGCCAGC - GGAA 3254 TAAGTCCCCAGGCTAT - TAATGTGCGA - GAAGGCATTCGAGCCCCGCCCGCCAGC - GGAA 3254 TAAGTCCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCCAGC - GGAA 3254 TAAGTCCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCCGC - GGAA 3257 TAAGTCCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGGCCCGCCAGC - GGAA 3269 TAAGTCCCCAGCCTAT - TAATGTCCGA - GAAGGCATTGGAGCCCCGCCCGCCAGC - GGAA 3269 TAAGTCCCCAGGCTAT - TAATGTCCGA - GAAGGCATTGGAGCCCCGCCCAGC - GGAA 3257 TAAGTCCCCAGGCTAT - TAATGTCCGA - GAAGGCATTGGAGCCCCGCCCAGC - GGAA 3258 TAAGTCCCCAGGCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGCCCGCCCAGC - GGAA 3258 TAAGTCCCCAGCCTAT - TAATGTGCGA - GAAGGCATTGGAGCCCCGCCCGCCAGC - GGAA 3258 TAAGTCCCCAGCCTAT - TAATGTGCGA - GAAGGCATGGAGCCCCGCCCGCCCAGC - GGAA 3258 TAAGTCCCCAGCCTAT - TAATGTGCGA - GAAGGCATGGAGCCCCGCCCGCCAGC - GGAA 3258 TAAGTCCCCAGCCTAT - TAATGTGCGA - GAAGGCATGGAGCCCCGCCAGC - GGAA 3258 TAAGTCCCCAGCCTAT - TAATGTGCGA - GAAGGCATCGGAGCCCCGCCCGCCAGC - GGAA	A 3282 A 3299 A 3283 A 3307 A 3329 A 3272 A 3268 A 3306 G 703 A 3374 A 3322 A 3310 A 3310 A 3310 A 3306 A 3309
3287       ACT - TGCGAATACTAGAGACTACA - CCACAATC - ATCCTCCCCCCCCCGCCACGCCCAAATCG         3283       ACT - TGCGAATACTAGAGACTACA - CCACAATC - ATCCTCCCCCCCCCCCCCCC	3359 3328 3324 2827 3324 3324 3324 3324 3324 3324 3324 33
3343 T       CATCAGCTACGATTGGC       A AACCCTTCCGTTCTGTACAGGCCGGCATGACTACGG         3339 T       CATCAGCTACGATTGGC       A AACCCTTCCGTTCTGTACAGGCCGCATGACTACGG         3356 ACATCAGCTACGATTGGC       A AATACTTCCGTTCTGTACAGGCCGACATGACTACGG         3340 ACATCAGCTACGATTGGC       A AATACTTCCGTTCTGTACAGGCCGACATGACTACGG         3340 ACATCAGCTACGAATGGC       A AATACTTCCGTTCTGTACAGGCCGACATGACTACGG         3360 ACATCAGCTACGATTGGC       A AACCCTTCCGGTCTGCACACCCCGACATGACTACGG         3380 ACATCAGCTACGATTGGC       A AACCCTTCCGGTCTGCACACTCCGACATGACTACGG         3329 ACATCAGCTACGAATGGC       A AATCCTTCCGGTCTGTACAAGGCCGACATGACTACGG         3328 ACATCAGCTACGAATGGC       A AATCCTTCCGTTCTGTACAGGCCGACATGACTACGG         3328 ACATCAGCTACGAATGGC       A AATCCTTCCGTTCTGTACAGGCCGACATGACTACGG         3328 ACATCAGCTACGAATGGC       A AATCCTTCCGTTCTGTACAGGCCGACATGACTACGG         3328 ACATCAGCTACGAATGGC       A AATCCTTCCGTTCTGTACAGGCCGACATGACTACGG         3329 ACATCAGCTACGAATGGCA       A AATCCTTCCGTTCTGTACAGGCCGACATGACTACGG <td>C 3395 C 3403 C 3441 C 3384 C 3384 C 3380 C 2883 C 3419 C 3486 C 3418 C 3418 C 3444 C 3424 C 3422 C 3423 C 3418</td>	C 3395 C 3403 C 3441 C 3384 C 3384 C 3380 C 2883 C 3419 C 3486 C 3418 C 3418 C 3444 C 3424 C 3422 C 3423 C 3418
3399       TTCGATGTAATCCGGAG ACTCGAAGAAGACAAGCCATTCGACTATGAC TTTTA         3395       TTCGACGTGATCCGGAG ACTCGAAGAAGACAAGCCATTCGACTATGAC TTTTA         3412       TTCGACGTGATCCGGAG ACTCGAAGAAGACGACGACCGATTCGACTATGAC TTTTA         3404       TTCGACGTGATCCGGAG ACTCGAAGAAGACGACGACCGATTCGACTATGAC TTTTA         3404       TTCGACGTGATCCGGAG ACTCGAAGAAGACGACGACCGATTCGACTATGAC TTTTA         3404       TTCGATGTGATCCGGAG ACTCGAAGAAGACGACGACCGATTCGACTATGAC TTTA         3404       TTCGATGTGATCCGGAG ACTCGAAGAAGACGACCGATCGACTATGAC TTTA         3412       TTCGATGTGATCCGGAG ACTCGAAGAAGACGACCGATTGGACTATGAC TTTA         3424       TTCGATGTGATCCGGAG ACTTGAACAAGACGACGACCGATTCGACTATGAC TTTA         3435       TTCGATGTGATCCGGAG ACTTGAACAAGACGACGACCGATTCGACTATGAC TTTA         3436       TTCGATGTGATCCGGAG ACTTGAACAAGACGACCGATCGACTATGAC TTTA         3431       TTCGATGTGATCCGGAG ACTTGAACAAGACGACCGATCGACTATGAC TTTA         3432       TTCGATGTGATCCGGAG ACTTGAACAAGACGACCAACGACCATTCGACTATGAC TTTA         3431       TCGATGTGATCCGGAG ACTTGAACAAGACGACCATCGACTATGAC TTTA         3432       TTCGATGTGATCCGGAG ACTTGAACAAGACGACCATCGACTATGAC TTTA         3433       TCGATGTGATCCGGAG ACTTGAACAAGACGACGACCATTCGACTATGAC TTTA         3433       TCGATGTGATCCGGAG ACTTGAACAAGACGACGACCATTCGACTATGAC TTTA <td>C 3448 C 3465 C 3449 C 3495 C 3495 C 3436 C 3434 C 3434 C 3434 C 3434 C 3434 C 3434 C 3434 C 3472 C 3434 C 3540 C 3488 C 3498 C 3498 C 3498 C 3477 C 3472 C 3472</td>	C 3448 C 3465 C 3449 C 3495 C 3495 C 3436 C 3434 C 3434 C 3434 C 3434 C 3434 C 3434 C 3434 C 3472 C 3434 C 3540 C 3488 C 3498 C 3498 C 3498 C 3477 C 3472 C 3472
3453       ATCTTTCC - TAG TGAAT - ACATC ATTCACTACATCACACAC - TG	- 3488 - 3505 - 3480 - 3535 - 3476 - 3476 - 2977 - 2477 - 3474 - 3513 - 3474 - 3513 - 3474 - 3517 - 897 - 897 - 3580 - 3529 - 3527 - 3517 - 3517

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gansuensis\_e7080\_ Epichloë\_enebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_gestucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E4668\_ Epichloë\_brancians\_E46804\_ Epichloë\_brancians\_AL04344\_ Epichloë\_brancians\_AL04262\_ Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_typhina\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1

Epichloë\_uncintar\_CD\_105040\_ Epichloë\_amarillans\_E57\_ Epichloë\_antarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_branicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_gasuensis\_e7080\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobilis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_uncintata\_CB5\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

3489       -       A GT       -       T T G T C A       -       -       A         3506       -       A GT       -       T T G T C A       -       A         3490       -       A GT       -       T T G T C A       -       A         3490       -       A GT       -       T T G T C A       -       A         3470       -       A GT       -       -       T G T C A       -       -       A         3477       -       A GT       -       -       T G T C A       -       -       A         3475       -       A GT       -       T T G T C A G       -       -       -       A         3475       -       A G T       -       T T G T C A G       -       -       -       A         3475       -       A G T       -       T T G T C A G       - <t< th=""><th>AACAACCCAT      </th></t<>	AACAACCCAT
3523       CAGTCGGTC -AGTGCCAGTT -CAAGT         3540       CAATCGGTC -AGTGCCAGTT -CAAGT         3569       CCTCGGTC -AGTGCCAGTT -CAAGT         3509       CCTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3511       CAGTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3012       CCGTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3509       CGTCGGTC -AGTGCCAGTT -CAAGT         3548       CGTCGGTC -AGTGCCAGTT -CAAGT         3547       CATGACGCGTACGCT - AGTGCCAGTT -CAAGT         3548       CGTCGGTC -AGTGCCAGTT -CAAGT         3547       CAGACGGTC -AGTGCCAGTT -CAAGT         3561       CAGA	CGCAG - AACTGGA - TAA - GAGCC - TACACAA 3577         CGCAG - AACTGGA - TAA - GAGCC - TACACAA 3573         CACAG - AGCTGGA - TAA - GAGCC - TACACAA 3573         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3574         GCAAC - AGCTGGA - TGA - GAGCC - TACACAA 3574         GCAAC - AGCTGGA - TGA - GAGCC - TACACAA 3574         GCAAC - AGCTGGA - TGA - GAGCC - TACACAA 3520         CGCAG - AGCTGGA - TAA - GAGCC - TACACAA 3520         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3520         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3520         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3559         CGCGG - AGCTGGA - TGA - GAGCC - TACACAA 3653         CGCGG - AGCTGGA - TGA - GAGCC - TACACAA 3623         CGCAG - AACTGGACCA - TAA - GAGCC - TACACAA 3623         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3623         CGCGAG - AGCTGGA - TGA - GAGCC - TACACAA 3601         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3601         CGCAG - AGCTGGA - TGA - GAGCC - TACACAA 3601         CGCAG - AGCTGGA - TGA - GAGCC - TACA
3774 T G T C C T T G A A - T A C A C C A T A - C A - T G G 3591 T G T C C T C G A A - T A C A C C A T A - T A - T G G 3775 T G T C C T C G A A - T A C A C C A T A - C A - T G G 3624 G T A A C T C G C A T G T A T A A C C T A C - T G G 3621 T G T C C T C G A A - T A C A C C A T A - C A - T G G 3562 T G T C C T C G A A - T A C A C C A T A - C A - T G G 3560 T G T C C T C G A A - T A C A C C A T A - T A - T G G 3560 T G T C C T C G A A - T A C A C C A T A - T A - T G G 3560 T G T C C T C G A A - T A C A C C A T A - T A - T G G 3560 T G T C C T C G A A - T A C A C C A T A - T A - T G G 3599 T G T C C T C G A A - T A C A C C A T A - T A - T G G	- CGCAGGA - GCC AAT - T - ACA - CC - 3620 - CGCAGGA - GCC AAT - T - ACA - CC - 3616 - CGCAGGA - GCC AAT - T - ACA - CC - 3617 ACCTAGTAAGCT T T AGTAATAT - ATA 3675 - CGCAGGA - GCC AAT - T - ACA - CC - 3663 - CGCAGGA - GCC AAT - T - ACA - CC - 3663 - CGCAGGA - GCC AAT - T - ACA - CC - 3604 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3602 - CGCAGGA - GCC AAT - T - ACA - CC - 3604 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC AAT - T - ACA - CC - 3644 - CGCAGGA - GCC
3621 - ATCGGGCTCAATCCTGG CGTGGCCAT         3617 - ATCGGGCTCAATCCTGG CGTGGCCAT         3634 - ATCGGGCTCAATCCTGG CGTGGCCAT         3618 - ATCGGGCTCAATCCTGG CGTGGCCAT         3676 - AGTGCCCCTATACTCTG CGTGGCCAT         3686 - ATCGGGCTCAATCCTG CGTGGCCAT         3603 - ATCGGGCTCAATCCTG GCGTGGCCAT         3604 - ATCGGGCTCAATCCTG GCGTGGCCCAT         3645 - ATCGGGCTCAATCCCG GCGTGGCCCAT	GACATTGTCTTCGAACAAGAGCA-3669
3670       -       GCACATCTTGGGTTTTGGA-CT         3666       -       GCACATCTTGGGTTTTGGA-CT         3683       -       GCACACCTTGGGTTTTGGA-CT         3667       -       GCACACGTTGGGTTTTGGA-CT         3729       -       TCTCCTACTAGCTACCTAAGT         3713       -       GCACATCTTGGGTTTTGGA-CT         3654       -       GCACATCTTGGGTTTTGGA-CT         3652       -       GCACATGTTGGGTTTTGGA-CT         3652       -       GCACATGTTGGGTTTTGGA-CT         3691       -       GCACATGTTGGGTTTTGGA-CT         3691       -       GCACATGTTGGGTTTTGGA-C	GC GG GAAAGTT GG AC G       - GG GA GAACAT CGT G 3719         GC GG GAAAGTT GG AC G       - GC GA GAACAT CGT G 3715         GC GG GAAAGTT GG AC G       - GC GA GAACAT CGT G 3732         GC GG AAAGTT GG AC G       - GC GA GAACAT CGT G 3732         GC GG AAAGTT GG AC G       - GC GG ACAAC AT CGT G 3732         GC GG AAAGTT GG AC G       - GC GG ACAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GG ACAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GG ACAAC AT CGT G 3701         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3701         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3701         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3701         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3701         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3703         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3739         GC GG AAAGTT GG AC G       - GC GG AG AAC AT CGT G 3739         GC GG AAAGTT GG AC G       - GC GA GAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GA GAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GA GAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GA GAAC AT CGT G 3763         GC GG AAAGTT GG AC G       - GC GG AG AC AT CGT G 3744         GC GG AAAGTT GG AC G       - GC GG AG AC AT CGT

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_alyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_uncinata\_Cs5\_102040\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_fromicola\_AL04262\_ Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_festucae\_F11 Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_mobins\_AL9924 Epichioe\_typhina\_ATCC\_200747\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_s205\_Poae\_P31 Epichloë\_uncinata\_C85\_102646\_

3720       ATTGAAGC - C C AG - GC - CGTCT CAATC TCAGGT TT - CCG - GGT 3757         3716       ATTGAAGC - C C AG - GC - CGTCT CAATC TCAGGT TT - CCG - GGT 3753         3733       ATTGAAGC - C C G G - GC - CGTCT CAATC TCAGGT TT - CCG - GGT 3753         3717       ATTGAAGC - C C G G - GC - CGTCT CAATC TCAGGT TT - CCG - GGT 3763         3775       ATTGAAGC - C CAGT CAATC TAGGGT TT - CCG - GGT 3763         376       ATTGAAGC - C CAATC TAAGGGCTACC TT - CCG - GGT 3823         3763       ATTGAAGC - C CAATC TAAGGGCTACC TT - CCG - GGT 3743         3704       ATTGAGGC - C	
3758       GA - AC - CTGGC - AGA CCACTAC - TTC GAATCGATGTTCATCTGCAAACCC - 3803         3754       GA - AC - CTGGC - AGA CCACTAC - TTC GAATCGATGTCATCTCATCTGCAAACCC - 3803         3754       GA - AC - CTGGC - AGA CACTAC - TTC TG AGATCGATGTCATCTGCAAACCC - 3803         3755       GA - AC - CTGGC - AGA CACTAC - TTC AGATCGATGTCATCTTCAAACCC - 3803         3751       GA - AC - CTGGC - AGA CACTATA - TTC AGATCGATGTCATCTCCAAACCC - 3803         3755       GA - AC - CTGGC - AGA CACTTA - TTC AAATCGATGTCATCTCCAAACCC - 3803         3824       AATACTATAGCTAAGAATACACTATA - TTC AAATCGATGTCATCTGCAAACCC - 3803         3801       GA ATC - TGGC - AGA CACTTA - TTC GAATCGATGTCATCATCTGCAAACCC - 3843         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAATCGATGTCATCTGCAAACCC - 3785         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA CACCTTA - TTC GAGTCCATGCTCATCTGCAAACCC - 3824         3740       GA - AC - CTGGC - AGA C	
3804 - AACGGCGAGATCCT GAAAGTGGACTTT GGCAAGA - AAACCACTTTCTCA TAAA - 3856         3800 - AACGGCGAGATCCT GAAAGTGGACTTT GGCAAGA - AAACCACTTTCCCCA TAAA - 3859         3817 - AACGGCGAGATCCT ACAAGTGGACTTTT GGCAAGA - AAACCACTTTCCCCA TAAA - 3859         3801 - AACGGCGAGATCCT AGAAGTGGACTTTC CCAAGA - ACACCACTTTCCCCA AAA - 3859         3801 - AACGGCGAGATCCT AGAAGTGGACTTTCCCAAGA - AGACCACTTTCCCCA TAAG - 3853         3882 TAA CTATA TATACCI ATATTAI AGTAATGTAAAGTAAAATTACCIACCT - AAA - 3849         3874 - AACGGCGAGATCCT AAAGTGGACTTTCCCAAGA - AAACCACTTTCCCCA TAAA - 3849         3788 - AACGGCGAGATCCT CAAAGTGGACTTTCCCAAGA - AAACCACTTTCCCCA TAAA - 3849         3788 - AACGGCGAGATCCT CAAAGTGGACTTTCCCAATAG - AAACCACTTTCCCCA TAAA - 3849         3786 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3849         3786 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3847         3786 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3873         3825 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3826 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3827 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3824 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3824 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3824 - AACGGCGAGATCCT ACAAGTGGACTTTCCCAATAG - AACCACTTTCCCCA TAAA - 3874         3824 - AACG	
3857 GG - AAGATGCT - GC CAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3905         3853 GG - AAGATGCT - GC CAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGGAT 3918         3870 - GG - TAGATGCT - GCAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGGAT 3918         3854 - GG - AACTTGCT - GCAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGATT 3918         3935 - G - AACTT AAGAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3920         3935 - G - AACTT AAGAA ACTATAAGAAACGAGTAAACTCACAGAGCAT 3948         3841 - G - GAAGATGCT - GCCAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3849         3841 - G - GAAGATGCT - GCCAA GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3849         3841 - G - GAAGATGGT - CAAG GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3849         3842 - G - GAAGATGGT - CACAG - GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3849         3843 - G - GAAGATGGT - CACAG - GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3847         3878 - G - GAAGATGGT - CACAG - GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3847         3877 - G - GAAGATGGA - CAGGG GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3847         3877 - G - GAAGATGGA - CAGGG GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3847         3877 - G - GAAGATGGA - CAGGG GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3847         3877 G GAAGATGGA - CAGGG - GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3933         3883 G GAAGATGGA - CAGGG - GT - ACCAG - GGAGGAAGCCAGAAACTCACAGAGCAT 3933         3893 - G - GAAGATGGA - CAGGG - GT - ACCCAG - GGAGGAAGCCAGAAACTCACAGAACTCACAGAGCAT 3933	
3906	8 ; 0 + ; 5 + 7 + 8 + 2 + 0 8 8 5 7 2

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_brachvelytri\_E4804\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_gansuensis\_e7080\_ Epichloë\_enebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_gestucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_P51 Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atotaraae\_MYA-1229\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_tophina\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_subsp\_poae

Epichloë\_uncintar\_CDS\_10c0rd\_ Epichloë\_amarillans\_ES7 Epichloë\_antarillans\_ES7 Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F1 Epichloë\_festucae\_F1 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_unbis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_typhina\_MYA-1228\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_uncinata\_C85\_102646\_

3949 3966 3950 4035 3996 3937 3935 3935 3974 3935 3974 3935 3973 1395 4041 3989 3999 3997 3978 3977	) GTTGGACAAG - TACACAACGCCCGC ; GCTGGACAAG - TACACAACGCCCGC ; GCTGGACAAG - TACACAACGCCCGC ; GCTGGACAAG - TATACAACGCCCGC ; GTGGACAAG - TATACAACGCCCGC	C - T G G C C A G T C T G T T
4001 4018 4002 4078 4048 3989 3987 4026 3987 4026 3987 4025 1449 4093 4041 4051 4029 4030 4025	L CAA - CCGACGAGCAAGCGTCTTCTC G GAA - CCGACGAGCAAACGTCTTCTC G GAA - CCGACGAGGCAAACGTCTTCTC G GAA - CCGACGAGCAAACGTCTTCTC G GAA - CCGACGAGCAAACGTCTTCTCC G GAA - CCGACGAGCAAACGTCTTCTCCC G GAA - CCGACGAGCAAACGTCTTCTCCC G GAA - CCGACGAGCAAACGTCTTCTCCCCCCCCCCCCCCCCCCC	CTACACC - ATCGATGC G - CCTGAAGA - ATTGACC 4058 CTACACC - ATCGATGC G - CCTGAAGA - ATTGACC 4058 CTACACC - ATCGATGC T - CCTGAAGA - ATTGACC 4071 CTACACC - ATCGATGC T - CCTGAAGA - ATTGACC 4071 CTACACC - ATCGATGC G - CCTGAAGA - ATTGACC 4010 CTACACC - GTCGATGC G - CCTGAAGA - ATTGACC 4040 CTACACC - GTCGATGC T CCTGAAGA - ATTGACC 4040 CTACGCC - ATCGATGC T CCTGAAGA - ATTGACC 4040 CTACGCC - ATCGATGC T CCTGAAGA - ATTGACC 4079 CTACACC - GTCGATGC T CCTGAAGA - ATTGACC 4078 CTACACC - ATCGATGC T CCTGAAGA - ATTGACC 4078 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 4074 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 4078 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 4078 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 4078 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 408 CTACACC - ATCGATGC C CCTGAAGA - ATTGACC 408
4055 4072 4056 4124 4102 4043 4041 3544 4041 4080 4041 4079 1496 4147 4095 4105 4083	AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGTGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCAGGTTGGTGCCCT AACAGTGCGCCAGGTTGGTGCCCT AACAGTGCGCCAGGTTGGTGCCCT AACAGTGCGCCAGGTTGGTGCCCT AACAGTGCGCCAGGTTGGTGCCCT	GACT GC TG AA GGAAAAGTCTTCTTTTA 4108 GACT GC TG AA GGAAAAGTCTTCTTTTA 4104 GACT GC TG GA GGAAAAGTCTTCTTCTA 4121 TAATAAGG TA AA GAAAAAGTCTTCTTCTA 4125 GACT GC TG GA GAAAAGTCTTCTTCTA 4151 ACAACT GC TG GA GAAAAGTCTTCTTCTA 4195 GACT GC TG CA GAAAAGTCTTCTTCTA 4195 GACT GC TG CA GAAAAGTCTTCTTCTA 4195 GACT GC TG CA GAAAAGTCTTCTTCTA 4199 GACT GC TG CT GGAAAAGTCTTCTTCTA 4199 GACT GC TG CT GGAAAAGTCTTCTTCTA 4128 TG GTCTACGC CA TGTCAGTG CGAAAGTCTTCTTCTA 4128 GACT GC TG CT GGAAAAGTCTTCTTCTA 4128 GACT GC TG CA GGAAAAGTCTTCTTCTA 4128 GACT GC TG CA GGAAAAGTCTTCTTCTA 4124 GACT GC TG AAGGAAAAGTCTTCTTCTA 4128 GACT GC TG AAGGAAAAGTCTTCTTCTA 4128 GACT GC TG AAGGAAAAGTCTTCTTCTA 4128
4105 4122 4106 4171 4152 4096 4091 3594 4091 4130 4091 4129 1554 419 4145 4155 4133 4134 4129	C AAT - AC AGAACATT CT CCCA         C CAAT - AC AGAACATACCCCCCCCA         C CAAT - AC AGAACATCCCCCCCCA         L TAAT T ACTAAAAAATCCTTTTTACA         C CAAT - AC AGAACATCCTTTTACA         C CAAT - AC AGAACATTGTCCCA         C CAAT - AC AGAACATGTCCCA         C CAAT - AC AGAGCATAGTCCCA         C CAAT - AC AGAACATAGTCCCA	A T C T G G C G T G G A G T T T C G A C C G G A G T C T G C G A 4163 AT C T G G C G T T G G A G T T T C G A C C G G A G T C T G C G A 4159 AT T T G G C G T T G G A C G T T T C G A C C G G A G T C T G C A 4160 A C T T A A C T A T A T A T A T A T A T
4164 4160 4177 4161 4229 4151 4146 4185 4146 4185 4146 4184 1592 4200 4210 4188 4189 4184	GCAAAATTACCGTGACCTATGCCC         GCAAAGTACCGTGACCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTGTGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC         GCAAAGTACCATGCCCTATGCCC <td>GT C TTTTTAAAAG       ACCC       T - TCAC 4208         GT C TTTTAAAAG       ACCC       T - TCAC 4204         GT C TTTTAAAAG       AACCC       T - TCAC 4204         GT C TTTTAAAAG       AACCC       T - TCAC 4204         GT TTTTAAAAG       AACCC       T - TCAC 4204         GT TTTTTAAAAG       AACCC       T - TCAC 4204         GT TTTCTAAAAG       AACCC       T - TCAA 4221         GATTTTCTAAAAGA       AACCC       T - TCAA 4278         GATTTTCTAAAAGA       AACCC       T - TCAA 4278         GATTTTCTAAAAGA       ACCC       T - TCAA 4274         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 4294         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 3693         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 4228         GTTTTCTAAAAGA       ACCC       T - TCAA 4229         GTTTTCTAAAAGA       AACCC       T - TCAA 4228         GTTTTCTAAAAGA       AACCC       T - TCAA 4228         GTTTTCTAAAAGA       AACCC       T - T CAA 4228         GTTTTCTAAAAGA       AACCC       T - T C</td>	GT C TTTTTAAAAG       ACCC       T - TCAC 4208         GT C TTTTAAAAG       ACCC       T - TCAC 4204         GT C TTTTAAAAG       AACCC       T - TCAC 4204         GT C TTTTAAAAG       AACCC       T - TCAC 4204         GT TTTTAAAAG       AACCC       T - TCAC 4204         GT TTTTTAAAAG       AACCC       T - TCAC 4204         GT TTTCTAAAAG       AACCC       T - TCAA 4221         GATTTTCTAAAAGA       AACCC       T - TCAA 4278         GATTTTCTAAAAGA       AACCC       T - TCAA 4278         GATTTTCTAAAAGA       ACCC       T - TCAA 4274         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 4294         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 3693         GTTTTCTAAAAGA       ACCC       T - TCAA 4290         GTTTTCTAAAAGA       ACCC       T - TCAA 4228         GTTTTCTAAAAGA       ACCC       T - TCAA 4229         GTTTTCTAAAAGA       AACCC       T - TCAA 4228         GTTTTCTAAAAGA       AACCC       T - TCAA 4228         GTTTTCTAAAAGA       AACCC       T - T CAA 4228         GTTTTCTAAAAGA       AACCC       T - T C

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F36 Epichloë\_festucae\_F36 Epichloë\_gansuensis\_e7080 Epichloë\_gansuensis\_e7080 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200736\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E3368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveriae\_ATCC\_200747\_ Epichloë\_molrians\_MYA-1228\_ Epichloë\_molrians\_MYA-1228\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Paea\_P51 Epichloë\_uncinata\_C85\_102646\_\_

Epichioe\_uncinata\_CBS\_102646\_ Epichioe\_amarillans\_ES7\_ Epichioe\_amarillans\_E4668\_ Epichioe\_brachvelytri\_E4804\_ Epichioe\_brachvelytri\_E4804\_ Epichioe\_bromicola\_AL0434\_ Epichioe\_francovalue Epichioe\_festucae\_AR1 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_F11 Epichioe\_festucae\_F11 Epichioe\_festucae\_F11 Epichioe\_glyceriae\_ATCC\_200747\_ Epichioe\_moltis\_AL9924\_ Epichioe\_typhina\_subsp\_Poae Epichioe\_typhina\_subsp\_Poae Epichioe\_typhina\_CS120646\_ Epichioe\_uncinata\_CBS\_102646\_

Epichloe\_uncinata\_CBS\_102646\_ Epichloe\_amarillans\_E4668\_ Epichloe\_amarillans\_E4668\_ Epichloe\_brachyelytri\_E4804\_ Epichloe\_brachyelytri\_E4804\_ Epichloe\_bromicola\_AL0434\_ Epichloe\_fromicola\_AL04262\_ Epichloe\_fromicola\_AL04262\_ Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_E368 Epichloe\_festucae\_F01 Epichloe\_gaveraise\_e7080\_ Epichloe\_giveraise\_e7080\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_ATCC\_200736\_ Epichloe\_typhina\_subsp\_poae Epichloe\_typhina\_subsp\_poae Epichloe\_typhina\_subsp\_poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_glasvensis\_e7080\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_Poae\_PS1 Epichloë\_typhina\_Sub2646\_

4209       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATCATT - CA - TTC AAGT T/         4205       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATCATT - CA - TTC AAGT T/         4222       AGCG - TCTGGG - TAGATGGTAGGT - GGA - ATCGTT CA - TAC AAGT T/         4222       AGCG - TCTGGG - TAGATGGTAGGT - GGA - ATCGTT CA - TAC AAGT T/         4206       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTGTT CA - TAC AAGT T/         4206       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTGTT CA - TAC AAGT T/         4207       AGTAACTAGGTAGATAAATTTTAGAC CTAGATACTTACACTAC - AAGTACTTAGA AAGT - GCG -         429       AGTAACTAGGGTAGGTAGGT - GGA - ATCATTT CA - TTC AAGT - GCG -         4196       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATCATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4191       CGCG - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT         4229       CGC - TCTGGG - TAGATGGTAGGT - GGA - ATTATTT CA - TAC AAG - TAGCGT	AC 4247 AC 4249 AC T A 4334 4296 - T 4296 - T - 4240 AC CT 4240 AC CT 4240 AC CT 4240 AC CT 4279 AC CT 4279 AC CT 4279 AC CT 4278 AC CT 4278 AC CT 4278
4252       - G       - T AATTCTGTCACTAA       - T TGTTTATCCAG G G G ACATCAGAT         4268       - G       - T AATTCTGTCACTAA       - T TGTTTATCCAG G G G ACAATCAGAT       - C - C / A         4266       - G       - T ACTCTGCGCACTAA       - T TGTTATCCAG C G G ACAATCAGAT       - C - C / A         4250       - G       - T ACCTCTGTCACTAA       - T TGTTATCCAG T G G ACAATCAGAT       - C - C / A         4250       - G       - T ACCTCTGTCACTAA       - T TGTTATCCAG T G G ACAATCAGAT       - C - C / A         4250       - G       - T ACCTCTGTCACTAA       - T TGTTATCCAC C G CACAAATCAGAT       - T - C / A         4250       - G       - T ACCTCTGTCACTAA       - T TGTTTATCCAC C G CACAATCAGAT       - T - C / A         4237       - T ACCTCTGTCACTAA       - T TGTTTATCCAC C G CACAATCAGAT       - T C / A         4241       - G       - T ACCTCTGTCACTAA       - T TGTTTATCCAC T GACAATCAGAT       - T C / A         4241       - G       - T ACCTCTGTCACTAA       - T TGTTTATCCAC T GACAATCAGAT       - T C / A         4280       - G       - T ACCTCTGTCACTAA       - T TGTTTATCCAC T GACAATCAGAT       - T C / A         4241       - G       - T ACCTCTGTCACTAA       - T TGTTTATCACA G GACAATCAGAT       - T C / A         4241       - G       - A CCTCTGTCACTAA       - T TGTTTATCAC	A C G C 4294 A C G C 4312 A C G C 4296 A A C G C 4393 A C G C 4342 A C G C 4287 A C G C 4287 A C G C 4287 A C G C 4287 A C G C 4326 A C G C 4326 A C G C 4330 A C G C 4330 A C G C 4330 A C G C 4326
4299	ATTA 4342 ATTA 4360 ATTA 4364 AGAA 4453 ATTA 4390 ATTA 4334 ACTA 4335 ACTA 4335 ACTA 4374 ACTA 4374 ACTA 4374 ACTA 4374 ACTA 4378 ATTA 4378 ATTA 4378
4347       TG - TCCTCACCGCCG ATAGC - ATGAACCT CATTGCATGGTTGGAAGCC         4343       TG - TCCTCACCGCCG ATAGC - ATGAACCT CATTGCCATGGTTGGAAGCC         4343       TG - TCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4345       T - ATCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4454       AAGACCTATACC - CTTATATTCTTTATAAAGCTACTTCCTAACCTA - TAGGA         4391       T - ATCCTCACCGCCG ATAGC - ATGAACCT TGTTGCCATGGTTGGAGGCC         4345       T - ATCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4335       T - ATCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4336       T - ATCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4337       T - ATCCTCACCGCCG ATAGC - ATGAACCT CGTTGCCATGGTTGGAGGCC         4338	C T G 4392 C T G 4394 C T G 4394 C T G 4394 C T G 4384 C T G 4385 C T G 4385 C T G 4385 C T G 4424 C T G 4425 C T G 4425 C T G 4425 C T G 4428
4397       CCTTG - CTTGCCAAGCTCAAT GG - ACTAGAC AAGTGG ACAG - GCGAGG         4393       CCTTG - CTTGCCAAGCTCAAT GG - ACTAGAC AAGTGG ACAG - GCGAGG         4411       CCTTG - CTTGCCAAGCTCCAT GA - AGAAGAC AAGTGG ACAG - GCGAGG         4395       CCTTG - CTTGCCAAGCTCCAT CA - AGAAGAC AAGTGG ACAG - GCGAGG         4395       CCTTG - CTTGCCAAGCTCCAT CA - AGAAGAC AAGTGG ACAG - GCGAGG         4307       TATTAACCT ATATACCTCTATTACTATAAGAACACCTAAGAAGAGCG ACAG - GCGAGG         4307       TATTAACCTATATACCTCTATTACATAAGAAGACCTAAGAAGAGCG ACAG - GCGAGG         4411       CCTTG - CTTTGCCAAGCTCCATG GACTAGAC AAGTGG ACAG - GCGAGG         4385       CCTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4386       CCTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4386       CCTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4386       CCTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4386       CTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4424       CTTG - CTTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4424       CTTG - TTGCCAAGCTCCATG AAGAAGAC AAGTGG ACAG - GCGAGG         4438       CTTG - TTGCCAAGCTCCATG AGAGAAGAC AAGTGG ACAG - GCGAGG         4438       CTTG - TTGCCAAGCTCCATG AGAGAG	G C T A 4444 G C T A 4440 G C T A 4458 G T T A 4442 C T A 4456 G C T A 4482 G C T A 4433 G C T A 4433 G C T A 4433 G C T A 4433 G C T A 4471 G C T A 4471 G C T A 4471 G C T A 4472 G C T A 4476 G C T A 4476 G C T A 4476 G C T A 4476

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_A817 Epichloë\_festucae\_A837 Epichloë\_festucae\_A837 Epichloë\_festucae\_A838 Epichloë\_festucae\_F31 Epichloë\_festucae\_F368 Epichloë\_festucae\_F368 Epichloë\_glyceriae\_A7CC\_200747\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasusensis\_e7080\_ Epichloë\_gasusensis\_e7080\_ Epichloë\_mollis\_AL3924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_uncinata\_C85\_102646\_

Epichioe\_uncinata\_CBS\_102646\_\_ Epichioe\_amarillans\_E57\_ Epichioe\_amarillans\_E4668\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434\_ Epichioe\_francoina\_AL0434\_ Epichioe\_france\_AR1 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F11 Epichioe\_festucae\_F11 Epichioe\_galveeriae\_ATCC\_200747\_ Epichioe\_alise.e7080\_ Epichioe\_alise.e7080\_ Epichioe\_galveeriae\_ATCC\_200747\_ Epichioe\_inebrians\_MYA-1228\_ Epichioe\_inebrians\_MYA-1228\_ Epichioe\_typhina\_subsp\_poae Epichioe\_typhina\_subsp\_poae Epichioe\_typhina\_subsp\_poae Epichioe\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E5668\_ Epichloë\_atearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR3 Epichloë\_festucae\_AR3 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mollis\_AL9924 Epichloë\_mollis\_AL9924 Epichloë\_mollis\_AL9924 Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_102646

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4441 A - ACTTCTACTGGAAGA - CTACT A - CACTATCCAAG CG - AGATGA         4459 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4445 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4457 CTAGTTCTA CGAGGCTATACT A - CACTGGGCCGAG CG - AGCTTA         4458 C - ACTTCTACTGCAAGA - CTACT A - CACTGGTCGACAAA CG - AGCTTA         4430 A - ACTTCTACTGCAAGA - CTACT A - CACTGATCAAG CG - AGATGA         4431 C - ACTTCTACTGCAAGA - CTACT A - CACTGGTCGAC CG - AGATGA         4432 A - ACTTCTACTGGAAGA - CTACT A - CACTGGCCGAG CG - AGATGA         4433 A - ACTTCTACTGGAAGA - CTACT A - CACTGGCCCGAC CG - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGCCCGAG CG - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGCCCGAG CG - AGCTTA         4437 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4438 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG CG - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG C - G - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG C - G - AGCTTA         4434 C - ACTTCTACTGGAAGA - CTACT A - CACTGGGCCGAG C - G - AGCTTA         4434 C - ACTTCTAC	T C 4533 T C 4477 G C 4478 G C 3981 G C 4478 G C 4478 G C 4517 G C 4478 G C 4516 G C 4516 G C 4516 G C 4516 G C 4522 T C 4522 T C 4542
4490       TCA       AGC CCAAGACAT - TC       GGATCCCTAA       CTGG       AGC       CGATAT         4486       TCA       AGC CCAAGACAT - TC       GGATCCCTAG       CTGG       AGC       CGATAT         4504       TCA       AGC TCAAGCCAT - TC       GATCCCTAG       TGG       AGC	
4531       - GAT       - GCGGCG       - ATAGA       - TCCTCATA       - TGATTTTTG       CTGGGACCAAAA         4527       - GAT       - GCGGCG       - ATAGA       - TCCTCATA       - TGATTTTTG       CTGGGACCAAAA         4529       - GAT       - GCGGCG       - ATAGA       - TCCTCATA       - TGGTTTTTTG       CTGGGACCAAAA         4529       - GAT       - GCGGCG       - ATAGA       - TCCTCATA       - TGGTTTTTG       CTGGGACCAAAA         4664       CTAT       - AAGTTT       - A A ACCTTATA AAGATTAAAGAAAAAAGACTAAGGA       - AAAG         4575       - GAT       - CGGCGC       - ATAGA       - TCCTCATA       - TGATTTTCTG       CTGGGACCAAAA         4519       - GAT       - CGCCGC       - ATAGA       - TCCTCATA       - TGATTTTTG       CTGGGACCAAAA         4520       - GAT       - CGCCGC       - ATAGA       - TCCTCATA       - TGATTTTTG       CTGGGACCCAAAA         4520       - GAT       - CGCCCG       - TCCTCAT       - TGATTTTTG       CTGGGACCCAAAA         4520       - GAT       - CGCCCG       - TCCTCAT       - TGATTTTTG       CTGGGACCCAAAA         4520       - GAT       - CGCCCG       - TCCTCAT <td>LGA 4573           LGC 4575           LGC 4575           LGC 4575           LGC 4575           LGC 4575           LGC 4566           LGC 4660           LGC 4660           LGC 4609           <t< td=""></t<></td>	LGA 4573           LGC 4575           LGC 4575           LGC 4575           LGC 4575           LGC 4575           LGC 4566           LGC 4660           LGC 4660           LGC 4609           LGC 4609 <t< td=""></t<>
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Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804, Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_C85\_102646\_

Epichioe\_uncinata\_Cs5\_102040\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_fromicola\_AL0434. Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR38 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_mobins\_AL9924 Epichioe\_typhina\_ATCC\_200747\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Paae\_Ps1 Epichioe\_typhina\_subsp\_Paae\_Ps1

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_galveeriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_s205\_Poae\_P31 Epichloë\_uncinata\_C85\_102646\_

4691 CAGATGGCAGTGATGAACAATTTTGCTTCTTCTCACACAAAGCAA CACA - AGCTCGTC 474 4687 CAGATGGCAGTGATGAACACTGTTTTGCTTCTTCTCATACAACAACAA CACA - AGCTCGTC 474 4689 CAGATGACAGTGGTGCAACTGTTTTGCTTCTTCTCATACAATCAA CACA - AGCTCGTC 474 4818 AAGAGTCCTAAGGAGGCCTAGAAAACTCCCTAAGGTAAAAGTAAATGCCTTACCTCTAC 4818 AAGAGTCCTAAGGAGGCCTAGAAAACTCCCTAAGGTAAAAGTAAATGCCCTTACCTCTAC 4818 AAGAGTCCTAAGGAGGCCTAGAAAACTCCCTAAGGTAAAAGTAAATGCCCTTACCTCTAC 4818 AAGAGTCCTAAGGAGGCCTAGAAAACTCCCCTAAGGTAAAAGTAAATGCCCTTACCTCTAC 4827 CAGATGCCAGTGGTGAACAGTTTTGCTTCTTCTCACACAAGGAA CACA - AGCTCGTC 473 4830 CAGATGCCAGTGGTGAACAGTTTTGCTTCTTCTCATACAATCAA CACA - AGCTCGTC 473 4183 CAGATGCCAGTGGTGAACTGTTTTGCTTCTTCTCATACAATCAA CACA - AGCTCGTC 473 4194 CAGATGCCAGTGGTGAACTGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4185 CAGATGCCGCGTGGACACTGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4186 CAGATGGCGCGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4186 CAGATGGCGCGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4744 CAGATGGCGCGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4723 CAGGTAGCAGTGGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4723 CAGGTGACAGTGGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4723 CAGGTGACAGTGGTGAACAGTTTTGCTTCTTCTCCATACAATCAA CACA - AGCTCGTC 477 4723 CAGGTGACAGTGGTGAACTGTTTTGCTTCTTCTCATACAATCAA CACA - AGCTCGTC 477 4722	12 15 14 77 90 34 55 88 57 4 57 90 85 79 97 88 57 99 88 57 99 88 87 87 87 87 87 87 87 87 87 87 87 87
4747       A T C C AA C AA G G AA A C G C A G A C G G - C AA A C AA A C C AA G C C T G T C A C G A T T T - C C 4 80         4743       A T C C AA C G A G G A A A C G C C AA A C G G C AA A AA A	97 99 99 99 99 99 99 99 99 99 99 99 99 9
4802       CC CAGTTCGGAA CC - ATCTCAA - ACTTCTTCTGTGTGTCAACAATGACTTG 484         4798       CC CAGTTCGGAA CC - ATCTCAA - ACTTCTTCTGTGTGTCAACAATGACTTG 484         4801       CC CAGCTCGGGAA CC - ATCTCAA - ACTTCTTCTGGCGCAATAATAACTTG 484         4800       CC CAGCTCGGGAA CC - ATCTCAA - ACTTCTTCTGCGCCCAATAATAACTTG 484         4935       GC ATAAACATATTAAAATAGTGC TAATCAA - ACTTCTTCTGCGCCCAATAATAACTTG 484         4936       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCCAATAATAACTG 489         4790       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCCAATGATAATTG 439         4791       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCCAACAATAACTTG 433         4791       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCCAACAATAACTTG 434         4830       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTTG 483         4830       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTTG 483         4830       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTTG 483         4830       CC CAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTG 483         4830       C GAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTG 483         4830       C GAGCTCGGAA CC - ATCTCAA - ACTTCTTCTGCGCCAACAATAACTG 483         4830       C GAGCTCGGAA CC - ATCTCAA - ACTTCTTC	14 147 146 149 149 149 149 149 149 149 149 149 149
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4906 CT GGAAGCC GT AAAT CAT CAGT GGCT CGAGCACT GC GGAAAAGGACGT ACAT GGCGGCCCC 496 4902 CT GGAAGCC GT CAACAT CAT CAGT GGCT CGAGCACT GCGAAAAGGACGT AGAT GGCGGCGCCC 496 4904 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GCGAAAAGGAC GT AGACGGCGGCCCC 496 4950 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GCGAAAAGGAC GT AGACGGCGGCCCC 496 4950 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GCGAAAAGGAC GT AGACGGCGGCCCC 496 4950 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GCGAAAAGGAC GT AGACGGCGGCCCC 495 4895 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT AGACAGGACGT AGACGGCGGCCCC 495 4895 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4938 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4934 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4933 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4934 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4933 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4933 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4934 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 495 4935 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT ACGAAAAGGACGT AGACGGCGGCCCC 241 4995 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GGAAAAGGACGT AGACGGCGGCCCC 241 4995 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GGAAAAGGACGT AGACGGCGCCCC 241 4995 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GGAAAAGGACGT AGACGGCGCCCC 241 4995 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GGAAAAGGACGT AGACGGCGCCCC 241 4938 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACT GGAAAAGGACGT AGACGGCGCCCC 249 4938 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACC GGCAAAGGACGT AGAT GGCGGCCCC 249 4938 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACC GGCAAAGGACGT AGAT GGCGGCCCC 249 4938 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACC GGCAAAAGGACGT AGAT GGCGGCCCC 249 4937 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACC GGCAAAAGGACGT AGAT GGCGGCCCC 249 4937 CT GGAAGCC GT CAACCAT CAGT GGCT CGAGCACC GGCAAAAGGACGT AGAT GGCGGCCCC 249	51 54 53 99 53 54 57 54 53 54 55 54 55 54 55 54 55 54 55 54 55 54 55 54 55 54 55 54 55 54 55 54 55 56 55 56 56 56 57 56 56 57 56 56 57 56 56 57 56 56 56 57 56 56 57 56 56 57 56 56 56 56 56 56 56 56 56 56 56 56 56

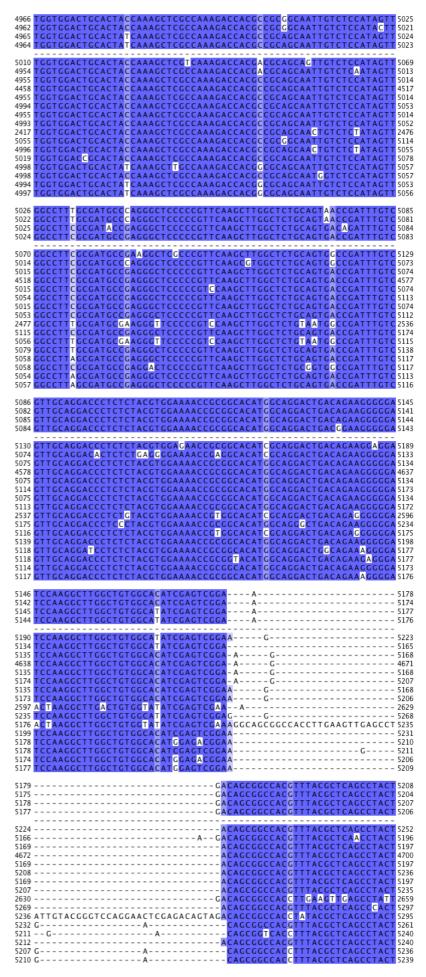
Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_E368 Epichloë\_festucae\_F11 Epichloë\_glavenise\_e7080\_ Epichloë\_glavenise\_e7080\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_ES7\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_SL05\_Poae\_Ps1 Epichloë\_uncinata\_C85\_102646\_\_

Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04362\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_mollis\_AL9324\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9324\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_foae\_Ps1 Epichloë\_tynetaacaes

Epichloë\_amarillans\_ES7\_ Epichloë\_amarillans\_E468\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachveltri\_E4804\_ Epichloë\_brachveltri\_E4804\_ Epichloë\_branicola\_AL0434\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR3 Epichloë\_festucae\_AR3 Epichloë\_festucae\_AR3 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F1 Epichloë\_festucae\_F1 Epichloë\_festucae\_F1 Epichloë\_festucae\_F1 Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924 Epichloë\_typhina\_asubsp\_poaae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_typhina\_Subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mohis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae



Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_branicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_uncinata\_C85\_102646\_\_ Epichloë\_anarillans\_E57\_ Epichloë\_aotearoae\_MYA-1228\_

Epichioē\_aotearoae\_mtA-1222\_ Epichioē\_brachveļtri\_E4804\_ Epichioē\_brachveļtri\_E4804\_ Epichioē\_bromicola\_AL0434\_ Epichioē\_bromicola\_AL04262\_ Epichioē\_festucae\_AR1 Epichioē\_festucae\_AR37 Epichioē\_festucae\_AR37 Epichioē\_festucae\_F31 Epichioē\_festucae\_F31 Epichioē\_gansuensis\_e7080\_ Epichioē\_gansuensis\_e7080\_ Epichioē\_glyceriae\_ATCC\_200747\_ Epichioē\_moblis\_AL9924\_ Epichioē\_typhina\_subsp\_poae Epichioē\_typhina\_subsp\_poae\_Ps1 Epichioē\_typhina\_subsp\_foae\_Ps1 Epichioē\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_moltis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_Subsp\_Poae Epichloë\_typhina\_Subsp\_Poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_uncinata\_CBS\_102646\_ Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mobilis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_Subsp\_PaeaPs1 Epichloë\_uncinata\_CBS\_102646\_\_

Epichloë\_uncinata\_CBS\_102646\_\_ Epichloë\_amarillans\_E54 Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mobilis\_AL9924\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae

5205 5208	GTACGGAA GTACGGGG	C <mark>G</mark> AGGAAC CAAGGAAC	TCGAGAC	AGTATTT AGTATTT	GATCCCA CGTCTCA	ATGCTCC	ATTTGTCAA ATTTGTCAA	A G C T G A A 5268 A G C T G A A 5264 A G C T G A A 5267 A G C T G A A 5266 G C T G A A 5266
5197 5198 4701 5198 5237 5198 5236 2660 5298 5296 5262 5241 5241 5237 5240	GTACCGCA GTACCGAC GTACCGAC GTACCGAC GTACCGAC GTACCGAC GTACCGAC GTACCGCAC GTACCGCC GTACCGCC GTACCGCC GTACCGCC GTACCGCC GTACCGCC GTACCGCC GTACCGCC		TCGATAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC TCGAGAC	AGTATTT AGTATTT TGTATTT AGTATTT TGTATTT TGTATTT GGTATTT AGTATTT AGTATTT AGTATTT AGTATTT AGTATTT AGTATTT		AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC AGGCTCC	ATTTGTCAA ATTCGTCAA ATTCGTCAA ATTCGTCAA ATTCGTCAA ATTCGTCAA ATTTGTCAA ATTTGTCAA ATTTGTCAA ATTTGTCAA ATTTGTCAA ATTTGTCAA ATTTGTCAA	AGCTGAA 5312 AGCTGAA 5256 AGCTGAA 5257 AGCTGAA 5257 AGCTGAA 5257 AGCTGAA 5295 AGCTGAA 5295 AGCTGAA 5295 AGCTGAA 5357 AGCTGAA 5357 AGCTGAA 5357 AGCTGAA 5350 AGCTGAA 5300 AGCTGAA 5300 AGCTGAA 5299
5265	GCCGTGCC	AGATGGT AGATGG <mark>C</mark>	CGT C GC GT	GCTCGTA	GAGCACO GAGCACO	CATTCAA CATTCAA	GACAGTAGC	TTT G 5325 TTT G 5321 TTT G 5324 TTT G 5323
5257 5258 4761 5258 5297 5258 5296 2720 5358 5356 5356 5322 5301 5301 5297	GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC GCCGTGCC	A G A T G G T ( A G A	GT AGCGT GT GGCGT GT GGCGT GT GGCGT GT GGCGT GT GGCGT GT AGCGT GT AGCGT GT AGCGT GT GGCGT GT GGCGT GT GGCGT	ACTCGTA GCTCGTA GCTCGTA GCTCGTA GCTCGTA GCTCGTA GCCCGAA GCTCGAA GCTCGAA GCTCGTA GCTCGTA GCTCGTA	GAGCACC         GAGCACC	CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA CATTCAA	GACAGTAGC GACAGTAGC GACAGTAGC GACAGTAGC GACAGTAGC GACAGTAGC GACAGTAGC	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
5322 5325	ACAGAGGG ACAGAGGG	CGGATT <mark>G</mark> CGGATTAC	GGTACAC GGTACAC	CACCAAA CACCAA <mark>C</mark>	GATGGAC GATGGAC	TTGTCCT	GATTCTTGC GATTCTTGC	A A G C G A G 5385 A A G C G A G 5381 A A G C G A G 5384 A A G C G A G 5383
5314 5315 4818 5315 5354 5315 5353 2780 5415 5416 5379 5358 5358		C G G A T T A C C G G A A T T G C G G A A T T G C G G A A T T G C G G A T T G C G G A T T G C G G A T T A C G G A T T A	GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC GGTACAC	CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA CACCAAA	GATGGAC GAAGGAC GAAGGAC GAAGGAC GAAGGAC GAAGGAC GATGGAC GATGGAC GATGGAC GATGGAC GATGGAC GAAGGAC GAAGGAC	TTGTCCT TTGTCTT TTGTCTT TTGTCTT TTGTCTT TTGTCTT TTGTCCT TTGTCCC TTGTCCCT TTGTCCT TTGTCCT TTGTCCT TTGTCCT	GATTCTTGC GATTCTTGC GATTCTTGC GATTCTTGC GATTCTTGC GATTCTTGC GATCTTGC GATCTTGC GATCTTGC GATCTTGC GATTCTTGC GATTCTTGC GATTCTTGC	A A G C G A C A A G C G A C S 374 A A G C G A G S 374 A A G C G A G S 374 A A G C G A G S 4877 A A G C G A G S 4877 A A G C G A G S 413 A A G C G A G S 413 A A G C G A G S 414 A A G C G A G S 474 A A G C C A G S 474 A A G C C A G S 474 A G C C A G S 476 S 47
5382 5385	AGAAGTGC AGAAGTGC AGAAGTGC	GAGGCTC1 GAGGCTC1 GAGGCTC1	TACGGCGT TACGGCGT TACGGTGT	CGACAAA CGACAAA CGACAAA	GTCTGGC GTCTGGC GTCTGGC	AG <mark>AAT</mark> AA AGCAGAA AGCAGAA	T CGGGGCCA CCGGAGCGA T CGGAGCGA	TCTC 5441 TCTC 5440
5374 5375 4878 5375 5414 5375 5413 2840 5475 5476 5476 5478 5418 5418 5414	AGAAGTGC AGAAATAT AGAAGTAT AGAAGTAT AGAAGTAT AGAAGTAT CGAAGTAT CGAAGTAT CGAAGTGC AGAAGTGC AGAAGTGC AGAAGTGC	GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTCT GAGGCTTT	ACGGT GT ACGGT GT		GT CT G G G GT AG G G G GT CT G G G	GGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA AGCAGAA		TC TC 5486 C 5418 TC TC 5431 TC TC 5431 TC TC 5431 TC TC 5431 TC TC 5431 TC TC 5431 TC TC 5453 TC TC 5532 TC TC 5495 TC TC 5474 TC TC 5473
5442 5442	AGTGCAGA. AGTGCAGA. AGTGCAGA.	ACTCGCG/ ACTCGCG/ ACTCGCG/	ACTTTG <mark>T</mark> T ACT <mark>CTA</mark> GT ACTTTGGT	GAA <mark>G</mark> ACA GAATGCA GAATGCA	TGGAGCO TGGAGCO TGGAGCO	ATGGAGA ATGGAGA ATGGAGA	AACCGTCGT AACCGTCGT	C A T G T T G 5505 C A T G T T G 5501 C A T G T T G 5501 C A T G T T G 5500 C A T G T T G 5500
5419 5432 5432 5471 5432 5470 2897 5532 5533 5496 5475 5475 5471	ACCECAGA ACTECAGA ACTECAGA ACTECAGA ACTECAGA ACTECAGA ACTECAGA ACTECAGA ACTECAGA AATECAGC ACTECAGA ACTECAGA ACTEGAGA	A CT C G C G A G T C G C G C A CT C G C G A A CT C G C G A	ACTTTGGT GCATAC GCTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT ACTTTGGT	AAATACA GTATATA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAATACA GAAGACA GAAGACA GAAGACA	T G G A G C C A T G C C T G C A G C C T G C A G C C T G C A G C C T G G A G C C	AT GGAGA AC GGAGA AC GGAGA AC GGAGA AC GGAGA AC GGAGA AC GGAGA AT GGAGA AT GGAGA AT GGAGA AC GGAGA AC GGAGA AC GGAGA	AACCGTCGT GA- GACCGTCGT GACCGTCGT GACCGTCGT GACCGTCGT GACCGTCGT AATCGTCGT AATCGTCGT AATCGTCGT AACCGTCGT AACCGTCGT AACCGTCGT	CATGTTC 5546 

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434 Epichloë\_fromicola\_AL04262\_ Epichloë\_fostucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brancharter Epichloë\_brancharter Epichloë\_brancharter Epichloë\_brancla\_AL0434\_ Epichloë\_brancla\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR3 Epichloë\_festucae\_AR3 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080\_ Epichloë\_typhina\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1

Epichloë\_antarillans\_E57 Epichloë\_amarillans\_E57 Epichloë\_antarillans\_E57 Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_gasuensis\_e7080\_ Epichloë\_gasuensis\_e7080\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_moliis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_formicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gansuensis\_e7080 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_CS\_102646\_

5506 GGTTCCGAACCGCCGCAATGGTATTTGACGTCTTCTAGAAAGATTCTCGCGGCAGCAAAG 5565 5502 GGTTCCGAACCGCCGCAATGGTATTTGACGTCTTCTAGAAAGATTCTCGCGCAGCAAAG 5561 5501 GGTTCCGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCAGCAACA5560
S547 GGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAAAG 5606 S467 C - CTCT A CGCTCCATT - CTCTC - CACGACC - CTGACAC CCTCCTAACA - CATG 5516 S478 CCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5528 4995 GGTTCTGAACCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5531 S531 GGTTCTGAACCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5531 S532 GGTTCTGAACCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5531 S533 GGTTCTGAACCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5531 S534 CGTTCTGAACCGCCGCAATGGTATCTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5531 S535 GGTTCTGAACCGCCGCAGTGGTATTTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAGAG 5539 S536 CGTCTGGAACCGCCGCAGTGGTATTTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAACAAC 5652 S556 CGCTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAACAAC 5652 S556 CGCTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGGCAGCAACAAG 5539 SGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCCAGCAACAAG 5539 SGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCCAGCAACAAG 5539 SGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCGCAGCAAAG 5539 SGTTCTGAACCGCCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCGCAGCAAAG 5539 SGTTCTGAACCGCCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCGCAGCAAAG 5539 SS34 GGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCGCGCGCAGCAAAG 5590 SS34 GGTTCTGAACCGCCGCAATGGTATTTGACGTCTTCTGGAAAGATTCTCTCGCAGCAGAAG 5593
5566 GCG-ACTTTTACTTGGCTCGATGCCCCCACGTGGCTTGGTGC-CG-ATCCCAGCGG 5618 5562 GCG-ACTTTTACTTGGCTCGATGCCCCCACGTGGCTTGGTGC-CG-ATCCCAGCGG 5614 5562 GCG-ACTTTTACTTGGCTCGATGCTCCCACGTGGCTTGGTGC-CG-ATCCCAGCGG 5613 5561 GCG-ACTTTTACTTGGCTCGATGCTCCCACGTGGCTTGGTGC-CG-ATCCCAGCGG 5613
5607       CCG - ACTTTTACTTGG CTCGATGCT CCCACGTGCCTGGTGC - CG - ATCCCAGCGG 5659         5517       GTGCAC CCCGCGGCACCCCATGC - CCTA - G - GCCTAGGTCC - CG - ATCCCAGCGG 5570         5529       GCG - ACTTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5581         5055       GCG - ACTTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5581         5055       GCG - ACTTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5604         5510       GCG - ACTTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5643         5525       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5643         5526       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5643         5527       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5643         5528       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5642         5507       GCG - ACTTTACTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5643         5652       GCG - ACTTTACTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5704         5635       GCG - ACTTTACTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCGG 5668         5595       GCG - ACTTTACTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAGCG 5664         5595       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGGCTTGGTGC - CG - ATCCCAACGG 5647         5595       GCG - ACTTTACTTGG CTCGATGCCCCCACGTGCGCTGGTGC - CG - ATCCCAACGG 5643
5619 TGCAAGAG - GATACGCCTACGTCGCCGCCACAGAGTAATCTACGAATTGGGAGAGAGT 5677 5615 TGCAAGAG - GATACGCCTACGTCGCCGCCACAGAGTAATCTACGAATTGGGAGAGAGT 5673 5615 TGCAAGAG - GATACGCCTACGTCGCCGCCACAGAAGAATCTACGAATTAGGAGAGAGT 5673 5614 TGCAAGAG - GATACGCCTACGTCGCCGCCACAGAAGAATCTACGAATTAGGAGAGAGT 5672
5660       TGCAAGAG - GATACGCCTACGTCGCCCCCCGGCAAGAATCTACGAATTAGGAGAGAGA
5678 CTGCAGAGGAAGAAAAGTCGGCCGCGCCCCAGGAAGTCGCCTTCGCCTCACGCT - TGCAG 5736 5674 CTGCAGAGGAAGAAAAGTCGGCCGCGCCCCAGGAAGTCGCCTTCGCCTCACGCT - TGCAG 5732 5674 CTGCAGAGG AAAAGTCGGCTGTGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5729 5673 CTGCAGAGG AAAAGTCGGCTGCCTCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5728
5719       CT GCAGAGG AAAAGTCGGCTGCCTGCCCAGGAAGTCGCCTTTGCGTCACGCT - TCCAG 5774         5625       CT TCAGTT AATATACGAGTGG CCCATACCT C TTGCGTCACGCT - TCCAG 5693         5641       CT GCAGAGGAAAAAGTCGGCTGCGTTCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5693         5167       CT GCAGAGGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5223         5703       CT GCAGAGGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5722         5703       CT GCAGAGGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5720         5704       CT GCGGAGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5720         5703       CT GCGGAGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5720         5704       CT GCGGAGGAAAAAGTCGGCTGCGCTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5720         5702       CT GCGGAGGAAAAAGTCGGCTGCGCTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5720         5702       CT GCGGAGGAAAAAGTCGGCTGCGCCCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5760         3129       CT GCAGAGG AAACGTCGACTGCGCTCCCAGGAACTCGCCTTTGCCTCACGCT - TCCAG 5760         5728       CT GCAGAGG AAGCGTCGACTGCGCTCCCAGGAACTCGCCTTTGCCTCACGCT - TCCAG 5819         5728       CT GCAGAGGAAAAAGTCGGCTGCGTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5786         5707       CT GCAGAGGAAAAAGTCGGCTGCTGCTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5785         5707       CT GCAGAGGAAAAAGTCGGCTGCTGCTCCCAGGAAGTCGCCTTTGCCTCACGCT - TCCAG 5765         5703       CT GCAGAGGAAAAAGTCGGC
5737 GATGTTCTGGCTGTCAGGCCCAGCCC GGGAGCAAGCTTCCGTCAGTTTGCCCTGGAGA 5794 5733 GATGTTCTGGCTGTCAGGCCCAGCCC GGGAGCAAGCTTCCGTCAGTTTGCCCTGGAGA 5790 5730 GATGTTCTGGCTGTCAAGCCCAGCCC GGGAGCAAGCTTCCGTCAGTTTGCCCTGGAGA 5787 5729 GATGTTCTGGCTGTCAAGCCCAGCCC GGGAGCAAGCTTCCGTCAGTTTGCCCTGGAGA 5786
5775 GATGTTCTGGCTGTCAAGCCCAGCCC - GGGAGCAAGATTCCGTCAGTTGCCCTAGACA 5832 573 - TGTTCTCACA-TCGGC - CAGCCATAAGATCTTC - TACCGC GAAACGCCTTGCCGAS 5726 5700 GATGTTCTGGCTGTCAAGCCCAGCCA - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5780 5723 GATGTTCTGGCTGTCAAGCCCAGCCC - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5832 5723 CATGTTCTGGCTGTCAAGCCCAGCCC - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5780 5723 CATGTTCTGGCTGTCAAGCCCAGCCC - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5780 5723 CATGTTCTGGCTGTCAAGCCCAGCCC - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5819 5723 CATGTTCTGGCTGTCAAGCCCAGCCC - GGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5819 5723 CATGTTCTGGCTGTCAAGCCCAGCCC - AGGAGCAAGCTTTCGTCAGTTTGCCCTGGAGA 5818 5725 CATGTTCTGGCTGTCAAGCCCAGCCC - AGGAGCAAGCTTCCGTCGTTGCCCTGGAGA 5818 5726 CATGTTCTGGCTGTCAAGCCCAGCCC - AGGAGCAAGCTTCCGTCGTTGGCCTGGGAG 5818 5727 CATGTTCTGGCTGTCAAGCCCAGCCC - AGGAGCAAGCTTCCGTCGTTGGCGTGGGAGA 5824 5728 CATGTTCTGGCTGTCAAGCCCAGCCC - AGGAGCAACCTTCCGTCGTTGGGCTGGGAGA 5878 5737 CATGTTCTGGCTGTCAAGCCCAGCCC - GGGAGCAAGCTTCCGTCGTTGGGCTGGGAGA 5844 5766 CATGTTCTGGCTGTCAAGCCCCAGCCC - GGGAGCAACCTTCCGTCAGTTGGCCTGGAGA 5823 5766 CATGTTCTGGCTGTCAAGCCCAGCCC - GGGAGCAAGCTTCCGTCAGTTGCCCTGGAGA 5823

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR38 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_mobrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae

Epichioe\_uncinata\_Cs5\_102040\_ Epichioe\_amarillans\_E57\_ Epichioe\_antarillans\_E57\_ Epichioe\_aotearoae\_MYA-1229\_ Epichioe\_brachyelytri\_E4804\_ Epichioe\_bromicola\_AL0434. Epichioe\_fromicola\_AL0434. Epichioe\_festucae\_AR3 Epichioe\_festucae\_AR37 Epichioe\_festucae\_AR38 Epichioe\_festucae\_AR48 Epichioe\_festucae\_F31 Epichioe\_festucae\_F31 Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_mobins\_AL9924 Epichioe\_typhina\_ATCC\_200747\_ Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Poae\_Ps1 Epichioe\_typhina\_subsp\_Paae\_Ps1 Epichioe\_typhina\_subsp\_Paae\_Ps1

Epichloe\_anarillans\_E57 Epichloe\_amarillans\_E57 Epichloe\_anarillans\_E57 Epichloe\_aotearoae\_MYA-1229 Epichloe\_branicola\_AL04262 Epichloe\_bromicola\_AL04262 Epichloe\_festucae\_AR1 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR37 Epichloe\_festucae\_AR48 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_festucae\_F31 Epichloe\_gasuensis\_e7080 Epichloe\_alyceriae\_ATCC\_200747\_ Epichloe\_inebrians\_MYA-1228\_ Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_typhina\_subsp\_Poae Epichloe\_uncinata\_C55\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_gasuensis\_e7080\_ Epichloë\_giveriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_C85\_102646\_

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mobrins\_MYA-1228\_ Epichloë\_typhina\_atCC\_200736\_ Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_subsp\_Poae Epichloë\_typhina\_s205\_Poae\_P31 Epichloë\_uncinata\_C85\_102646\_

5795 AT GT CCT GT AT A C CA T A C T G - T C T C A T T T T G A G G G C G A T A C T A G C 5791 AT GT C C T GT AT A C CA T A C T G - T C T C A T T T T G A G G G C G AT A C T A G C 5788 AT GT C C T T AT A C CA T A C T G - T C T C A A T T T G A G G G C G AT A C T A G C 5787 AT GT C C T GT AT A C CA T A C T G - T C T C A A T T T G A G G G C G AT A C C A G C	5832
\$833       ATGTCCTGTATACCA TACTG - TCCCAGTTTGAGGGCGATACTAG C         \$727       ATATTAAAATAACAAATAATCCCCTCCAACTCCGAATGAAT	
5840 TCATGGAGTGTGTTGTTCCGAGCTCCAGCTGGGAGTCCTTGGCAGG 5836 TCATGGAGTGTGTTGTTCCGAGCTCCAGCTGGGAGTCCTTGGCAGG 5833 TCATGGAGTATGGTGTTCCGAGCTCCAGCTGGGAGTCCTTGAGTGG 5832 TCATGGAGAATGTTGTTCCGAGCTCCAGCTGGGAGTCCTTGAGTGG	<mark>A A T T G T</mark> 5887 A C T T G T 5884
5878 TCATCGAGTATGTTGTTCCCAGGCCCAGCTGGGAGTCCGTGGATGG         5779 T TGTACGATCTTGT-CCGAGTTCCCCGGGACATTTCGTG         5803 TCATCGAGTGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5829 TCATCGAGTGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTTGTTCCCAGCTCCAGCTGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTTGTTCCCAGCTCCAGCTCGGGAGTCCTTGACAGG         5826 TCATCGAGTGTGTTGTTCCCAGCTCCAGCTCGGGAGTCCTTGACAGG         5828 TCATCGAGTGGTGTTGTTCCCAGCTCCAGCTGCGGAGTCCTTGACAGG         5828 TCATCGAGTACGTATTTTCCGAGCTCCAGCTGGGAGTCCTTGCATGG         5923 TCATGGAGTAGTTGTTGTTCCCAGCTCCAGCTGCGGGAGTCCTTGCATGG         5890 TCATCGAGTAGCTATTTCCCAGCTCCAGCTCCAGCTGGGAGTCCTTGCATGG         5869 -TC ATGGATTATATATTGTTCCCAGCTCCAGCTGCGGAGTCCTTGCATGG         5869 -TC ATGGATTATATATGTTCCCAGCTCCAGCTGCGGAGTCCTTGAGTGG         5868 TCATCGAGTTATATATATGTTCCCAAGCTCCAGCTGCGAGTCCTTGAGTGG         5868 TCATCGAGTTATATATATGTTCCCAAGCTCCAGCTGGGAGTCCTTGAGTGG         5868 TCATCGAGTTATATATATGTTCCCAAGCTCCAGCTGCGGAGTCCTTGAGTGG         5868 TCATCGAGTTATATATATGTTCCCAAGCTCCAGCTCGGAGTCCTTGAGTCGG	TTGTCTGCS830 
5892 CATTG AGTG - GAAGGATCAGG - GT - C - A - AGTTCA GATTGAGGGTTG 5888 CATTG AGTG - GAAGGATCAGG - GT - C - A - AGTTCA GATTGAGGGTTG 5885 CATTG AGTG - GAAGGATCAGG - GT - C - A - AGTTGA GATTGAGGGTTG 5884 CATTG AGTG - GAAGGATCAGG - GT - C - G - AGTTGA GATTGAGGGTTG	C A C G A G G C 5937 C A C G A G G C 5934 C A C G A G G C 5933
\$930       CATTG AGTG - GAAGGATCAGG - GT - C CAGTTGA GATTGAGGGTT         \$831       CTCTG AT AGTC C GG ACAATCTG GT GTT CTGGTGTTGCCCC C GATAAGTGT         \$855       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$878       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$878       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$878       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$878       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$917       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$918       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$919       CATTG AGTG - GAAGGATCAGG - GT - C AAGTTCA GATTGAGGGTT         \$910       CATTG AGTG - GAAGGATCAGG - GT - C AGTTGA GATTGAGGGTT         \$911       GATTG AGTG - GAAGGATCAGG - GT - C GAGTTGA GATTGAGGGTT         \$912       CATTG AGTG - GAAGGATCAGG - GT - C GAGTTGA GATTGAGGGTT         \$915       CATTG AGTG - GAAGGATCAGG - GT - C GAGTTGA GATTGAGGGTT         \$920       CATTG AGTG - GAAGGATCAGG - GT - C GAGTTGA GATGGAGGGTT         \$921       CATTG AGTG - GAAGGATCAGG - GT - C CAGTTGA GATGGAGGGTT         \$922       CATTG AGTG - GAAGGATCAGG - GT - C CAGTTGA GATGGAGGGTT	$ \begin{array}{c} CA = CA \subset CG \subset S979 \\ CA = CA \subset CT \\ S887 \\ TA \subset CG \subset CS \\ S941 \\ TA \subset CG \subset CS \\ S941 \\ TA \subset CG \subset CG \subset S942 \\ TA \subset CG \subset CG \subset S965 \\ TA \subset CG \subset CG \subset CS \\ CG \subset CG \subset CG \subset CS \\ CG \subset CG \subset CG \subset CG \\ CG \subset CG \subset CG \subset CG \\ CG \subset CG \subset CG \subset CG \\ CG \subset CG \subset CG \\ CG \subset CG \subset CG \\ CG \subset CG \\ CG \subset CG \subset CG \\ CG \\ CG \subset CG \\ CG \subset CG \\ CG \\ CG \subset CG \\ $
5942 ATCCGAGACCGGCTGACTCGTTTCTCG - GAAAGAGATTG - GGCGACGATCT 5938 ATCCGAGACCGGCTGACTCGTTTCTCG - GAAAGAGATTG - GGCGACGATCT 5935 ATCCGAGACCGGCTGGCTCGTTTTTCG - GAAAGAGATTG - GGCGACGATCT 5934 ATCCGAGACCGGCTGGCTCGTTTATCG - GAAAGAGATTG - GGCGACGATCT	T <mark>AATAATGA</mark> 5994 T <mark>AATAATGA</mark> 5991
\$980       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGACATTGGGCGACGATC         \$888       ATCGGACGCGCTCCCCGCTTCTGGACGAAAGA-ATCGTGCGCCGTTC         \$995       ATCCGACACCGGCTGACTCGTTCTCGAGAAAGAGATTGGACGACGATC         \$995       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$995       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$995       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$967       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$988       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$998       ATCCGACACCGGCTGACTCGTTTCTCGGAAAGAGATTGGACGACGATC         \$998       ATCCGACACCGGCTGACTCGTTTTCTCGGAAAGAGATTGGACGACGATC         \$998       ATCCGACACCGGCTGCTCCTTTTCCGGAAAGAGATTGGACGACGATC         \$998       ATCCGACACCGGCTGGCTCGTTCTTCCGGAAAGAGATTGGACCACGATC         \$992       ATCCGACACGGCCTGCTCCTTTTTCCGGAAAGAGATTGGCCCACGATC         \$992       ATCCGACACCGCCTGCTTGCTTTTTCCGGAAAGAGATTGGCCCACGATC         \$992       ATCCGACACCGCCTGCTTGCTTTTTCCGCAAAGAGATTGGCCCACGATC         \$992       ATCCGACACCGCCTGCTTGTTTTTCCGGAAAGAGATTGGCCCACGATC         \$992       ATCCGACACCGCCTGCTTGCTTGTTTTCTCGCAAAGAGATTGGCCCACGATC         \$992       ATCCGACACCGCCTGCCTTGCTTGTTTCTCG	C A T A A T G A 6036 C A T A A T G A 5951 C A T A A T G A 5951 C A T A A T G A 5951 C A T A A T G A 5954 C A T A A T G A 6023 C A T A A T G A 6022 C A T A A T G A 6042 C A T A A T G A 6081 C A T A A T G A 6082 C A T A A T G A 6082 C A T A A T G A 6042 C A T A A T G A 6042
5999 TGGAAATTTTGA - CTGGTCGCTTCTTAAAAAT TTCCAGAGGA - C 5995 TGGAAATTTCCA - CTGGTCGCTTCTTAAAAAT TTCCAGAGGA - C 5992 TGGAAATTTCGA - CTGGCCGCTTCTTGAAAAT TTCCAGAGGA - C 5991 TGGAAATTTCGA - CTGGTCGCTTCTTGAGGAT TTCCAGAGGA - C	C - GTTATG 6044 C - GTTATG 6041 C - GTTATG 6040
6037 T G G A A A T T T C G A - C T G G T C G C T C T T G A A A A T T T C C A G A G G A - C 5940 C C T G C C A A G G C A T C T G A G G C C T T G T A A G T G T G G T T A C A T T G G A G C T T A 5962 - T G G A A A T C T C G A - C T G G T C G T T C T T A A A A A T - T T C C A G A G G C 5985 - T G G A A A T A T C C A - C T G G T C G T T C T T A A A A A T - T T C C A G A G G C 6024 - T G G A A A T A T C C A - C T G G T C G T T C T T A A A A A T - T T C C A G A G G C 5985 - T G G A A A T A T C C A - C T G G T C C T T C T T A A A A A T - T T C C A G A G G C 6024 - T G G A A A T A T C C A - C T G G T C C C T C T T A A A A A T - T T C C A G A G G C 5985 - T G G A A A T C T C G A - C T G G T C C C T T C T T A A A A T - T T C C A G A G G C 6023 - T G G A A A T C T C G A - C T G G T C C C T C T T A A A A T - T T C C A G A G G C T 6043 - T G G A A A T T C G A - C T G G T C C C T T C T T G A G A G T - T T C C A G G G C C T 6083 - T G G A A A T T C G A - C T G G T C C C T T C T T G A G G G T - T T C C A A G C C A 6049 - T G G A A A T T C G A - C T G G T C C C T T C T T G A G A G T - T T C C A A G C C A 6049 - T G G A A A T T C G A - C T G G T C C C T T C T T A C A A A T - T T C C A A G G C A 6049 - T G G A A A T T C G A - C T G G T C C C T T C T T A C A A A T - T T C C A A G C C A 6043 - T G G A A A T T A C A - C T G G T C C T T C T T T G A A A T - T T C C A A G C A A T 6043 - T G G A A G T T A C A - C T G G T C C T T C T T T G A A A T - T T C C A G C G A C T 6043 - T G G A A G T T A C A - C T G G T C C T T C T T T G A A A T - T T C C A G C G A C T 6024 - T G G A A G T T A C A - C T G G C C C T T C T T G A A A T - T T C C A G C G A C T 6027 T G G A A G C T C G A - C T G G C C C T T C T T G A A A T - T T C C A G C G A C T 6027 T G G A A G C T C G A - C T G G C C C C T T C A T A A A A A T - T T C C A G C C A T	C - CTTATC 6086 GTCACATA 5997 C - CTTATC 6011 C - CTTATC 5537 C - CTTATC 6034 C - CTTATC 6034 C - CTTATC 6034 - CCTTATC 6034 - CCTTATC 6034 - CCTTATC 6131 TCCCTATC 6131 TCCCTATC 6131 TCCCTATC 6132 C - CTTATC 6098 - CCTTATC 6098 - CCTTATC 6098 - CCTTATC 6098

Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atoeraoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_E3368 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_festucae\_F31 Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mohis\_AL9924\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae\_Ps1 Epichloë\_typhina\_SE57

Epichloë\_uncinata\_CBS\_102646\_\_ Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atoearoae\_MYA-1229\_ Epichloë\_brachyelytri\_E4804\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_fromicola\_AL04262\_ Epichloë\_festucae\_AR1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_F01 Epichloë\_festucae\_F01 Epichloë\_festucae\_F10 Epichloë\_festucae\_F10 Epichloë\_festucae\_F10 Epichloë\_festucae\_F10 Epichloë\_festucae\_AR1 Epichloë\_festucae\_F10 Epichloë\_festucae\_AR1 Epichloë\_festucae\_F10 Epichloë\_festucae\_F10 Epichloë\_molis\_AL9924\_ Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_typhina\_subsp\_poae Epichloë\_uncinata\_CBS\_102646\_\_

6045 GATCC-TTCGTGTGTGATT 6042 GACCC-TTCGTGTGTGATT	- CGGTTCACTG ATAAGCTACT GACCGTGGGCA 6097 - CGGTTCACTG ATAAGCTACT GACCGTGGGCA 6093 - CGGTTCACTG ATAAGCTACT GACCGTGGGCA 6090 - CGGTTCACTG ATAAGCTACT GACCGTGGGCA 6089
5998         GATTC         ATACGGATTACTTCCTT           6012         GATCC         TTCG	
6098 ATGGCAGCGCTGGAAATCGGT- 6094 ATGGCAGCGCTGGAAATCGGT- 6091 ATGGGAGCGCTGGAAATCGGT- 6090 ATGGGGGCGCTGGAAATCGGT-	AG 6116 AG 6113
6136 AT G C C A C C C T G G A A T A C C T 6058 A A C C A A C C A T G A T C C T G G A T G 6061 AT G C G A C G A T G A A T T C A C T 5587 AT G C G A C C A T G A A T T C A C T 6084 AT G C G A C C A T G A A T T C A C T 6123 AT G C G A C C A T G A A T T C A C T 6124 AT G C G A C C A T G A A T T C A C T 6125 AT G C G A C C A T G A A T T C A C T 6126 AT G C A C C A T G A A T C A C T 6127 AT G C G C C C C A G C A A T C A C T 6148 AT G C G C C C C C A G G A A T C A C T 6148 AT G C G C C C C C A G G A A T C A C T 6148 AT G C G C C C C C A G G A A T C A C T 6148 AT G C G C C C C T G G A A T C A C T 6142 G T A T C G C C C C T G G A A T C A C T 6142 G T A T C G C C C C T G G A A T C A C T 6142 G T A T C G C C C C T G G A A T C A C T 6142 G T A T C G C C C C T G G A A T C A C T 6126 AT G C G C C C C T G G A A T C A C T 6126 AT G C G C C C C T G G A A T C A C T 6126 AT C G C C C C C T G G A A T C A C T 6126 AT C G C C C C C T G G A A T C A C T 6126 AT C G C C C C C T G G A A T C A C T 6126 AT C G C C C C C T G G A A T C A C T 6126 AT C G C C C C C T G G A A T C A C T 6126 AT C G C C C C C C T G G A A T C A C T 6126 AT C G C C C C C C T G G A A T C A C T 6126 AT C G C C C C C C T G G A A T C A C T 6126 AT C G C C C C C C T G G A A T C A C T 6126 AT C G C A C C C C T C G A A T C A C T 6126 AT C C C A C C C C T C G A A T C A C T 6126 AT C C C A C C C C T C G A A T C A C T 6126 AT C C C A C C C C T C G A A T C A C T 6126 AT C C C A C C C C T C C C A A T C A C T 6126 AT C C C A C C C C T C C C A A T C A C T 6126 AT C C C A C C C C T C C C T C C C A A T C A C T 6126 AT C C C A C C C C C T C C C A A T C A C T 6126 AT C C C A C C C C C C C C C C C C C C	AC         6081           AC         6083           AC         6083           AC         6106           AC         6145           AC         6144           AC         3568           AC         6203           AC         6203           AC         6149           AC         6149           AC         6144           AC         6149           AC         6144           AC         6145

Appendix 20: Alignment of makes caterpillars floppy (*mcf*) genes from a range of *Epichloë* strains across multiple species using the alignment function of Geneious. The *E. gansuensis* sequence is cut short due to loss of sequence identity.

Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_E3368 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR1 Epichloë\_amarillans\_E57\_ Foichloë\_amarillans\_E6668 Epichloē\_amarillans\_E57\_ Epichloē\_amarillans\_E4668\_ Epichloē\_aotearoae\_ Epichloē\_brachyelytri\_ Epichloē\_bromicola\_AL0434\_ Epichloē\_bromicola\_AL04262\_ Epichloē\_gromicola\_AL04262\_ Epichloē\_glyceriae\_ATCC\_200751\_ Epichloē\_glyceriae\_ATCC\_200747\_ Epichloē\_inebrians\_MYA-1228\_ Epichloē\_mollis\_AL9924\_ Epichloē\_typhina\_ATCC\_200736\_ Epichloē\_typhina\_subsp\_poae\_E5819\_ Epichloē\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë, typhina, subsp. Poo Epichloë, festucae, AR48 Epichloë, festucae, AR48 Epichloë, festucae, Eg1 Epichloë, festucae, E2368 Epichloë, festucae, AR37 Epichloë, festucae, AR37 Epichloë, amarillans, E4668 Epichloë, acateareae Epichloē\_amarillans\_E4668\_ Epichloē\_aotearoae\_ Epichloē\_brachyelytri\_ Epichloē\_brachyelytri\_ Epichloē\_bromicola\_AL0434\_ Epichloē\_bromicola\_AL04262\_ Epichloē\_elymi\_ATCC\_201551\_ Epichloē\_gloveriae\_ATCC\_200747\_ Epichloē\_gloveriae\_ATCC\_200747\_ Epichloē\_mollis\_AL9924 Epichloē\_mollis\_AL9924 Epichloē\_typhina\_subsp\_.poae\_E5819\_ Epichloē\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichioë\_estucae\_AR48 Epichioë\_festucae\_AR48 Epichioë\_festucae\_Fg1 Epichioë\_festucae\_Fg1 Epichioë\_festucae\_E2368 Epichioë\_festucae\_AR37 Epichioë\_festucae\_AR37 Epichioë\_amarillans\_E4668\_ Epichioë\_atearcae Epichloē\_amarillans\_E4668\_ Epichloē\_aotearoae\_ Epichloē\_brachyelytri\_ Epichloē\_brachyelytri\_ Epichloē\_bromicola\_AL0434\_ Epichloē\_bromicola\_AL04262\_ Epichloē\_elymi\_ATCC\_201551\_ Epichloē\_gloveriae\_ATCC\_200747\_ Epichloē\_gloveriae\_ATCC\_200747\_ Epichloē\_mollis\_AL9924 Epichloē\_mollis\_AL9924 Epichloē\_typhina\_subsp\_poae\_E5819\_ Epichloē\_uncinata\_C8S\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E4668 Epichloë\_amarillans\_E4668 Epichloë\_atearoae Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_elymi\_ATCC\_201551\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_Z368 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR1 Epichloë\_amarillans\_E4668 Epichloë\_amarillans\_E4668 Epichloē\_amarillans\_E4668\_ Epichloē\_actearoae\_ Epichloē\_brachyelytri\_ Epichloē\_brachyelytri\_ Epichloē\_bromicola\_AL0434\_ Epichloē\_gelymi\_ATCC\_201551\_ Epichloē\_glymi\_ATCC\_2005747\_ Epichloē\_glyceriae\_ATCC\_200747\_ Epichloē\_inebrians\_MYA-1228\_ Epichloē\_inebrians\_MYA-1228\_ Epichloē\_mollis\_AL9924\_ Epichloē\_mollis\_AL9924\_ Epichloē\_typhina\_ATCC\_200736\_ Epichloē\_typhina\_subsp\_poae\_E5819\_ Epichloē\_uncinata\_C85\_102646\_

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Epichloë\_uncinata\_CBS\_102646\_ Epichloë\_trybhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachvelytri Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200736\_ Epichloë\_trybhina\_subsp\_poae\_E5819\_ Epichloë\_trobina subsp\_Poae\_Ps1

Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_F11 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_antarillans\_E57 Epichloë\_antarillans\_E57 Epichloë\_antarillans\_E5668 Epichloë\_antearoae Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_

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<ul> <li>Hand DK EERG FTAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS SCYNAVES VE I P GYEH 402</li> <li>STARKEE RG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 398</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 398</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 393</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 399</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 399</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 399</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 399</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 399</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 398</li> <li>SG KEERG FAAVVREGEHRGTP LGGT AGGR LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 402</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS RCYNYT SS VEI SGYEH 416</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS TGYKT YG SVEI SGYEH 416</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS TGYKT YG SVEI SGYEH 416</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS TGYKT YG SVEI SGYEH 416</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS TGYKT YG SVEI SGYEH 420</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE FS TGYKT YG SVEI SGYEH 421</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI SGYEH 423</li> <li>SG KEERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI SGYEH 436</li> <li>SG MK ERG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI SGYEH 432</li> <li>SG KERKG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI SGYEH 439</li> <li>SG KERKG FAAVVREGEHRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI SGYEH 433</li> <li>SG KERKG FAAVVREGE HRGAP LGGT AGER LS EGR FR EL LACDNE SGYNATS SVEI S</li></ul>
<ul> <li>359 QK I RKGF FAVV RG I GLQAP LGG TAGER LSEQR FS DLLAGDNEF STG Y EKYGSY EI SCY EH 432</li> <li>342 QK E ERGF AAVV REGEHRQT P LGG TAGQR LSEQR FRELLAGDNDF SSG YN AY ESY EI P CYEH 422</li> <li>403 DMT LSE I VQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SI WQMT NDV VAM FQ EMGGYTK 463</li> <li>399 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 459</li> <li>399 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 459</li> <li>310 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 451</li> <li>311 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 311</li> <li>315 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 459</li> <li>400 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 459</li> <li>400 DMT LSE MVQLF DR SST SL TQ T EQGALLRR I DLARK LF NE SVWQK TNDV I AM FQ EMGGYTK 459</li> <li>400 DMT LSE MVQLF DR SST SL TQ T ELGALLRR VD LARK LF NE SVWQK TNDV I AM FQ EMGGYTK 450</li> <li>417 DMT LSE MVQLF DR SST SL TQ T ELGALLRR VD LARK LF NE SI WQK TNDV I AM FQ MGGYTK 450</li> <li>418 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 457</li> <li>419 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 457</li> <li>410 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 477</li> <li>412 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 478</li> <li>430 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 450</li> <li>441 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 478</li> <li>453 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 451</li> <li>445 DMPQ DLLHA I PD EYGGG CY PLVY AM SVALAS S DF A I DLACAK LVCL SP NNAADMK 520</li> <li>350 DMT LSE MVQLF DR SST SL TQ T ELGALLRR I DLARK LF NE SI WQK TNDV I AM FQ MGGYTK 453</li> <li>446 DMPQ DLLHA I PD EYGG</li></ul>
<ul> <li>403 DMT LS EI VQL F DR SST S LTQ T EQGALLRR I DLARK KL F NE S I WQMT ND VVAM FQEMGGYTK 453</li> <li>399 DMT LS EM VQL F DR SST S LTQ T EQGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 459</li> <li>251 DMT LS EM VQL F DR SST S LTQ T EQGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 311</li> <li>255 DMT LS EM VQL F DR SST S LTQ T EQGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 460</li> <li>400 DMT LS EM VQL F DR SST S LTQ T EQGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 460</li> <li>400 DMT LS EM VQL F DR SST S LTQ T EQGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 460</li> <li>400 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 460</li> <li>400 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SVWQKT ND VI AMF QEMGGYTK 476</li> <li>400 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 476</li> <li>400 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 477</li> <li>417 DMT LS MVQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 477</li> <li>418 DMT LS MVQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 477</li> <li>419 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 477</li> <li>410 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 477</li> <li>410 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 478</li> <li>420 DMT LS EM VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 480</li> <li>231 DMT LS EI VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 480</li> <li>233 DMT LS EI VQL F DR SST S LTQ T ELGGALLRR I DLARK EL F NE SI WQKT ND VI AMF QEMGGYTK 463</li> <li>464 PMPQ DI LLMAI PD EY GGG C Y PL VY AM SVALAS S O FAI EQ LCAK L VGL S P NNAADMK 516</li> <li>465 PM M D DI LLMAI PD EY GGG C Y PL VY AM SVALAS S O FAI EQ LCAK L VGL S P NNAADMK 516</li> <li>460 PM M D DI LLMAI PD EY GGG C Y PL</li></ul>
<ul> <li>270 DMT LS EM I K LFDR SST S LTQT EQGALLRR I ELARK DLF NE S VWQKTNDV I AMFQEMGGYTK 480</li> <li>233 DMT LS EI VQLFDR SST S LTQT EQGALLRR I DLARK ELFNE S I WQKTNDV VAMFQEMGGYTK 480</li> <li>403 DMT LS EI VQLFDR SST NLTQT EQGALLRR I DLARK ELFNE SI WQKTNDV VAMFQEMGGYTK 463</li> <li>464 PMPQD I LLAA I P DKYGGGR CYP LVYAMSVALASS DFA I DALCAK LVGLSP NNAADMK 520</li> <li>460 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNAADMK 516</li> <li>460 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>312 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>312 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>314 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>461 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>461 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>461 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>461 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I EQLCAK LVGLSP NNQADMK 516</li> <li>462 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNAADMK 516</li> <li>463 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNSADMK 516</li> <li>464 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNSADMK 513</li> <li>478 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNSADMK 534</li> <li>479 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNSADMK 534</li> <li>479 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNAADMK 534</li> <li>479 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNAADMK 534</li> <li>479 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNAADMK 534</li> <li>479 PMPQD I LLHA I P DEYGGGR CYP LVYAMSVALASS DFA I DQLCAK LVGLSP NNAADMK 534</li> <li>479 PMPQD</li></ul>
464 PMPQDILLNAIPDKYGGGRCYPLVYAMSVALASS NFAIDALCAKLVGLSPNNAADMK 520 460 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNAADMK 516 460 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNAADMK 516 312 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS NFAIEQLCAKLVGLSPNNAADMK 368 316 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS NFAIEQLCAKLVGLSPNNAADMK 516 461 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNAADMK 516 461 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNAADMK 516 461 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNAADMK 516 461 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIEQLCAKLVGLSPNNESDMK 517 460 PMPQDILLHAIPDEYGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 517 461 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 517 460 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 516 477 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 513 478 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 534 479 PMPQDILLHAIPDEHGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 534 478 PMPQDILLHAIPDEHGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 534 478 PMPQDILLHAIPDEHGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNESDMK 534 478 PMPQDILLHAIPDEHGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNEADMR 534 331 PMPQDILLHAIPDEHGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNEADMK 537 294 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS DFAIDQLCAKLVGLSPNNAADMK 537 294 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS NFAIDQLCAKLVGLSPNNAADMK 537 294 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASS NFAIDQLCAKLVGLSPNNAADMK 530 521 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGCTK 577 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGCTK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGCTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGCTK 578 518 NAKLLKRCLEDLHTSYPAAEASRP
478 PMPQDILLHAVPDEYGGGRCYPLVYAMSVALASSDFAIDQLCAKLVGLSPNNEADMR 534 331 PMPQDILLRAVPDEHGGGRCYPLVYAMSVALASSDFAIDFVIDQLCAKLVGLSPNNEADMR 534 481 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASSFFAIDQLCAKLVGLSPNNAADMK 537 294 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASSNFAIDQLCAKLVGLSPNNAADMK 537 464 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASSNFAIDQLCAKLVGLSPNNAADMK 520 521 NAELLKRCLEDLHTSYPAAEASRPIGNMTLAEAVSVLEKSTETTTLAMHTEVHAMLLGLK 577 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 577 518 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 429 369 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 429 373 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 577 516 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 577 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTILAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLESSTETTILAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLESSTETTILAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLESSTETTILAMHTEVHAMLLGLK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLESSTETTILAMHTEVHAMLLGLK 577
464 PMPQDILLHAIPDEYGGGRCYPLVYAMSVALASSNFAIDQLCAKLVGLSPNNAADMK 520 521 NAELLKRCLEDLHTSYPAAEASRPIGNMTLAEAVSVLEKSTETTTLAMHTEVHAMLLGTK 581 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 577 516 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 429 369 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 429 373 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 429 374 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 433 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGTK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTILAMHTEVHAMLLGTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVGMLEKSTETTILAMHTEVHAMLLGTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTILAMHTEVHAMLLGTK 578 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLEKSTETTILAMHTEVHAMLLGTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLEKSTETTILAMHTEVHAMLLGTK 578
517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 577 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 429 369 NAVLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 429 373 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 433 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 577 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLERSTETTTLAMHTEVHAMLLGLTK 578 518 NAKLLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLEKSTETTILAMHTEVHSMLLGLTK 578 517 NAELLKRCLEDLHTSYPAAEASRPIGNMTLEEAVSMLEKSTETTILAMHTEVHSMLLGLTK 578
514 NAELLKRCLEDLHT SYPAAEASRP I GNMTLEEAV SVLEKST ETTTLAMHT EVHAMLLGLTK 574 535 NSKLLKRCLEDLHT SYPAAEASRP I GKMTLEEAV SMLEKST ETTTLAMHT EVHAMLLGLTK 595 536 NAKLLKRCLEDLHT SYPAAEASRP I GKMTLEEAV SMLEKST ETTTLAMHT EVHAMFLGLTK 597 537 NAKLLKRCLEDLHT SYPAAEASRP I GNMTLEEAV SWLEKST ETTTLAMHT EVHAMLLGTK 577 538 NAQLFKRCLEDLHT SYPAAEASRP I GNMTLEEAV SVLEKST ETTTLAMHT EVHAMLLGVTK 595 392 NAELLKRCLEDLHT SYPAAEASRP I GNMTLEEAV SILEKST ETTTLAMHT EVHAMLLGVTK 595 338 NAQLLKRCLEDLHT SYPAAEASRP I GNMTLEEAV SVLEKST ETTTLAMHT EVHAMLLGVTK 598 351 NAELLKRCLEDLHT SYPAAEASRP I GNMTLEEAV SVLEKST ETTTLAMHT EVHAMLLGVTK 581

Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_E2368 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR1 Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_atearoae Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_elymi\_ATCC\_201551\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mollis\_AL9924 Epichloë\_mollis\_AL9924 Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_R48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Z368 Epichloë\_festucae\_A87 Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_ganzensource\_Z01551\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_ganebrans\_MYA-1228\_ Epichloë\_ganebrans\_MYA-1228\_ Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp.Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_R18 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR1 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_ Epichloë\_aotearoae\_ Epichloë\_bachvelvtri Epichloë\_aotearoae\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_drymi\_ATCC\_201551 Epichloë\_gansuensis\_e7080 Epichloë\_gansuensis\_e7080 Epichloë\_mebrians\_MYA-1228\_ Epichloë\_mollis\_AL9924 Epichloë\_mollis\_AL9924 Epichloë\_typhina\_stubsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_L2368 Epichloë\_festucae\_AR37 Epichloë\_estucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_actearoae\_ Epichloë\_aotearoae\_ Epichloë\_brachyelytri\_ Epichloë\_brachyelytri\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_dromicola\_AL04262\_ Epichloë\_glyceriae\_201551\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_mollis\_AL9924\_ Epichloë\_typhina\_Subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Z368 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR1 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_aotearoae\_ Epichloë\_aotearoae\_ Epichloë\_brachyelytri, Epichloë\_brachyelytri\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_elymi\_ATCC\_201551\_ Epichioe\_etymi\_AICC\_201551\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_gansuensis\_e7080\_ Epichioe\_inebrians\_MYA-1228\_ Epichioe\_inoplins\_AL9924\_ Epichioe\_typhina\_AISP\_poae\_E5819\_ Epichioe\_typhina\_s045p\_poae\_E5819\_ Epichioe\_inoplina\_s045p\_102646\_

582 TR NST SWHFY DP NFA I VT FNS GEALLEAATK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 430 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 430 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 430 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 579 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 579 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 579 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 579 SG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 596 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 597 SG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 596 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GQ TP SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F 578 NG NST SWHFY DP NFA I AT FK SG EALLEATTK FF EE SG FAK VY EAQ GY P SF DF F	R I DAERV 638 R I DAERV 490 R I DAERV 490 R I DAERV 490 R I DAERV 494 R I DAERV 638 H I DAERV 639 R I DAERV 638 R I DAERV 638 R I DAERV 638 C I DAERV 656 C I DAERV 657 C I DAERV 657 C I DAERV 657
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657 SRIGFDHNLTVADLVQPETLLETIASEHKTATFIQDPAQLTASRAFSAQTELLE 514 SGIILDYNLTVADLVRPETLLETVASQQQTATFIQDPAQLTASDAFSAQTELLE 660 SRIGFDHNLTVADLVEPETLLETIASEHKRATFVQDPAQLTSSRNFSAQTELLE 473 SRIGFDHNLTVADLVEPDTLLETIAADRKTATLYHDPAQLTSSRTFSAQTELLE	TLGLGEA 574
643 LASDLTTT	650
704 AWR DATAR LEEST GG EHWMP I LET LK EGG EEGSY EVQ F I NLKNKN ET KWIST E 700 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKGSY EVQ F I NLKNKN ET KWIST E 520 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKGSY EVQ F I NLKNKN ET KWIST E 552 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKN ET KWIST E 554 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKN ET KWIST E 556 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKN ET KWIST E 700 AWH DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKN ET KWIST E 701 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKN ET KWIST E 701 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLNKNG TKWIST E 701 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLNKNG TKWIST E 700 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLNKNG TKWIST E 700 AWR DATAR LEESL GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 718 AWR DATAR LEESL GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E 719 AWR DATAR LEEST GMG EHWMP I LET MK EGG EKG SY EVQ F I NLKNKNET KWIST E	S P A I K D F 760 S P A I K D F 760 S P A I K D F 612 S P A I K D F 612 S P A I K D F 616 S P A I K D F 761 S P A I K D F 761 S P A I K D F 761 S P A I K D F 757 C H A I K D F 778 S H A I K D F 778 S P A I K D F 760
718 AWR DATAR LEESTGVGEHWMP I LETMK EGGEKGSY EVQ F I NLKNK NETKWI STE 575 AWR DATAR LEESTGMGEHWMP I LETMR EGDEKGSY EVQ F I NLKNK NETKWI STE 721 AWR DATAR LEESTGMGEHWMP I LETMK EGGEKGSY EVQ F I N I ENK NETKWI STE 534 AWR DATAR LEESLGI GEHWMP I LETLK EGGEEGSY EVQ F I NLKNM ETKWI STE	SPAIKDF 778 SPAIKDF 635 SPAIKDF 781 SPAIKDF 594
765 KAR LDEHLETLSKTHEFESGSFMREENLAHAEAIDGLNAMFIMRTLIEHFAGKK 761 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 761 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 613 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 614 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 617 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 618 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 761 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 762 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 763 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 764 KARLDEHLETLSKTYEFESGSFMRKENLAYAEAIDGLNAMFIVRTLIEHFAGKK 765 KARLDEHLETLSKTYEFESGSFMRKENLAYAEAIDGLNAMFIVRTLIEHFAGKK 765 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGLNAMFIVRTLIEHFAGKK 778 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGCNAMFIVRTLIEHFAGKK 778 KARLDEHLETLSKTYEFESGSFMRKENLAHAEAIDGCNAMFIVRTLIEHFAGKK 760 KARLDEHLKTLSKTYEFESGSFMRKENLAHAEAIDGCNAMFIVRTLIEHFAGKK	T E E S T - 819 T E E S T - 819 T E E S T - 671 T E E S T - 671 T E E S T - 671 T E E S T - 819 T E E S K - 820 T E E S K - 820 T E E S K - 819 T E E S K - 811 T E E S K - 838 T E E S K - 831
779 KAR LDEHLGT LSKTY EFESGSFMRKENLAHAEA I DGLNAMLI VRALI EHFSGKK 636 KAR LDKHLKSLSETY EFESGSFMRKENLPHAEA I DGLNAMS I VQT LI EHFSSKE 782 KAR LDEHLETLSKTY EFESGSFMRKENLEHAEA I DGLNAWFI VRT LI EHFTAKK 595 KAR LDEHLETLSKTHEFESGSFMREENLAHAEA I DGFNAMFI VRT LI EHFAGKK	TEESK 694 TEEST 840 TEESKKS 655
824 - T NA E LADA LK I H SY L N L TQMGQQ T L G D V G KM V N L V K D M L E T GQ V A K S S L T T L V 820 - T NA E LADA LM I H SY L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 820 - T NA E LADA LK I H SF L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 672 - T NA E LADA LM I H SY L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 672 - T NA E LADA LM I H SY L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 676 - T NA E LADA LM I H SY L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 820 - T NA E LADA LM I H SY L N L TQ I G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 821 - T NA D L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 821 - T NA D L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V N L V K D M L E T GQ V A K S S L ST L V 820 - T NA E LADA LK I H SY L N L TQ M G HQ T L G D V G KM V D L V K D M L E T GQ V A K S S L ST L V 821 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V D L V K D M L E T GQ V A K S S L ST L V 838 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V D L V K M L E T GQ V A K S S L ST L V 839 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V D L V K M L E T GQ V A K S S L ST L V 839 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V N L V K M L E T GQ V A K S S L ST L V 830 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G KM V N L V K M L E T GQ V A K S S L ST L V 831 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G K M V L V K M L E T GQ V A K S S L ST L V 841 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G K M V L V K M L E T GQ V A K S S L ST L V 695 - T N Q L ADA LK I H SY L N L TQ M G HQ T L G D V G K M V L V K M L E T G Q V A K S S L ST L V 641 - T NA E L ADA LK I H SY L N L TQ M G HQ T L G D V G K M V L V K M L E T G Q V A K S S L ST L V 656 K T NA E L ADA LK I H SY L N L TQ M G Q T L G D V G K M V L V K D M L E T G Q V A K S S L ST L V 656 K T NA E L ADA LK I H SY L N L T	/ K G L G N A S 879 / K G L G N A S 879 / K G L G N A S 731 / K G L G N A S 731 / K G L G N A S 735 / K G L G N A S 879 / K G L G Y A S 880 / K G L G Y A S 879 / K G L G Y A S 879 / K G L G Y A S 897 / K G L G Y A S 898 / K G L G Y A S 897 / K G L G Y A S 879 / K G L G Y A S 754 / K G L G N A S 754 / K G L G N A S 754 / K G L G N A S 716

Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_E368 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668 Epichloë\_aotearoae\_ Epichloë\_aotearoae\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_gansuensis\_e7800\_ Epichloë\_gansuensis\_e7800\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_ATCC\_200745\_ Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_

Epichloë\_uncinata\_C85\_102646\_ Epichloë\_festucae\_AR48 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F11 Epichloë\_festucae\_F13 Epichloë\_festucae\_R37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachcyelytri\_ Epichloë\_bromicol\_AL0434\_ Epichloë\_bromicol\_AL0434\_ Epichloë\_glyceriae\_ATCC\_201551\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_

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Epichloë\_uncinata\_C85\_102646\_ Epichloë\_festucae\_AR48 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F11 Epichloë\_festucae\_F13 Epichloë\_festucae\_R37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachcyelytti Epichloë\_bromicol\_AL0434\_ Epichloë\_bromicol\_AL0434\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_typhina\_subsp\_poae\_P51

Epichloë\_uncinata\_C85\_102646\_ Epichloë\_tryphina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fl1 Epichloë\_festucae\_Fl3 Epichloë\_festucae\_R37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_amarillans\_E4668\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromicola\_AL04262\_ Epichloë\_bromics\_ATCC\_200747\_ Epichloë\_moliis\_AL9924\_ Epichloë\_tryphina\_ATCC\_200736\_ Epichloë\_uncinata\_C85\_102646\_ Epichloë\_uncinata\_C85\_102646\_

Epichloë\_uncinata\_CBS\_102646\_ Epichloë\_trybhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_F11 Epichloë\_festucae\_F13 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E57\_ Epichloë\_amarillans\_E4668\_ Epichloë\_brachvelytti Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_inebrians\_MYA-1228\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_uncinata\_CBS\_102646\_ Epichloë\_uncinata\_CBS\_102646\_

Epichloë\_typhina\_subsp\_Poae\_Ps1 Epichloë\_festucae\_AR48 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_Fg1 Epichloë\_festucae\_F11 Epichloë\_festucae\_AR37 Epichloë\_festucae\_AR37 Epichloë\_marillans\_E57\_ Epichloë\_amarillans\_E568\_ Epichloë\_marillans\_E668\_ Epichloë\_brachyelytri\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_bromicola\_AL0434\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_gansuensis\_e7080\_ Epichloë\_glyceriae\_ATCC\_200747\_ Epichloë\_typhina\_ATCC\_200736\_ Epichloë\_typhina\_subsp\_poae\_E5819\_ Epichloë\_uncinata\_C85\_102646\_

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1364 FWIRMSDRTV 1509 FWIRMSDRTV 1326 FWIRMSDRTV	I KP HP D I H LNY L LVGT I A ST DGR G EQ F C F F I KAHP D I H LDY L LVG I LNST DG S G EQ F C F F I KP HP D I H LEY L LVGT I A ST DG S D EQ F C F F I KP HP D I H LNY L LVGT I A ST G D S G E L F C F F	SHKEHKLVIQQGNGKQAKEPR 1424 SYKEHKLVIQQGNGKQAKKPR 1569 SYNQHKLVVQRGNGTQAEKPR 1386
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1732 DGRGVLEKHP 1607 DGRSVPELHP 1734 DGRGVLVEHP 1551 DGRGVLVEHP	FKTVALT – EGGLRYTTKDGVVLI LASER SA FKTVALT – EGGLRYTTKDGVVLI LASER SA FKTVALT – EGGLRYTTKDGVVLI LASER SA FKTVALT – EGGLRYTTKDGVVLI LASER SA FKTVALT – EGGLRYTTKEGVVLI LASER SA	R LYGVDKVWQQNRSDLS-AEL 1790 R LYGVDKVWQQNQSDVN-AAL 1666 R LYGVDKVWQQNRSDLS-AEL 1792 R LYGVDKVWQQNQSDLS-GEL 1609

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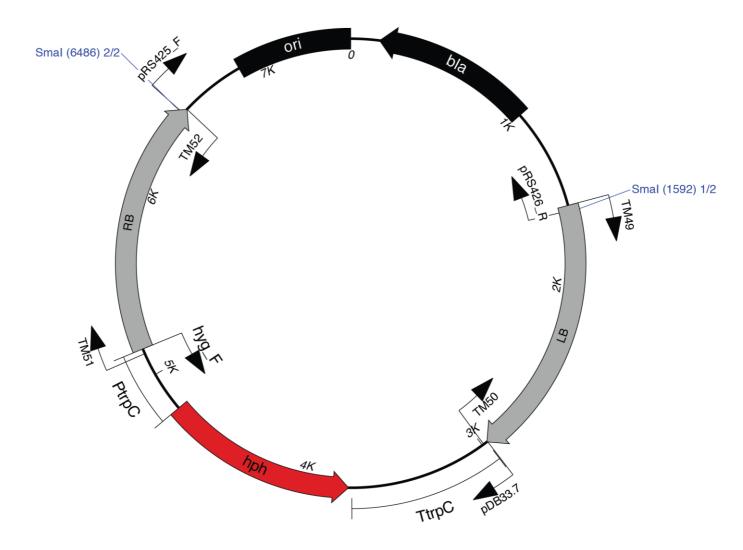
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9_	1895 1895 1747 1746 1751 1842 1897 1889 1913 1913 1912 1788 1915 1732 1960 1956 1808 1807 1812 1874 1958 1807 1812 1874 1958 1952 1974 1973 1849					ECC///			5 SW 5 SW	ESSERTION FOR THE STREET STREE	LTTTLLASS	GGGGGGGGGGG-GGGGGG HRRRRRRRRRRR-R	VIIVII	EEEEE EEEE E E E E E E E E E E E E E E	KKKKK - KKKK - K KNKK LLLLLL - LLLL - LLL		QQQQQ QQQX X X X X X X X X X X X X X X	Q   E E Q   E E   E   E   E   E   E   E	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	R         HP	R P P R P P R R P P R R P P R R P P R R P P R R P P R R R P P R R R P P G P	AD AD AD AD AD AD AD AD AD AD AD AD AD A	S F S F S F S F S F S F S F S F S F S F	L G K L G K L G K L G K L G K L G K F G K F G K L G K L G K L G K L G K	R L R L R L R L R L R L R L R L R L R L		)DL )DL )DL )DL )DL )DL )DL )DL )DL )DL	I MM I MM I MM I MM I MM I MM I MM I MM	E I E I E I E I E I E I E I E I E I E I	1955 1955 1807 1806 1811 1873 1957 1949 1951 1973 1972 1848 1975 1792 1996 1992 1992 1894 1843 1848 1994 1994 1994 1987 2010 2009 1885 2012
	1895 1895 1747 1746 1751 1842 1897 1897 1897 1913 1913 1913 1912 1788 1915 1956 1956 1956 1808 1807 1812 1874 1958 1952 1954 1952 1974					ECC///			5 SW 5 SW	ESSERTION FOR THE STREET STREE	LTTTLLASS	GGGGGGGGGGG-GGGGGG HRRRRRRRRRRR-R	VIIVII	EEEEE EEEE E E E E E E E E E E E E E E	KKKKK - KKKK - K KNKK LLLLLL - LLLL - LLL		QQQQQ QQQX X X X X X X X X X X X X X X	Q   E E Q   E E   E   E   E   E   E   E	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	R         HP	R P P R P P R R P P R R P P R R P P R R P P R R P P R R R P P R R R P P G P	AD AD AD AD AD AD AD AD AD AD AD AD AD A	S F S F S F S F S F S F S F S F S F S F	L G K L G K L G K L G K L G K L G K F G K F G K L G K L G K L G K L G K	R L R L R L R L R L R L R L R L R L R L		)DL )DL )DL )DL )DL )DL )DL )DL )DL )DL	I MM I MM I MM I MM I MM I MM I MM I MM	E I E I E I E I E I E I E I E I E I E I	1955 1955 1807 1806 1811 1873 1957 1949 1951 1973 1972 1848 1975 1792 1996 1992 1844 1843 1848 1894 1994 1994 1994 2010 2009 1885
9_	1895 1895 1747 1746 1751 1842 1897 1889 1913 1913 1912 1788 1915 1732 1960 1956 1808 1807 1812 1874 1958 1807 1812 1874 1958 1952 1974 1973 1849					ECC///			5 SW 5 SW	ESSERTION FOR THE SECOND FOR THE SEC	LTTTLLASS	GGGGGGGGGGG-GGGGGG HRRRRRRRRRRR-RRRRR	VIIVII	EEEEE EEEE E E E E E E E E E E E E E E	KKKKK - KKKK - K KNKK LLLLLL - LLLL - LLL		QQQQQ QQQX X X X X X X X X X X X X X X	Q   E E Q   E E   E   E   E   E   E   E	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	R         HP	R P P R P P R R P P R R P P R R P P R R P P R R P P R R R P P R R R P P G P	AD AD AD AD AD AD AD AD AD AD AD AD AD A	S F S F S F S F S F S F S F S F S F S F	L G K L G K L G K L G K L G K L G K F G K F G K L G K L G K L G K L G K	R L R L R L R L R L R L R L R L R L R L		)DL )DL )DL )DL )DL )DL )DL )DL )DL )DL	I MM I MM I MM I MM I MM I MM I MM I MM	E I E I E I E I E I E I E I E I E I E I	1955 1955 1807 1806 1811 1873 1957 1949 1951 1973 1972 1848 1975 1792 1996 1992 1992 1894 1843 1848 1994 1994 1994 1987 2010 2009 1885 2012

Appendix 21: Alignment of makes caterpillars floppy (Mcf) proteins from a range of *Epichloë* strains across multiple species using the alignment function of Geneious.

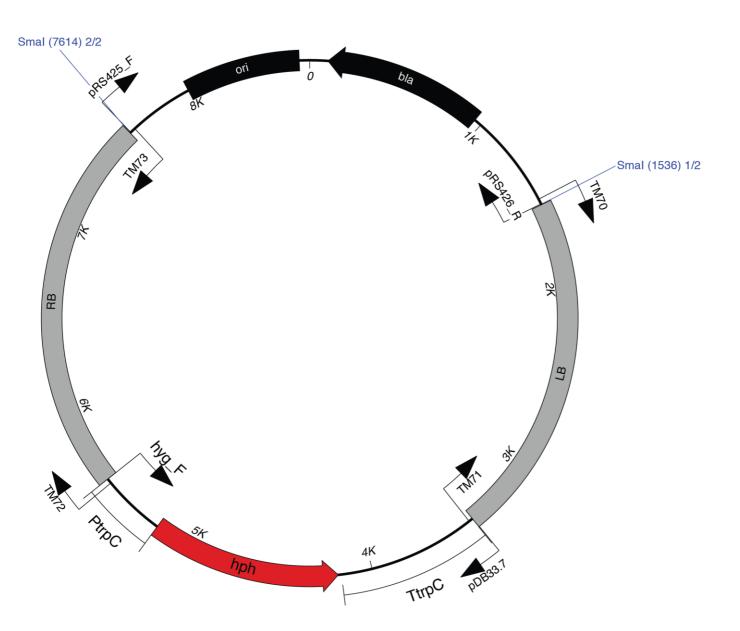
E.typhina_subsp_Poae_Ps1	1 MAHNT DE A FI VEF SKDP LA FI TKG E K G Y A V 30
Nditissima	1 M S L L Y V P F L L D P P D S V T N Y S L P S P Y K V I TK K T G D L NHK D I HQ L L S S P I A F L R T S P I G
Pgigantea	1 M S N I P A D P A L L S F V D D P Q K F L T P T A T V A T A 30
E.typhina_subsp_Poae_Ps1	31   S S DN I P C R P D I R I G DN G H R F E S V G NP DQ P A F E I R Y Y G S E A G A DT I S A Y H L - 82
Nditissima	61 L L D F H E Q R E A R K D G R V L P R K V F A K - L D K C Q NP L NP L V T W Y Q I K A R L S D D G S H N L L 114
Pgigantea	31 V S T Y Q M F S F G I NA R P G G - V P R E G Y F Q F V V N P T G K Q G V I S Y D I K W A G A T P S A NT V WA Y N L - 88
E.typhina_subsp_Poae_Ps1	83 – CYNGGAQT SWTPAQIDIP – – KINPEHNLLFTGSLSGCSVIVTN, NDDQYRVYHD SRQD S139
Nditissima	115 H SYLLQ SP SNKEPTYVDIPVHPAK DEPRLLVTEELGGCSVVVRRLDETTYRVYHDRGLNT 174
Pgigantea	89 – DYYAGAQTN SKPAFLDIP – – KTVAEKTLLFTGALTGCSVIVTSLDANTYRVFHD SRLES145
E.typhina_subsp_Poae_Ps1	140   S L L Y D D V V M A V D Y R D Y K H R D A S T G F A S T F M H Y – – R E G R W S L C F Q R Q T I V A N A R L V L Y E P A 197
Nditissima	175   S I C Y P D V V M A I D H F R Y G M L P H E G D L A T V L M H Y D P E A R Q W S L L V Q R – – L K R N L K V W S R E A – 231
Pgigantea	146   S L F Y D N V M A V D W S D Y S V F S R E – G L A L A F M Q F – – R D G Q W R L I T Q L Q T N S P T G A I L P R S S V 202
E.typhina_subsp_Poae_Ps1	198 P R D G P S I L G A E P L I E M V P G S Y N R E L V Q S R F E Q S R E K C L E R L – R D A R T K L S N D P F Q G E – D G 255
Nditissima	232 – – N A E R Y C A P V P L N I H T E E T Y P Q L A L R P G NM R D R – – – V R R K M K T W I N A Y A P E F T V E – D G 285
Pgigantea	203 L R T A L Q D T Y F P P – S V D S P G S Y D H A A R R A A F D S S R T E S G K R L I R V A T E A F S L S T V P N Q P D G 261
E.typhina_subsp_Poae_Ps1	256 D F Q – P F E E N R I S L D N E A V R Y S Q R L R – A D L H D L MK D R R H D R DH P G D D DMA F S A Q W L V S L L G 313
Nditissima	286 D F V H P G L K E K A T L A N D A V K S T Q V M R N – Y L Q G V L A D I K S D K L K P – – – E L I Q L R D W C Q D S G 341
Pgigantea	262 A F V – P F – G E T I S L N N P A V N H N T A I R NA V E H E I I V – M D D E R L – – – – A V M D K V P A L WQ V L N 313
E.typhina_subsp_Poae_Ps1	314 DQ L F L N E R F V G R A V A S <mark>S R N Y D F T Y L W L K</mark> Q K E E R G F T <mark>A V</mark> V R E G E H R Q T P L G G <b>T A G</b> Q R L S E Q 373
Nditissima	342 K E AM S R H -  –  L S D L I Q E S K D E D F T Y L R C R R E L I D R E K G V F ND L Q V Q S G L L G D T A G E R F A D L 399
Pgigantea	314 T Q H R P I H D L V E P V A L D <mark>S</mark> S K L D Y T Y L W L K Q K E A R G I D A I V V L D G R L R A P L G D T A G E R M T S Q 373
E.typhina_subsp_Poae_Ps1	374 R F R E L LAGDN D F S S GY NAY E S V E I P G – – Y E H DM T L S E I VQL F DR S S T S L T Q T E Q GAL L R R 431
Nditissima	400 H S R L L E L S NQ D L AQ GY LMD T A L V L P E D I V P R DM A I V DMK E L L T G D E V T L T D VQK GAL L K R 459
Pgigantea	374 E L E M L – S G NA D F A S GY D T Y K T VA I P G – – F T S DM D A K AM T L L F – – D S AQ L T D A E K GAL V HY 428
E.typhina_subsp_Poae_Ps1	432 I D L A R K K L F N E S I WQMT N D V VAM F Q EMGGYT K P M P Q D I L L NA I P D K Y G <mark>G G R C Y P L V</mark> Y AM S 491
Nditissima	460 I D D E C L R L Y R D E I W T E A D A V V R R F Q D A Q G I V T G A P Q V A L F E S A T D V A A G G R C Y P L V R A A A 519
Pgigantea	429 I S D A N A Q E Y R A S V W D K T N D V L G V F Q D S A T S T K P M P Q D L L H A I P D E Y – G G R C Y P L V R A M A 487
E.typhina_subsp_Poae_Ps1	492   VAL – A S S N F A I DAL C AK L V G L S P NNAADMK NA EL L K R C L ED L H T S Y P AA EA S R P I – C NMT 549
Nditissima	520   VAL D EQ G P R AM ED I MAK I V S M E R R T T ND –  –  – G E L L R G C L H R L H G N ED A R AA S N R L E G Q F T 576
Pgigantea	488   VAL – S Q S M F S V D Q L M V K L T A L T –  – T D AD L W NA T L F M R C L K D L H S S Y P AA E S S K H I – G K M E 543
E.typhina_subsp_Poae_Ps1	550 LA E A V S V L E – – K S T E T T T LAMHT E V HAM L L G V R K T R N S T – – SWH F Y DP N F A L VT F N S G E A 605
Nditissima	577 L E DAMA E L – – – – – – – – – – – – – – – – T L G D G Q S G A Q L – – Q Y H F Y DP N F A L L T L Q S Q E A 614
Pgigantea	544 L R D A I S L L D V S K P G A K V V Y A L N T E T HAM L L A A N D N G P T L P A S F H F Y DP N F L L A T F D S S E V 603
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	606 L L E A A T K F F E E S C F A K V Y E AQ -
E.typhina_subsp_Poae_Ps1	664 ET I TADR KT V T L Y H D P AQ L T S S R T F S AQ T E L L E T L R L G E A AWR D A T A R L E E S L G I G E H WM 723
Nditissima	675 E S V T L A V S D P F S S R L P R L S S S A D A F S G A L R L A E - G WR S A V P R L Q N E A G L D A H WM 727
Pgigantea	664 E S I T I K S A P E F N L P S P D R F T T Q G T L A A T S A L L D G F G L A E - G WR R A T E D L E T S L G L Q G N WM 722
E.typhina_subsp_Poae_Ps1	724 PILETLKEGGEEGSYEVQFINLKNMNETKWISTESSA-IKDFKARLDEHLETLSKTHEFE782
Nditissima	728 PILSTMERQPEGLQVRFLNLNNPGESERVATVTELDVLELRNHLDEQLQRLGQVATVE785
Pgigantea	723 PVLESLEDLG-GGHYQIQEVNLDDPNEKRIVSTDDER-IQQFKQYISDQLDALRKVYDLQ780
E.typhina_subsp_Poae_Ps1	783   S C S F M R – – E E N L A H A <b>E A I D G L NAM F</b> I M R T L I E H F A G K K – – – – – T E E S K T N A E L A D A L K I 834
Nditissima	786   E G G T V K L H E P K E V S G E P I D G L NAM F L VQ M V F E Y L N P S K K P P V D D S P P S E S T K T L E K A L Q A 845
Pgigantea	781   G <mark>C T F V E – – K E N V P E G E G I D G L NAM F</mark> V V K T V I DMY S G H A – – – – – – G G A N S N L AM A L K V 831
E.typhina_subsp_Poae_Ps1	835 H S Y L N L T Q M G Q Q T L G D V G K M Y N L V K D M L E T G Q V A K S S L T T L V E R L G N V S E G F G V L L 890
Nditissima	846 H S Y F NMAQ I T Y S T T Q D V G K M I S L T R E L I V A E Q A G A K T L S R F S S T L G R V L G S V G E A L G V L F 905
Pgigantea	832 Q S Y F N L A Q M G R T T L G D Y S H V Q L A Q S I L K N E Q V A F A E L S T I G K A F G R A S D G L G T L L 887
E.typhina_subsp_Poae_Ps1	891. G G <mark>AN V V L D A Y E L</mark> A H T D N D A Q K <b>A V F G T Q L A F D S V T F L A S A G T I G A G L I G A T T A V A V M G G</b> V S 950
Nditissima	906. A A G <mark>S V V L D I I E L</mark> A N S E D S A Q K <b>A V L T T Q L V F D</b> G V N L G V A T G G T V A S L M G A A T V G A M L G A G 965
Pgigantea	888. S A A S <mark>V V</mark> F <b>D</b> A Y <b>E L</b> S N A Q D D V Q R <b>A V F G T Q L G F D</b> S A S L L I G V A S L G A G <b>I G A S T</b> A A L L G G A G 947
E.typhina_subsp_Poae_Ps1	951 VILGGLAVGVGALASGFAQIAEKAKYVGRYFGDADEAYKAGGFKYDRDHGILVPLFGAVI
Nditissima	966 VLLAGVGIGVGALVQAFGEVAHKAEAVGKYFADAQESYRAGGYKYDAEKKWLMPLPGAVI
Pgigantea	948 AVVAGLAVGFMGLADAFGQVAEDAKTVGKYFGDAEKAYLAGGYKYDSANEALIPLAYAVI
E.typhina_subsp_Poae_Ps1	1011  S E I D – AAGN <mark>WK F D SQ R LY R</mark> TWHG ST G S <mark>G R I</mark> NY F FWY GDK P RM I QDK SQ A I NV R EG I GAP – 1068
Nditissima	1026  RQ L D L GANT VT F D SQ Y LY R – – – – AP D K G R I – – – – WPWN F P SV DH RK E L A I H VR E A LY AP N 1077
Pgigantea	1008  S S V N LAT GT Y S F D SQ Y I Y R T H S G ST G S G A I NY F FW AGD Y P T V V Y DK SQ A I NV R AG I G AP – 1066
E.typhina_subsp_Poae_Ps1	1069 A S G K L A N T G – – D Y T T I – V L P A T P K S F I S Y E WQ I L P F C T G R H D Y G F D V I R R L E E D E – – R F D 1123
Nditissima	1078 V E G T L P P E G E R D L T A V I I L P S V L K S Y I A V D W NQ L P F S L H R H D R G F D L L R Q I E K D N D P NWL 1137
Pgigantea	1067 A T G T L P R G Y – – N N S T I F V L P A T P K S Y I S Y G W Q M L P G A T T R G D Y G F D V I R R L E E D K – – R F D 1122
E.typhina_subsp_Poae_Ps1	1124 Y DFY I FP SEY I I HQ I T H E FVK TP I AVR LGNR SVR VQVA E L DE S L HNV L EYT I HGAGANYT 1183
Nditissima	1138 Y N FY SWP A EY I V HK L HQ YYAT TP V DYY L SQR SVR LAMP P L L KAQ ENY L EYT L HGAGAQHV 1197
Pgigantea	1123 Y DFY I FP A ERT I NT L SH EYVGT SVT I Q L SR NA I R VQVP E FP SVMQ GKMN <mark>YT L</mark> Q GAGAAYT 1182
E.typhina_subsp_Poae_Ps1	1184   CLNPGVAITLSSGWSNTCWVLDCGKLDGENIVIEARAVSISGFRVNLADT LF 1236
Nditissima	1198 VDLRRGSSLTLTTHADTRWVLDGRELPSDSVTFGDAPTGQRMLRIGGVSVTVDSTRNKD 1257
Pgigantea	1183 VGLETGASITLGSTNASTSWILDCTHLSFTTASVDSNGVTVGGVRVNIADHN F 1235
E.typhina_subsp_Poae_Ps1	1237   S S M L 1 Y K P N G E I L E V D F A N K T T F P I K E D G D K Y Q G – – G S Q K L T D H , ND L S D K H L L G T A L I V 1294
Nditissima	1258   S S L L V L A S N K A T L A I D L E S Q T I E P C E I P A D K WY G E D D G Q T L E Q H L H D L H S R N H
Pgigantea	1236   A S M I I A T P T N D M L K V N F T N P H T S I V S E D A S N F S G – – – S A A L Q A Q L D K L N D S Q L D S A F V L 1292
E.typhina_subsp_Poae_Ps1	1295  VDK Y T T P – – A G Q S V G R A F Y E P T S K R L L Y T I D A P E E L T N S A Q V G A L T A E G K V F F Y – – N T E H 1350
N_ditissima	1318  VDD Y T A P Y D S N Q E V G R A F Y E V S R E R M L Y S V G V P L D L T K E A Q L G C V F G D – V Y F Y C A T K E H 1376
P_gigantea	1293  LKK Y T T P – – S G E A V G D A Y Y E A S R R R F L Y S T G L P Q T L Q Q G N G I V V A T D D D E VY F Y – – N L G A 1348

E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1351 SA IWRVDVSTGVCEAKYHALCPFSKRTLQRVWVDADNQIHALFRHQLSENHFGT 1377 HALWRVDAPTGTLAAQYCPLFNSPEVTLKRVW-QHSHVLYATYFYRYADDDKGDQTAEL 1349 QA IWRVNPIDGSCTAKYRAYYPSAMRTLVQVWLEDDN-VFATYRHVLGFTGLYHSDL	T 1435
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1407 Y I L T A <mark>D SM</mark> N L V GM V G G P A L L A K L HQ E D K WT G E V R L L L E D - Y Y T G P S E L L P K L K L F E S L A ( 1436 C V V HA <mark>D SM V L A T I T D Q H L F E K L I S E K L I K G G D V E S L L K E T Y Q S T K S S S A L N F I T G Q R V I J 1406 Y I L G K <mark>D SM I L V S I I G D P P L L R Q L</mark> S F L D H V D Y P L D Q M I A D - <mark>Y</mark> A L Q E T S A D V S A P T S G P T G</mark>	K 1495
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1466 ADI DAAT DP HL I FYLGP K G K DHY F Y HQ FWLRMSDR TV I K P - HP D I H L NYLLVG 1496 AI I Q G AT L T V N NG ND NH D E AV NA S N S Q Q R F C W L R LQ D G MV I N PWD K WL S N D Y G F V G 1465 S D V Q A S L D A K L T A V L G K E D D G T A R C F W L R T G D G T V I T P - N F T P T P D I AY A G	S 1552
E.typhina_subsp_Poae_Ps1 N_ditissima Pgigantea	1519 I – – A S T G D S G E – – – – – L F C F F S Y NQ H K L V VQ R G NG T Q A E K P R P V T I S P E L G T I S N F F S 1553 V P I A A S E E S G E Q NAQ D Q E L I F Y N P K M H – I L R R L V D I H G D F T C K E V D I P P Q F S G L D H V I Q C 1516 T P P T S S G P E E – – – – – – F Y F Y S I K H Q T I T F Q A G S G T K A G P P Q T Y N F P S D F G N L A N L S	2 1611
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1571 NNNLFSITDAGFILRLTTHCTLALEAVNHQWLEHREK – DVDGGPWWTALSK LAKDHGAA 1612 ADRFILTTKAGYIMQLMPGGAPALVAVSRDWLSSQQDPSTSLLSWWDQLTGLAKQHNAP 1568 EGRMFAISDSGVVLRLTQGGVFFLEAVNQTWIANLKN – SADHLPWWTKLQALADSHSAT	5 1671
E.typhina_subsp_Poae_Ps1 N_ditissima Pgigantea	1630 V S I V CL S DA E G S P - V Q AW L C S D R F V - V A G P S L R G K P R HMA C L T E G G S K 1672 L A V M G L C V G A P P A E K S - V P V W C H D D R I I - I A S A E L H G R F L Q L L G L S P D - G E S 1627 V A V L G L T A T S S T S A P A D S A S A P A V P V W Y I D G K F V I V V S P S L D N K R L D V L G L S K G P V G D V	A 1721
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1677 W LWHMET E D S G H LY A Q P T V R G K E L E T V F R L K A P F V N A E -  – A V P D G R G V L V K H P F K T V A L 1722 L L C D Y G V P G D C K L F Q Q P L I P R G H V P K V L Q N L Y I N G T I A D G A V P K A Q A K F T K L T L S A VA S Y 1687 W I A Y K D G D G S G H LY A Q P L A T G A -  V T D L F S P T D P T V N L A T I S P A Q I L T S MQ S V P F K D V K M	V 1781
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1735 EGG LRYTTK EGVVL I LASE – R SAR LYGVDK VWQQ – NR SDL SGE I ATL V – KT – WDHGE SV 1782 GNRVECVDH EGVGLR LRGN F SSAT VVGVT RAWQKP VQASL ESSL FWLQ – RR RGGL SP VLN 1746 EDG LQYTALDGR I FI I TDT – QTVTLLGVDK TWQAANAANLEATL VTLATQT EWNHGE V F	1840
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1791 M L <mark>G S E P P Q WY</mark> L T S S G N I V S A A K A T F T W L D A P T W L G A D P S G A R G Y A Y V A ( 1841 V E A D E T Q S S G P A WY N L E T N R I Y R F T A D N K D A M I D P Y H A W L G V H A T D A T - I A Y V H S S ( 1805 L Q G D P <mark>S D P P A WY</mark> H I V L G K A V R P T N I P V T W A D Q P V W L G V S I E A I M G W F F <mark>V</mark> P V K N T A S	<b>1896</b>
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1840 Q G R I Y E L G E G S A E E K K S A A S Q E V A F A S R F Q D V L A V K P S P G A S F R Q F – A L E N V H Y T I L S Q 1897 N K V L Y Q V – – – – T S S G S R P L W R S P F I T R L D N T L A F – S I P A Q T N A N L P V I A G A D H C L V T – 1861 V G V T Y T V – R V D V P G G S S A P V D G L S F T K R L D D I L I L V P S P G N S F S Y L P G I WG V R T A V I L Q (	F 1949
E.typhina_subsp_Poae_Ps1 N_ditissima Pgigantea	1899 E G D T S F M D Y S V P S A <mark>S W</mark> E S L S G L V I – E W K D Q G R V E M E G S T M H P G P L G W Y L A K R L G D D L I I N 1950 D S T Q G G C V L S I S K E <mark>S W E</mark> Y Y K A I T V A N Y E A M – T E E R NA I Y L E T D H L A S F M A V E S N C A L M F 1920 A G – – A L M G Y R V P E A <mark>S W</mark> D Y Y E S I V V Q D H N A S V R T Q P D S V Y L D V T N P G V L L A K K I G E D L V V	2007
E.typhina_subsp_Poae_Ps1 Nditissima Pgigantea	1958 EGSTGR FMK I SRAMVMDPTCM I HMTGK LLTVGNG SAGNQ	2 2067
E.typhina_subsp_Poae_Ps1 N_ditissima P_gigantea	2068 LMETWLPQRVLMVLDQVPRSIFP 2038 TIAYAVTQPSS	2090 2048

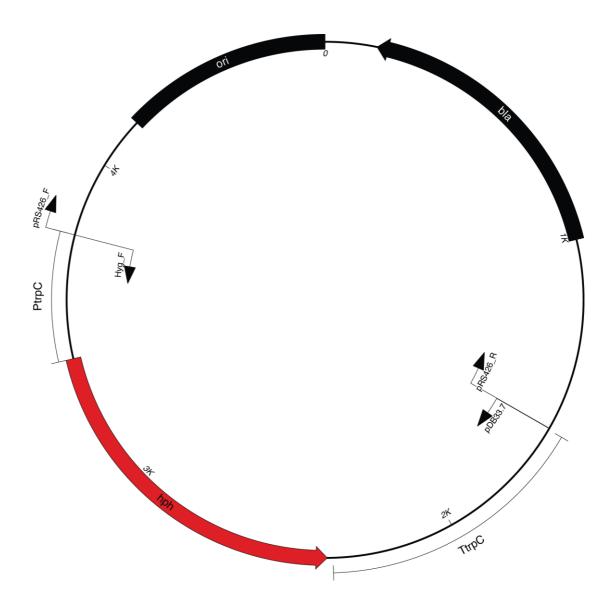
Appendix 22: Alignment of makes caterpillars floppy (Mcf) proteins from a range of fungal species across multiple species using the alignment function of Geneious.



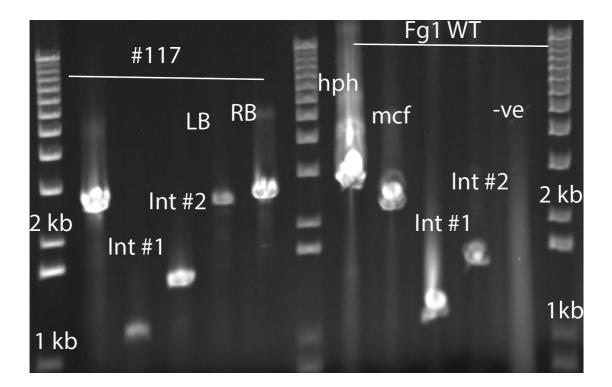
Appendix 23: Whole *Epichloë. Festucae* makes caterpillars floppy (*mcf*) gene deletion construct (pTM03, 7489 bp). Hph (hygromycin resistance gene). LB (left-border). RB (right-border). Ori (*E. coli origin* of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtrpC (terminator). Primers used to make plasmid are indicated by black arrows (all). Smal cut sites used to excise knock-out insert.



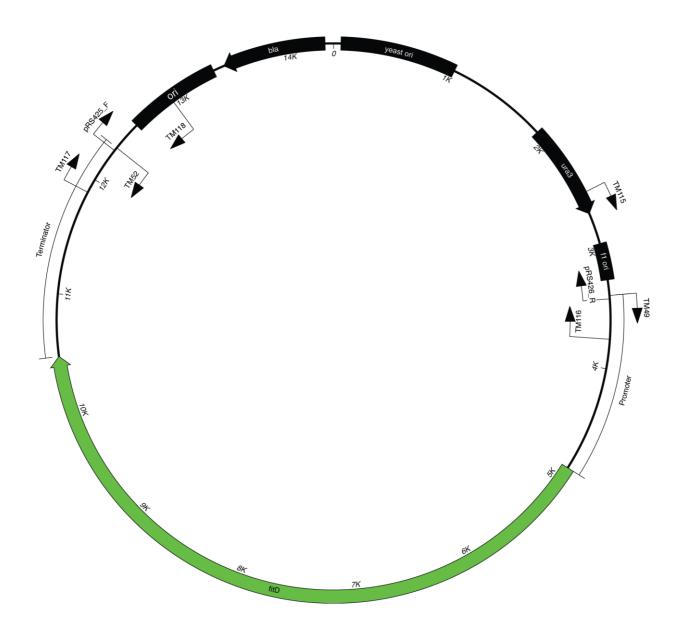
Appendix 24: 2 kb *Epichloë festucae* Fg1 5' of makes caterpillars floppy (*mcf*) gene deletion construct (pTM05, 8673 bp). Hph (hygromycin resistance gene). LB (left-border). RB (right-border). Ori (*E. coil* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtrpC (terminator). Primers used to make plasmid are indicated by black arrows (all). Smal cut sites used to excise knock-out insert.



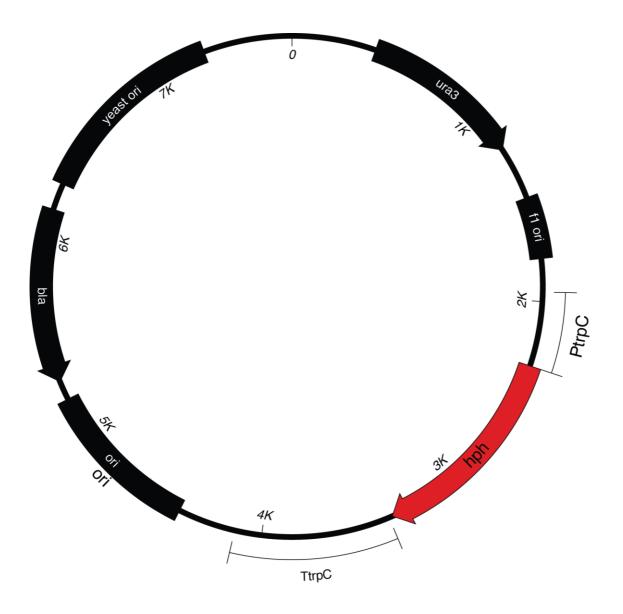
Appendix 25: Hygromycin resistant gene construct (pANS7-1, 4777 bp). Hph (hygromycin resistance gene). Ori (*E. coil* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtrpC (terminator). Primers used to amplify backbone (pANS425\_F/pANS425\_R) and resistant gene (Hyg\_F/DB33.7) are indicated by back arrows.



Appendix 26: PCR confirmation of #117 transformant ( $\Delta mcf$  Fg1/pTM05). DNA from either Fg1  $\Delta mcf$  mycelia or Fg1 wild type (WT) mycelia was subject to primers that amplify across the hygromycin gene (hph) (TM86/87), in the deleted region (int #1 and int #2) (TM111/112 and TM113/114), across the left border (LB) (TM89/109), and across the right border (RB) (TM90/110). –ve is water instead of primer



Appendix 27: *Epichloë festucae* var *lolii* AR48 makes caterpillars floppy (*mcf*) gene complementation construct (pTM04, 14377 bp). Hph (hygromycin resistance gene). LB (left-border). RB (right-border). Ori (origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtripC (terminator). Primers used to make plasmid are (TM49/TM52) and (pRS426\_F/pRS426\_R) indicated by black arrows. Primers used screen the transformants are (TM117/TM118) and (TM115/TM116) are indicated by black arrows



Appendix 28: Hygromycin construct (pDB48, 7702 bp). Hph (hygromycin resistance gene). Ori (*E.coli* origin of replication point). Bla (ampicillin resistance gene). PtrpC (promoter). TtypC (terminator).

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