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**Progress Towards Development of a Genetically Modified  
Strain of the Australia Sheep Blowfly *Lucilia cuprina*  
Suitable for a Sterile Release Program**

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## Abstract

The sterile insect technique (SIT) is concerned with the mass-rearing and release of sterilized insects which mate with "wild-type" females in the field, producing no viable offspring. The aim of this study was to use genetic engineering methods to make a strain of the Australia sheep blowfly *Lucilia cuprina* which is suitable for an area-wide sterile-male release program. The main objectives were: development of an efficient germline transformation system for introducing a target gene into *Lucilia* and development of an inducible female killing system to produce a male only population.

The *piggyBac* and *Minos* transposons were evaluated as transformation vectors for *L. cuprina*. Firstly, *Drosophila melanogaster* was used as a model system to determine if the frequency of both inter-plasmid transposition and germ-line transformation increases with the level of expression of the *piggyBac* transposase. Expression of the *piggyBac* transposase gene was controlled with either the  $\alpha 1$ -*tubulin*, *hsp83* or *hsp70* promoter, which have strong, intermediate and low constitutive activity respectively. The results show that the frequency of *piggyBac*-mediated germ-line transformation does increase with the level of expression of the transposase. In contrast, there does not appear to be a simple correlation between the level of expression the transposase and the frequency of transposition measured using an inter-plasmid transposition assay. This suggests that this widely used assay may not necessarily predict which is the best "helper" plasmid for germ-line transformation. Secondly, inter-plasmid transposition assays have shown that both *piggyBac* and *Minos* transposases are active in blowfly embryos. Thirdly, *Drosophila* eye color genes and the enhanced green fluorescent protein (*EGFP*) gene were tested as potential markers for identifying transgenic flies. The most promising marker based on transient expression appears to be *EGFP* driven by the *Drosophila polyubiquitin* gene promoter (*pUb-EGFP*). Fourthly, blowfly embryos were coinjected with the *piggyBac* helper driven by the *D. melanogaster*

*hsp70* promoter and the *PUBnlEGFP* marker gene. Two transgenic *L. cuprina* lines were isolated and characterised by Southern DNA hybridisation analysis and inverse PCR. The transformation frequency was 1.4 to 1.9%. Of the two transformant lines obtained, one had a single copy of the transgene and the other most likely has four copies. This is the first report of germ line transformation of *L. cuprina*.

A tetracycline regulated inducible expression system was adopted to develop a controllable female-killing genetic system based on the *D. melanogaster msl2* gene. One component of the system is the tetracycline dependent transactivator (*tTA*) gene controlled by a constitutive promoter. The other (*tetO-msl2*) is the *msl2* coding region controlled with a promoter bearing seven copies of the tetracycline operator (*tetO*) sequence. Female *D. melanogaster* carrying both a *promoter-tTA* and *tetO-msl2* gene constructs would be predicted to die in the absence of tetracycline due to expression of *msl2*.

In this study several *promoter-tTA* constructs were developed including *WH-arm* which uses the constitutive *armadillo* promoter. *Drosophila* carrying both *WH-arm* and *tetO-lacZ* transgenes were shown by spectrophotometric and histochemical staining assays to express  $\beta$ -galactosidase but only if raised on media that lacked tetracycline. There was a significant decrease in viability of females carrying both *WH-arm* and *tetO-msl2* gene constructs raised on media lacking tetracycline. However lethality was not 100%. Assembly of the MSL complex on female X chromosomes (due to expression of *msl2*) was confirmed by immuno staining of polytene chromosomes with anti-MSL3 antibody. Thus it appears that induction of 100% female lethality will require higher levels of *msl2* expression than obtained with the *WH-arm/tetO-msl2* system for controlling female viability in transgenic *Lucilia*.

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- Table 5.8.** Ratio of male (M) to female (F) offspring of crosses between *ypl-tTA* and a strain carrying *tetO-msl2* raised on instant food lacking tetracycline. 165

## ABBREVIATIONS

<i>arm</i>	<i>armadillo</i>
$\beta$	beta
$\Delta$	delta
$\lambda$	lambda
bp	base pairs
BSA	bovine serum albumin
BHI	brain heart infusion
<i>cn</i>	<i>cinnabar</i> gene
$^{\circ}\text{C}$	degrees Celsius
cDNA	complementary DNA
ddH <sub>2</sub> O	double distilled water
DNA	deoxyribonucleic acid
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
dNTPs	dinucleotide triphosphates
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediamine tetraacetic acid
EGFP	enhanced green fluorescent protein
F	female
g	gram
GFP	green fluorescent protein
<i>hsp</i>	heat shock protein
kb	kilobase pairs
mRNA	messenger ribonucleic acid
L	liter
<i>L. cuprina</i>	<i>Lucilia cuprina</i>
$\mu$	micro
M	male
M	molar

<b>Mb</b>	<b>million base pairs</b>
<b>min</b>	<b>minute</b>
<b><i>mle</i></b>	<b><i>maleless</i></b>
<b><i>msl</i></b>	<b><i>male specific lethal</i></b>
<b><i>mof</i></b>	<b><i>males absent on the first</i></b>
<b>nm</b>	<b>nanometer</b>
<b>OD</b>	<b>optical density</b>
<b>ORF</b>	<b>open reading frame</b>
<b>PBS</b>	<b>phosphate-buffered saline</b>
<b>PCR</b>	<b>polymerase chain reaction</b>
<b>RNase</b>	<b>ribonuclease</b>
<b>RNA</b>	<b>ribonucleic acid</b>
<b>rpm</b>	<b>revolutions per minute</b>
<b>TE</b>	<b>tris-EDTA</b>
<b><i>tetO</i></b>	<b><i>tetracycline operator</i></b>
<b>tetR</b>	<b>tetracycline repressor</b>
<b>tTA</b>	<b>tetracycline dependent transactivator</b>
<b>tub</b>	<b>tubulin</b>
<b>UV</b>	<b>ultra violet</b>
<b>U</b>	<b>units</b>
<b>UTR</b>	<b>untranslated region</b>
<b>V</b>	<b>volts</b>
<b>v/v</b>	<b>volume per volume</b>
<b>w/v</b>	<b>weight per volume</b>
<b>yp</b>	<b>yolk protein</b>

**LIST OF PUBLICATIONS ARISING FROM THIS THESIS**

X. Li, J.C. Heinrich and M. J. Scott (2001) *piggyBac*-mediated transposition in *Drosophila melanogaster*: an evaluation of the use of constitutive promoters to control transposase gene expression. *Insect Molecular Biology* **10(5)**: 447-455.

Jörg C. Heinrich\*, Xuelei Li\*, Rebecca A. Henry, Neville Haack, Leanne Stringfellow, Allen Heath and Maxwell J. Scott (2002) Germ-line transformation of the Australian sheep blowfly *Lucilia cuprina*. *Insect Molecular Biology* **11(1)**: 1-10.

\*These authors contributed equally to this study.