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**Transformation and Gene Targeting**  
**in *Aspergillus nidulans***

A thesis presented in partial fulfilment of  
the requirements for the degree of  
Doctor of Philosophy in Molecular Genetics  
at Massey University, Palmerston North,  
New Zealand

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**1996**

## Abstract

Transformation of a haploid *Aspergillus nidulans pyrG* auxotrophic strain (1-85) was optimised for the vector pGM32 containing the heterologous *Neurospora crassa pyr4* gene. The resulting uracil-independent transformants could be classified into two main groups based on morphology. The minority were morphologically very similar to the parental strain, easily purified and mitotically stable. The majority (10 times more frequent) were irregular in shape and shown to be heterokaryons that could not be resolved into transformed homokaryons. Analysis of the transformant types suggested regulation of multiple copies of the gene for OMPdecase (*pyr4* and *pyrG*) resulted in the titration/inactivation of essential trans-acting factors. The heterokaryon state was therefore a requirement for the survival of transformants containing multiple copies of the integrated vector.

The effect of altering the conditions of transformation on the efficiency of gene targeting in filamentous fungi was studied. The *A. nidulans niaD* and *amdS* genes, both involved in nitrogen source utilisation, were selected as target loci. Insertional inactivation vectors were constructed (based on pGM32) and parameters shown to have an effect on the targeting frequency at the *niaD* locus were subsequently tested at the *amdS* locus. A dramatic difference in targeting was observed between the *niaD* and *amdS* loci with targeting of *niaD* being much more efficient than *amdS* for the parameters tested. The level of gene targeting using circular DNA was found to correlate with the size of the homologous segment at both loci. Similarly the level of targeting was shown to increase at both loci when vectors were linearised within the region of homology. Unexpectedly the level of targeting was unaltered at the *niaD* locus when transcription was induced at different stages in the transformation procedure. Likewise targeting was unaffected by altering the amount of DNA in the reaction mix. The regeneration temperature, however, did appear to have an effect on targeting, with enhanced targeting observed at the lower temperature.

Gene replacement by transformation was used to disrupt the *cycA* gene in diploid and haploid *A. nidulans* strains. The first completely deficient *cyc* mutant in a filamentous fungus was isolated and shown to be non-lethal. Haploidisation analysis of the diploid transformant localised the chromosomal position of *cycA* to chromosome I.

## Acknowledgements

My sincerest thanks to my supervisor, Dr Rosie Bradshaw. Thank you for your time and energy, your support and encouragement, and, most of all, your friendship. I appreciate all the effort you went to on my behalf. Thank you also to my co-supervisor, Prof. Barry Scott. Your experience and guidance was of great value.

Lab work is not complete without lab mates with which to share the joys and frustrations. Thank you Karen, Carmel, Anita, Linda, Paul, Grant, Branwyn, Tania, Daniel and later Brendon. Individually you have all contributed to my thesis. I may not have shown it but your presence helped me a great deal. Thank you all.

Thank you also to the members of Scott Base, past and present, for providing all those emergency supplies and helpful tips. Particular thanks go to Carolyn, Austin and Mike.

I have a lot to thank the Department of Microbiology and Genetics for. Wonderful, if brief, technical assistance, thank you Shalome and Tash, and superb secretarial assistance, thank you Laura and, in the early days, Terri. A special thank you to Tim, for your advice and kind words, and to Max and Jan, for patiently putting up with my cynicism. To the rest of the Department, thank you for the pleasant, welcoming, and helpful environment you provided.

Financial assistance for this thesis was provided in the form of a Massey University Postgraduate Scholarship, William Georgetti Scholarship and New Zealand Federation of University Women's Fellowship. Without such support this thesis would not exist.

A special thanks to Shayne for being there for me, even if you didn't understand why I was doing it. Thank you also to my family and his, now mine also, for the much needed family support. Last but not least, thanks to my friends, particularly Cherie, for support, distractions and friendship, you are all treasured.

## Abbreviations

AMM	<i>Aspergillus</i> minimal media
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BSA	bovine serum albumin
bp	base pair(s)
$\chi$	chi squared or chromosome
CTP	cytidine triphosphate
DNA	deoxyribonucleic acid
FAD	flavin adenine dinucleotide
GTP	guanosine triphosphate
hr	hour(s)
kb	kilobase pairs(s)
min	minute(s)
NADP	nicotinamide adenine dinucleotide phosphate
PCR	Polymerase Chain Reaction
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
sec	second(s)
TE	Tris/EDTA buffer
TTP	thymidine triphosphate
UTP	uridine triphosphate

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