Transformation and Gene Targeting in *Aspergillus nidulans*

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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.

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Abbreviations

AMM	*Aspergillus* minimal media
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BSA	bovine serum albumin
bp	base pair(s)
χ	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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