

Transformation of *Neurospora pyr-4* with defective donor genes

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Using the Vollmer/Orbach transformation protocol, transformation frequencies of a *pyr-4* (OMP decarboxylase) strain of *Neurospora crassa* of circa $10(3)/\mu\text{g}$ are routinely achievable. At these levels of transformation, it is feasible to screen out ectopic integrations and look specifically for homologous integration events. Homologous integrants were sought by transforming a *pyr-4* recipient with interrupted or incomplete copies of the cloned *pyr-4* gene derived from the *pyr+* clone in plasmid pFB6, selecting by complementation of the pyrimidine auxotrophy in the recipient.

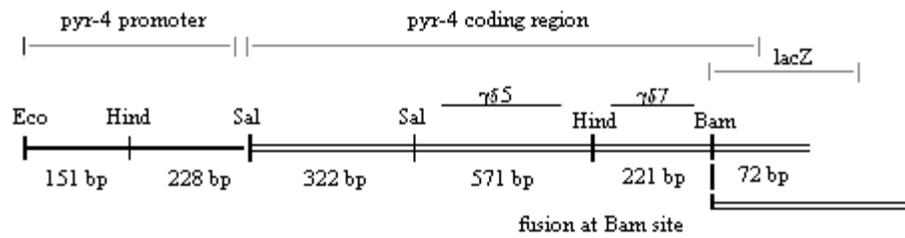
Defective clones used as donors were of two types: 1) Clones inactivated by the insertion of the transposon Tn1000 into the *pyr-4* gene, the method of construction employing the F-mediated transfer of the *pyr-4+*, *ampR* plasmid by conjugation into a *pyr-4-*, *ampR* recipient, and identifying *pyr-4-*, *ampR* exconjugants. 2) Inactivating *pyr-4+* clones by cutting with *Bam*H1 within the distal portion of the coding region and making a translational fusion with *lacZ*, this construct having β -galactosidase but not OMP decarboxylase activity.

Using two strains inactivated by Tn1000 (gd5 and gd7) and one *pyr-4/lacZ* fusion as donor DNA, transformation of a *pyr-4* recipient was carried out. Pyrimidine-independent transformants were obtained at low frequency with each of the donor clones (see table). Control reversion frequency was circa $5 \times 10(-8)$. With the three defective donors, restoration of an intact and functional *pyr-4* gene requires integration at the homologous chromosomal locus, with a homologous recombination event between the site of the mutant lesion in the recipient and the transposon integration site or the fusion site in the donor. This was confirmed by Southern analysis.

Transformation of *pyr-4*

plasmid	phenotype	DNA used	plasmid size	transfo. per μg	transfo. per spheroplast
pFB6	<i>pyr+</i>	5 μg	8.6 kb	900	$2380 \times 10(-7)$
gd5	<i>pyr-</i>	10 μg	17.5 kb	1.1	$6 \times 10(-7)$
gd7	<i>pyr-</i>	10 μg	17.5 kb	0.9	$5 \times 10(-7)$
<i>pyr-4/lacZ</i>	<i>pyr-</i>	10 μg	11.5 kb	1.9	$11 \times 10(-7)$

The locations of the three donor lesions are shown in the figure, based on restriction mapping. The lesion in the recipient is clearly proximal to the fusion site with *lacZ*, although its location within the circa 1.5 kb of the coding region proximal to that site is not yet known.



The constraints on the site of the required crossover mean that only some of the homologous integrants will give rise to pyrimidine-independent transformants. However, all such transformants are truly wild type in growth rate, unlike heterologous transformants.