An improved plasmid for transformation of *Neurospora crassa* using the *pan-2* gene as a selectable marker

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The pOKE103 plasmid can be used to transform *Neurospora crassa* using the *pan-2* gene (NCU10048.3) as a selective marker. We mapped the *pan-2* fragment to the right arm of LG VI, contig 4, and found that it carries a *pan-2* linked gene, *atg-12* (NCU10049.3). We report the removal of *atg-12* to produce an improved plasmid, pOKE104.

Genetic transformation is a key tool to study fungi and the ability to create strains transformed with multiple plasmids is especially important for mechanistic cellular and developmental studies. In *Neurospora crassa*, a number of options are available: For reproducible expression, transgenes can be targeted as single copies to the *his-3* locus by vectors designed to repair rarely reverting alleles at *his-3* (Aramayo and Metzenberg, 1996; Margolin et al., 1997). Recently, a system has been reported, where integration at the *csr-1* locus produces cyclosporin A resistance and allows the selection of single copy integrants at *csr-1* (Bardiya and Shiu, 2007). Selection of transformants using Hygromycin B resistance is also common (Staben et al., 1989), however, the use of this marker for systematic deletion of *Neurospora* genes (Colot et al., 2006) is likely to preclude its use in many cases.

The *pan-2* marker provides an additional means of transformation. In this case transformation is ectopic and results in the integration of single or multiple copies of the transgene. The pOKE103 vector constructed by J. Groteluesch en and Bob Metzenberg contains a genomic fragment of *pan-2* in the pGEM3Z (+) vector backbone and this vector produces tight selection in *pan-2* mutant backgrounds (Wu and Glass, 2001). We examined the *pan-2* fragment and found that it carries an additional gene, *atg-12*, a conserved ubiquitin-related protein associated with cellular autophagy (Hanada and Ohsumi, 2005). Thus, transformation with pOKE103 may also result in variable overproduction of ATG-12. There is currently no evidence to suggest that transformation with pOKE103 produces any phenotypic transformation. Nevertheless, because the effects of additional copies of *atg-12* may depend on the genotype and may emerge only in certain mutant backgrounds, we have removed *atg-12* from pOKE103 to produce a simplified derived plasmid, pOKE104.

Plasmid pOKE 103 contains a unique *Nde*I restriction site 5' of the *atg-12* gene and we used site-directed mutagenesis with two complementary primers ("new-NdeI"; GTC GGA ACA TAT GTT ATG GAC AGC ATG) to insert a second *Nde*I site 3' of *atg-12* (Fig. 1). Digestion of this vector with *Nde*I removes a 1347 bp fragment containing the entire *atg-12* gene. The vector fragment from this digestion was circularized to produce the pOKE104 vector (Fig. 1). The complete sequence of pOKE104 can be found using NCBI accession number FJ599664 and the plasmid can be obtained from the Fungal Genetics Stock Center (http://www.fgsc.net/).

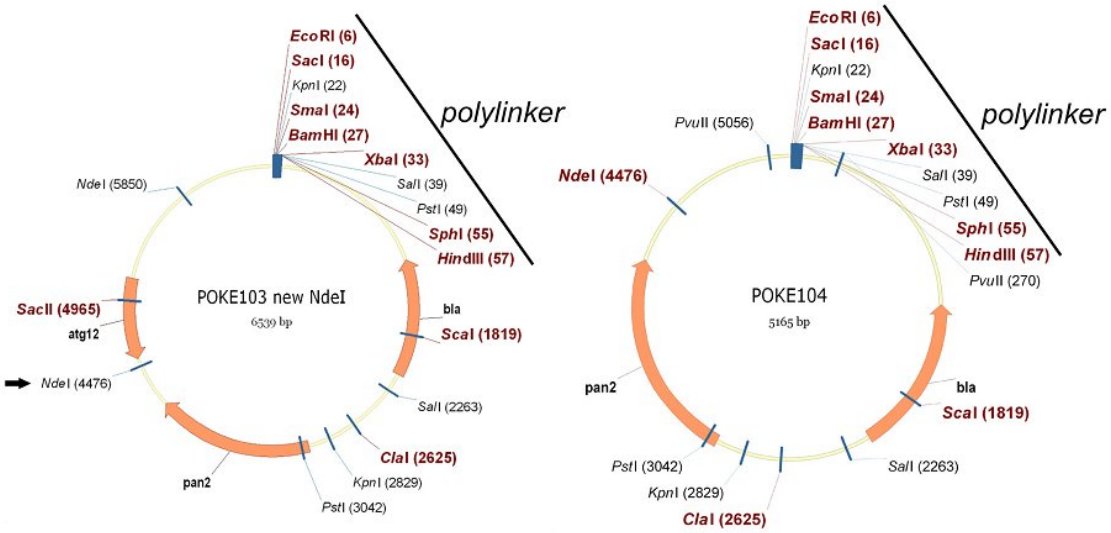

Figure 1. Partial restriction maps (drawn with Vector NTI) of plasmids pOKE103 and pOKE104. The introduced *Nde*I site is shown on the left in pOKE103 (arrow); the right map shows pOKE 104 after removal of *atg-12*. The polylinkers are indicated with unique restriction sites shown in red bold.

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