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Plasmid tagging for use in proving the identity of a complementing sequence in Aspergillus nidulans by tandem integration and loop-out repair

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This reports a convenient way for tagging a complementing sequence (gene or gene fragment) for use in tandem integration/loopout repair. It was used to verify cloning of the hypA locus. The backbone of a hypAI-complementing plasmid was tagged with pLH1, a Tn5 transposon having a diagnostic BamH1 fragment containing argB+. This was transformed into a hypAI, argB2 strain of A nidulans. Transformants with an integrated copy of the tagged plasmid were allowed to self-mate. Given integration at hypA, and that the plasmid sequence was from the hypA locus, this led to loopout repair of the hypAI defect with concomitant loss of argB+.

When a gene is cloned by phenotype complementation, it is necessary to demonstrate that the complementing fragment contains the gene of interest and not an extra-copy suppressor of the mutation. One strategy is to precisely map the locus and use appropriate cosmids from a chromosome-specific ordered cosmid library. However, it is possible that an extra-copy suppressor could be closely linked to the gene of interest. Another is to complement the mutant phenotype with a portion of the open-reading frame, either after sequencing a complementing genomic fragment or using a piece of a cDNA clone. However, identifying a portion of the open-reading frame that can complement the mutation might not be straightforward or the cDNA might not be available. In a more stringent method, transformants with tandem integrations of the mutant and putative wildtype allele are self \_mated and tested for loopout repair (Harris and Hamer 1995 EMBO J. 14:101-114). The transposon tagging method of Hamer and Gilger (1997 Fungal Genet. Newsl. 44:19-23) is a convenient way to tag a putative complementing gene or gene fragment with a selectable fungal marker for this purpose.

A plasmid containing a 1.8 kb genomic DNA fragment of hypA (hypA is ~ 4.3 kb) that complemented the A. nidulans hypA is mutation (Kaminskyj and Hamer 1998 Genetics 148:669-680), called pSKJ13, was tagged using the method of Hamer and Gilger (1997 Fungal Genet. Newsl. 44:19-23) with a To5 transposon containing argB as a selectable marker, pLH1 (Figure 1A). Transposition events were confirmed by comparing BamHI digests of pSKJ13 and three pLH1 Tn5 hops into pSKJ13 (Figure 1B). pLH1 contains a 4.7 kb BamHI fragment. pSKJ13 contains a 1.8kb SacI insert in pBluescript and is cut once by BamHI to give a 4.8 kb band. A BamH1 digest of Tn5 transpositions into pSKJ13 showed that each had the 4.7 kb fragment diagnostic of the pLH1 Tn5 transposon cassette, and two smaller bands. The relative sizes of the smaller bands related to the transposition site compared to the single BamHI site in pSKJ13. Transposon hop1 was named pSKJ13argB.

pSKJ13argB was transformed into ASK78 (hypA1<sup>ts</sup>, wA3, argB2), following the procedure of Osmani et al. (1990 J. Cell Biol. 111:543-551) and selected for arginine prototrophy at 28°C. All transformants were temperature sensitive due to hypA1<sup>ts</sup> in the recipient strain. Because ASK78 contains argB2, pSKJ13argB could integrate at the argB locus, or at the hypA locus, or ectopically. These can be distinguished by examining progeny phenotypes after self-mating. Self-mating leads to the chance for intra-chromosome crossover and looping-out (loss) of the region between the tandem repeats (putative repeats for hypA1<sup>ts</sup> and pSKJ13).

If pSKJ13argB integration occurred at the argB locus, then argB+ and argB2 would flank pSKJ13 (Figure 2A). If the integration occurred at the hypA locus, then hypA locus, then hypA locus (Figure 3A) would flank argB+ (Figure 3A) would produce progeny that were auxotrophic or prototrophic for arginine depending on the position of the crossover, but all progeny would be hypA locus (Figure 3B), then loopout repair could produce progeny that were auxotrophic for arginine and hypA locus (Figure 3B), then loopout repair could produce progeny that were auxotrophic for arginine and hypA or hypA locus (Figure 3B), then loopout repair could produce progeny that were auxotrophic for arginine and hypA or hypA locus (Figure 3B), then loopout repair could produce progeny that were auxotrophic for arginine and hypA or hypA locus on the position of the crossover. If pSKJ13 did not contain the fragment of hypA+ that corresponded to the lesion in hypA locus, or if the integration was ectopic, there could be no loopout repair at hypA. Finding argB2, hypA+ progeny would show there had been tandem integration at the hypA locus, loopout repair, and that pSKJ13 was derived from hypA rather than an extracopy suppressor of the hypA1 mutation.

Two transformants that had integrated copies of pSKJ13argB were streaked on minimal medium and allowed to form cleistothecia. One cleistothecium from each of these integrants was isolated and cleaned for random ascospore analysis. One hundred single ascospore isolates were analyzed for phenotype from each of these two cleistothecia: 26/100 and 16/100 progeny were hypA\*, argB2 (arginine auxotrophs). These transformants must have had pSKJ13argB integration at the hypA locus. Identification of hypA\* progeny indicated that pSKJ13 contains the genomic DNA region that spans the lesion in hypA1\* mutation.

In Aspergillus, this method can be used to prove complementation with genes or gene fragments. It could be adaptable to other fungal systems, for example using pLH2, Tn5 containing hph (Hamer and Gilger 1997 Fungal Genet. News). 44:19-23) for hygromycin resistance, or by substituting a different marker.

Figure 1. Creation of pSKJ13argB, containing part of the putative InpA sequence and an argB selectable marker

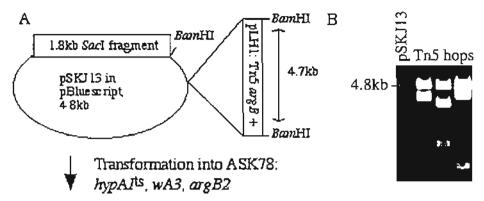


Figure 2. Single tandem integration events after transformation

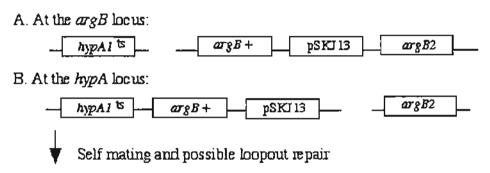


Figure 3. Effect of loopout repairs on single integrations at argB or hypA

