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Abstract

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 *hypA1*-complementing plasmid was tagged with pLH1, a Tn5 transposon having a diagnostic *Bam*H1 fragment containing argB+. This was transformed into a *hypA1, 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 *hypA1* defect with concomitant loss of *argB+*.

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 *hypA1*-complementing plasmid was tagged with pLH1, a Tn5 transposon having a diagnostic *Bam*H1 fragment containing *argB+*. This was transformed into a *hypA1*, *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 *hypA1* 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* *hypA1*[ts] 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 Tn5 transposon containing *argB* as a selectable marker, pLH1 (Figure 1A). Transposition events were confirmed by comparing *Bam*HI digests of pSKJ13 and three pLH1 Tn5 hops into pSKJ13 (Figure 1B). pLH1 contains a 4.7 kb *Bam*HI fragment. pSKJ13 contains a 1.8kb *Sac*I insert in pBluescript and is cut once by *Bam*HI to give a 4.8 kb band. A *Bam*H1 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 *Bam*HI site in pSKJ13. Transposon hop1 was named pSKJ13*argB*.

pSKJ13*argB* was transformed into ASK78 (*hypA1*[ts], *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*[ts] in the recipient strain. Because ASK78 contains *argB2*, pSKJ13*argB* 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*[ts] and pSKJ13).

If pSKJ13*argB* integration occurred at the *argB* locus, then *argB+* and *argB2* would flank pSKJ13 (Figure 2A). If the integration occurred at the *hypA* locus, then *hypA1*[ts] and pSKJ13 would flank *argB+* (Figure 2B). Integration could also occur at an ectopic site (not shown). Loopout repair after integration at the *argB* locus (Figure 3A) would produce progeny that were auxotrophic or prototrophic for arginine depending on the position of the crossover, but all progeny would be *hypA1*[ts]. If pSKJ13 contained the fragment of *hypA+* that corresponded to the lesion in *hypA1*[ts], and if integration occurred at the *hypA* locus (Figure 3B), then loopout repair could produce progeny that were auxotrophic for arginine and *hypA+* or *hypA1*[ts] depending on the position of the crossover. If pSKJ13 did not contain the fragment of *hypA+* that corresponded to the lesion in *hypA1*[ts], 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 pSKJ13*argB* 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 pSKJ13*argB* integration at the *hypA* locus. Identification of *hypA+* progeny indicated that pSKJ13 contains the genomic DNA region that spans the lesion in *hypA1*[ts] 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. Newsl. 44:19-23) for hygromycin resistance, or by substituting a different marker.

Figure 1. Creation of pSKJ13*argB*, containing part of the putative *hypA* sequence and an *argB* selectable marker
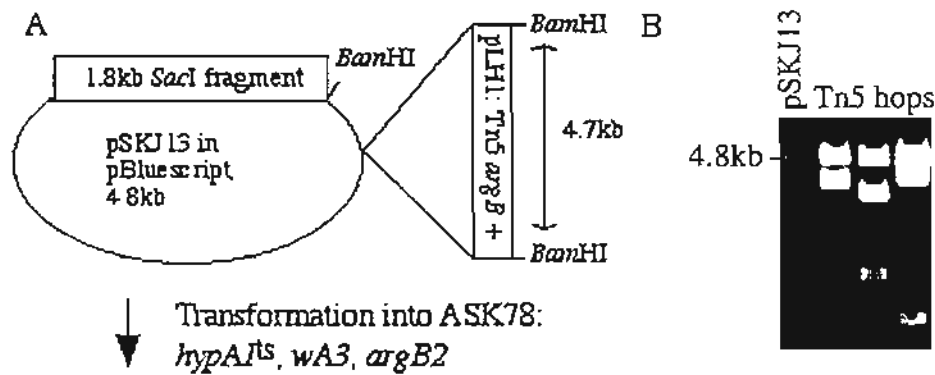
A

1.8kb *SacI* fragment   *Bam*HI

pSKJ 13 in pBluescript 4.8kb

pLH1: Tn5 *argB* +

*Bam*HI
4.7kb
*Bam*HI

B

pSKJ13

Tn5 hops

4.8kb —

Transformation into ASK78:
*hypAI*ts, *wA3*, *argB2*

Figure 2. Single tandem integration events after transformation

A. At the *argB* locus:

*hypAI*ts   *argB* +   pSKJ 13   *argB2*

B. At the *hypA* locus:

*hypAI*ts   *argB* +   pSKJ 13   *argB2*

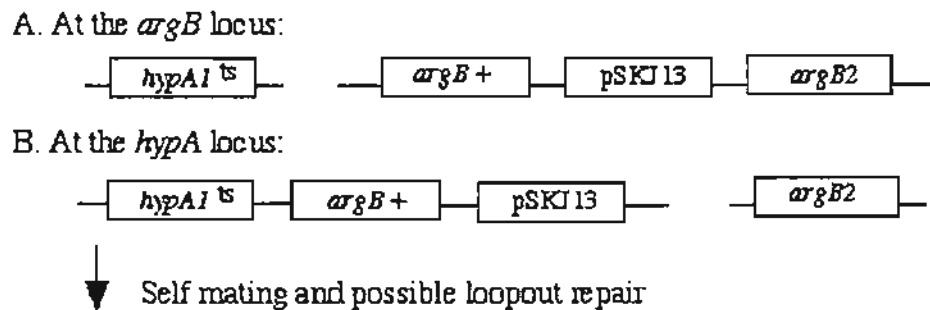Self mating and possible loopout repair

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

A. At the *argB* locus:

*hypAI*ts

argB+

argB2

pSKJ 13

*hypAI*ts

argB+ or *B2*

B. At the *hypA* locus:

argB2

*hypAI*ts

pSKJ 13

argB+

argB2

hypA+ or *AI*