

A deluxe cosmid vector for transformation of filamentous fungi

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The cosmid shuttle vector AN26 for the transformation of some filamentous fungi to hygromycin B resistance was constructed from the plasmid vector AN7-1. The features of the cosmid are: (1) a *Bam*HI cloning site, (2) *Not*I and *Sfi*I restriction sites surrounding the *Bam*HI site for easy removal of the cloned DNA insert, (3) T3 and SP6 RNA polymerase promoters outside the *Not*I and *Sfi*I sites for generation of end specific probes, and (4) dual *cos* sites separated by a unique *Cla*I site to facilitate cloning of non-size-selected DNA.

The addition of bacteriophage RNA polymerase promoters for probe synthesis, rare restriction enzyme sites for insert removal and dual λ packaging sites for cloning non-size-selected DNA have simplified the manipulation and analysis of cosmid clones and led to an increase in their use for genome analysis. We have incorporated these features into a cosmid shuttle vector for transformation of some filamentous fungi to hygromycin B resistance.

The cosmid AN26 (see Figure 1) is derived from the plasmid vector AN7-1 (Punt *et al.* 1987 Gene **56**:117-124). The plasmid was digested with *Bgl* II and *Hind* III and a 4.1 kb DNA fragment was cloned into phagemid TZ19R. The fragment contained the promoter of the glyceraldehyde-3-phosphate dehydrogenase (*gpdA*) gene from *Aspergillus nidulans* (Punt *et al.* 1988 Gene **69**:49-57) fused to the *Escherichia coli* hygromycin B phosphotransferase (*hph*) coding sequence (Gritz and Davies 1983 Gene **25**:179-188) followed by the *A. nidulans trpC* gene terminator (Mullaney *et al.* 1985 Mol. Gen. Genet. **199**:37-45). Site-directed mutagenesis was performed to remove a *Bam*HI site and 154 bps of *trpC* coding sequence that was part of the terminator fusion. Complementary oligonucleotides containing *Kpn*I adapters, T3 and SP6 RNA polymerase promoters (Basu *et al.* 1984 J. Biol. Chem. **259**:1993-1998, Brown *et al.* 1986 Nucl. Acids Res. **14**:3521-3526) and *Sfi*I, *Bam*HI and *Not*I restriction enzyme sites were annealed and ligated into the *Kpn*I site proximal to the *hph* gene fusion. The fragments COS 1 and COS 2 which contain the λ packaging sites (Miwa and Matsubara 1982 Gene **20**:267-279) were products of polymerase chain reaction amplification of λ DNA. COS 1 was blunt-end ligated to a filled-in *Hind*III site distal to the *trpC* terminator. COS 2 was ligated to an *Xba*I site in the *trpC* terminator and a reconstructed *Hind*III site proximal to COS 1. This operation removed 45 bps of *trpC* terminator and 19 bps of pUC18 sequence carried over from pAN7-1. The two packaging sites were now separated by a unique *Cla*I restriction enzyme site. This site is easily digested after propagation in a *dam*- *E. coli* strain.

The sequence of AN26 was assembled from the published sequences of its components and from the results of construct verification. It is deposited in GenBank as accession U09715. The modifications that were made in constructing AN26 from AN7-1 had no effect on transformation frequency, as judged by transformation of *Leptosphaeria maculans*. The cosmid is available to interested researchers upon request from the Fungal Genetics Stock Center and this

laboratory.

Figure 1. Cosmid AN26. The abbreviations denote the following: T3, SP6, bacteriophage RNA polymerase promoters; P_{gpd}, promoter of the *A. nidulans* *gpdA* gene; hph, *E. coli* *hph* gene coding sequence; T_{trpC}, terminator sequence from the *A. nidulans* *trpC* gene; COS1, COS2, sequences specifying λ phage packaging. The thin black line denotes the phagemid TZ19R.

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