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Abstract

Several selectable genes have been reported for construction of filamentous fungal transformation vectors. Among the most widely used is the *hygB* (also known as *hph*) gene of *E. coli*, which is generally useful because the corresponding selective agent (hygromycin B) is toxic to wild type strains of many fungi and because scoring of transformants is usually unambiguous. We, and others (Avalos et al. 1989 Curr. Genet. 16:369-372), have found that the same merits are evident using bialaphos (or phosphinothricin) as a selective agent and the *bar* gene (DeBlock et al. 1987 EMBO J. 6:2513-2518), which encodes phosphinothricin acetyltransferase, as a selectable marker. We report here the construction of three vectors which carry bar as the selectable gene and have easily exchangeable parts as well as convenient cloning sites.

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Versatile fungal transformation vectors carrying the selectable bar gene of *Streptomyces hygroscopicus*

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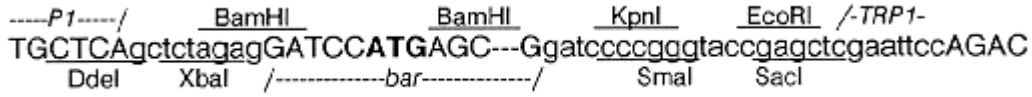
Several selectable genes have been reported for construction of filamentous fungal transformation vectors. Among the most widely used is the *hygB* (also known as *hph*) gene of *E. coli*, which is generally useful because the corresponding selective agent (hygromycin B) is toxic to wild type strains of many fungi and because scoring of transformants is usually unambiguous. We, and others (Avalos et al. 1989 Curr. Genet. 16:369-372), have found that the same merits are evident using bialaphos (or phosphinothricin) as a selective agent and the *bar* gene (DeBlock et al. 1987 EMBO J. 6:2513-2518), which encodes phosphinothricin acetyltransferase, as a selectable marker. We report here the construction of three vectors which carry bar as the selectable gene and have easily exchangeable parts as well as convenient cloning sites.

The first plasmid (pBP1) was constructed as follows. A 575 bp *Bam*HI fragment carrying the bar coding region was inserted into the *Bam*HI site of pUC18 (Yanish-Perron et al. 1985 Gene 33:103). A 631 bp *Sal*I-*Dde*I fragment carrying the *Cochliobolus heterostrophus* Promoter 1 element (Turgeon et al. 1987 Mol. Cell. Biol. 7:3297-3305) was end-filled, attached to *Xba*I linkers, and inserted into the pUC18 *Xba*I site immediately 5' of the *Bam*HI site, thus creating a Promoter 1::*bar* transcriptional fusion (Fig. 1). The second plasmid (pBP1T) was made by inserting a 470 bp *Acc*I fragment (blunt-ended and attached to *Eco*RI linkers) from the 3' untranslated region of the *C. heterostrophus TRP1* gene (Turgeon et al. 1986 Gene 42:79-88) into the *Eco*RI site of pBP1, thus providing a fungal terminator (Fig. 1). The junction regions were sequenced as shown below:



Restriction enzyme sites are underlined or overlined. Linker and vector sequences are in lower case letters. The 3' end of the Promoter 1 (P1) fragment is shown fused to the *Xba*I linker. Both ends of the *Bam*HI fragment carrying the *bar* gene are shown (internal sequences are omitted); the start codon is in bold type. The 5' end of the *TRP*1 terminator fragment is shown fused to the *Eco*RI linker.

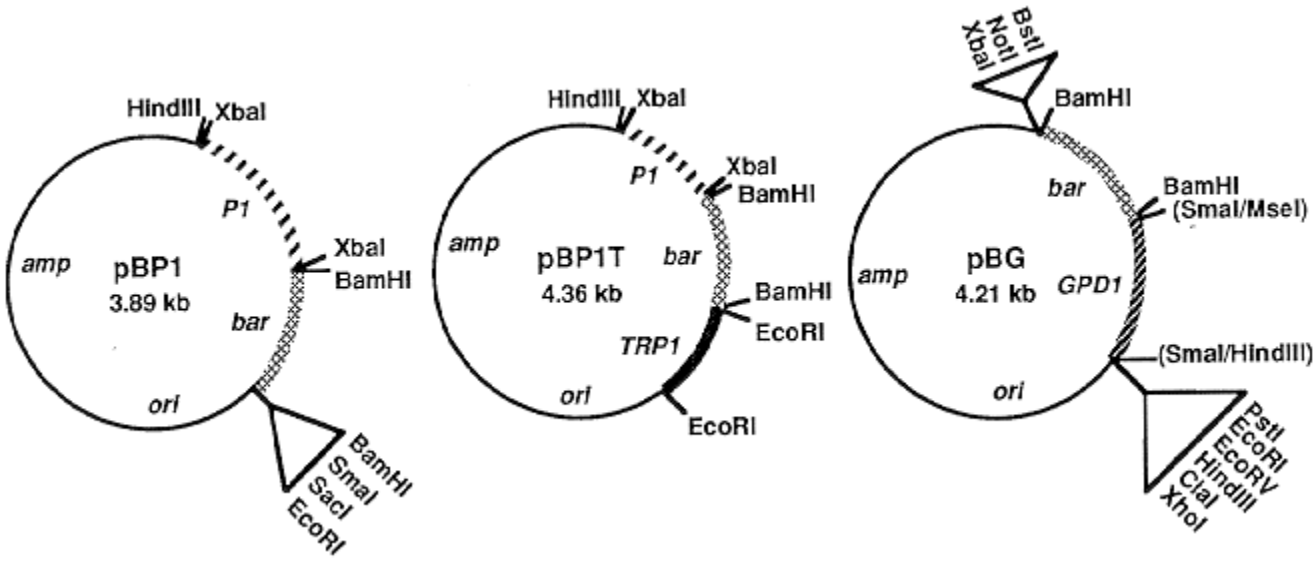


Figure 1. Construction of plasmids pBP1, pBP1T and pBG. Steps in construction are described in the text. Restriction enzyme sites shown are either unique (for cloning) or points at which the sequences indicated in the text were inserted. *amp = E. coli* ampicillin resistance gene; *ori = E. coli* origin of replication; *P1 = C. heterostrophus* Promoter 1; *bar = E. coli* bialaphos resistance gene coding region; *TRP1 = C. heterostrophus* tryptophan biosynthetic gene terminator; *GPD1 = C. heterostrophus* glyceraldehyde-3-phosphate dehydrogenase gene promoter.

An important feature of these plasmids is that their relevant parts (promoter, coding region, terminator) can be readily removed or exchanged with other sequences, simply by digestion with the appropriate enzyme (*Xba*I, *Bam*HI or *Eco*RI) and religation with or without a substitute fragment attached to the proper linkers. Note that some of the unused polylinker sites of pUC18 are no longer unique because they are also found in one or more of the inserts. Remaining cloning sites are *Eco*RI, *Sma*I, *Sac*I and *Hin*dIII for pBP1 and *Hin*dIII only for pBP1T.

The third plasmid (pBG) was made by inserting the 575 bp *Bam*HI *bar* fragment into the *Bam*HI site in the polylinker of the Bluescript vector pIIKS+. A 675 bp *Hin*dIII-*Mse*I fragment bearing the promoter of the *C. heterostrophus GPD1* gene (VanWert and Yoder 1992 Curr. Genet. in press), was end-filled and inserted into the *Sma*I site of pIIKS+, just 5' of the *bar* gene, thus creating a *GPD1* promoter::*bar* transcriptional fusion (Fig. 1). A combination of sequencing and restriction enzyme analysis confirmed the junction regions as shown below.

/----------*GPD1* promoter----------/ /----------*bar*----------/
cccAGCTTTTGCC--------AGTCTATTAggggGATCC**ATG**AGC--------CTAGgatcc
(SmaI/HindIII) (MseI/SmaI) BamHI BamHI

Conventions are as described above for pBP1T. Restriction enzyme sites in parentheses are nonfunctional.

Conventions are as described above for pBP1T. Restriction enzyme sites in parentheses are nonfunctional.

All three plasmids were used to transform *C. heterostrophus*, using standard procedures (Turgeon et al. 1987 Mol. Cell. Biol. 7:3297-3305). The protoplast regeneration medium for

selection of transformants was modified to contain only osmoticum and Cochliobolus minimal salts (Leach et al. 1982 J. Gen. Microbiol. 128:1719-1729), solidified with 1% agarose and containing either bialaphos or phosphinothricin at a final concentration of 50-100 ug/ml. Complex media were avoided since phosphinothricin (a synthetic compound also known as glufosinate-ammonium, an analog of L-glutamic acid) specifically inhibits glutamine synthetase. Bialaphos, a naturally-occurring tripeptide consisting of phosphinothricin and two residues of L-alanine, is toxic to cells after it is converted to phosphinothricin by endogenous cellular peptidases which remove its L-alanine residues.

The transformation frequency with each of the plasmids was 1-10 fast-growing and 50-500 slow-growing colonies/ug plasmid DNA, comparable to the frequencies obtained using similar plasmids but with the *hygB* gene substituted for *bar*. Integration of either pBP1 or pBP1T into chromosomal DNA occurred at both Promoter 1 and at ectopic sites. Single and multiple plasmid copies were observed at either type of site. When transformants were crossed to wild type, the bar gene segregated as a single mendelian element, indicating that integration occurred at a single site in each case. pBP1 and pBP1T were also used to transform *Colletotrichum graminicola*, using procedures similar to those described for *C. heterostrophus*.

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