A series of six compact fungal transformation vectors containing polylinkers with multiple unique restriction sites

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Abstract
In comparison with transformation vectors available for use in E. coli or yeast, there has been relatively little development of vectors for use in filamentous fungi. For example, expression yeast vectors carrying polylinkers flanked by promoters and terminators are available for various uses but such vectors have not been in the public domain for researchers working with filamentous fungi.

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A series of six compact fungal transformation vectors containing polylinkers with multiple unique restriction sites

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In comparison with transformation vectors available for use in E. coli or yeast, there has been relatively little development of vectors for use in filamentous fungi. For example, expression yeast vectors carrying polylinkers flanked by promoters and terminators are available for various uses but such vectors have not been in the public domain for researchers working with filamentous fungi. In addition, in E. coli, many of the most useful, compact vectors carry polylinkers into which insertion can be monitored using blue/white screening but we are only aware of one such vector designed for use in filamentous fungi (Nelson and Metzenberg, 1992 Fungal Genet. Newsl. 39:59-60).

The most compact available fungal selectable marker is the Ignite/basta-resistance (bar) gene (Avalos et al. 1989 Curr. Genet. 16:369-372; Straubinger et al. 1992 Fungal Genet. Newsl. 39:82-83; Pall, this volume). We report here on the construction of six compact fungal transformation vectors carrying the bar gene. Four of these carry polylinker regions subcloned from pGEM5Zf(+), pGEM7Zf(+), and pBluescriptII KS+ (Promega Biotech and Stratagene). The other two are expression vectors with numerous useful features.

The first four of the vectors are shown in Figure 1 and described in the text. Three of them (pBARGEM5-1, pBARGEM7-1, and pBARKS1) are each about 4.5 kb and all four carry the bar gene under control of the Aspergillus nidulans trpC promoter. The polylinkers from the pGEM and Bluescript vectors are shown at the top of each restriction map, with the four to eight sites that are unique in each construct underlined.

Two important points about these four vectors are as follows: Insertion of markers into these polylinkers will, in most cases, disrupt the function of the lacZ` gene which can be monitored by blue/white screening in medium containing Xgal using such E. coli host strains as DH5` or SURE. These vectors all replicate at relatively high copy number in E. coli, allowing relatively easy isolation of their DNA.

One vector, pBARGEM7-2, shown in (Figure 1) carries the lox NotI lox sequence derived from the pSE936 vector of Elledge et al. (1991, Proc. Natl. Acad. Sci. USA 88:1731-1735), as shown in the the left top part of its restriction map. This sequence has several important features that are described below.

Two expression vectors were constructed carrying the N. crassa mtr or, alternatively, the A. nidulans gpdA promoter and the A. nidulans trpC terminator flanking a polylinker with multiple unique sites. The BamHI to Kpnl region of the polylinkers was subcloned from pBluescriptII KS+. The mtr promoter cloned into pBARMTE1 (fig. 2) is composed of nucleotides 1 through
306 (SalI site) of the insert of the pCVN2.9 plasmid described previously by Koo and Stuart 
(1991, Genome 30:198-203). It was previously thought that the large open reading frame of the 
mtr gene started at position 975 (Koo and Stuart, op cit) of this sequence but Davin Dillon and 
David Stadler (personal communication) have recently shown that it actually starts upstream, 
possibly at position 347. The sequence of the gpdA promoter cloned into pBARGPE1 was from 
position -679 to position +53 (Punt et al. 1988 Gene 69:49-57). Both expression vectors are 
designed to express genes or cDNAs under their own initiation codons. In order to test the 
activity of these promoter/terminator combinations, precursor plasmids of pBARMTE1 and 
pBARGPE1, containing the same promoter/terminator sequences but without any fungal 
selectable markers were taken and the coding sequence of the bar gene devoid of any other 
promoter or terminator activity was inserted into their polylinkers. These were then used to 
transform N. crassa, selecting for Ignite/basta-resistance. Both of these constructs were active in 
generating basta-resistant transformants although about 2 to 3 times as many transformants were 
produced by the mtr construct as by the gpdA construct. We infer that both of these 
promoter/terminator combinations are active in N. crassa but that the mtr construct may be 
somewhat more active than is the gpdA construct.

The lox-NotI-lox sequence, found above in pBARGEM7-2, is present in the upper left region of 
the pBARMTE1 and pBARGPE1 plasmid maps (Figure 2). This sequence has been used by 
Elledge et al. (1991 Proc. Natl. Acad. Sci. USA 88:1731-1735) and by us (Brunelli and Pall, 
unpublished) to attach plasmids to the NotI containing arms of a derivative of lambda-gt7. This 
converts a plasmid vector into a lambda vector of a special type. Because the lox sites are sites of 
site-specific recombination catalyzed by the Cre protein, lambda infection of E. coli strains 
carrying a cre gene leads to excision of the plasmid sequence from the lambda vector. This 
"automatic excision" of the plasmid gives these vectors the advantages of both lambda and 
plasmids - the efficient packaging, infection and storage of large inserts in lambda - and the 
compact size and easy handling of plasmids. The EcoRI and XhoI sites in the polylinker of these 
vectors are unique not only in the plasmid versions but also in the lambda versions, allowing 
these to be used for construction of genomic and cDNA libraries. We expect to be inserting each 
of these three lox-NotI-lox-containing plasmids into lambda shortly.

The plasmids described are available through the Fungal Genetics Stock Center.

The bar gene in these constructs is active in E. coli, producing resistance to 400 micrograms/ml 
of Ignite/basta added to mineral salts/glucose media.

We plan to replace the bar gene in some these constructs with the hygromycin B resistance gene 
(hph or hyg) to provide an alternative selectable marker and also change the pattern of unique 
sites in the polylinkers.
Figure 1. Plasmid maps of four fungal transformation vectors. Each carries the bar gene as a selectable marker (lower left portion of map), a polylinker and lacZalpha sequence from pGEM or pBluescript vectors, including promoters from T3, T7 and/or SP6, and ampicillin-resistance gene and pUC type origin for growth and selection in E. coli. The pBARGEM7-2 plasmid also contains the lox-NotI-lox sequence allowing for attachment to lambda and site-specific recombination between lox sites.
Figure 2. Plasmid maps of two fungal expression vectors. Each plasmid carries a polylinker with 5 or 6 unique restriction sites flanked on the right by a fungal promoter directing transcription into the polylinker and flanked on the left by the trpC terminator. Both plasmids carry a bar selectable marker and a lox-NotI-lox site for insertion into lambda and for site-specific recombination between lox sites.