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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.

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 KpnI region of the polylinkers was subcloned from pBluescriptII KS+. The mtr promoter cloned into pBARMTE1 (fig. 2) is composed of nucleotides 1 through

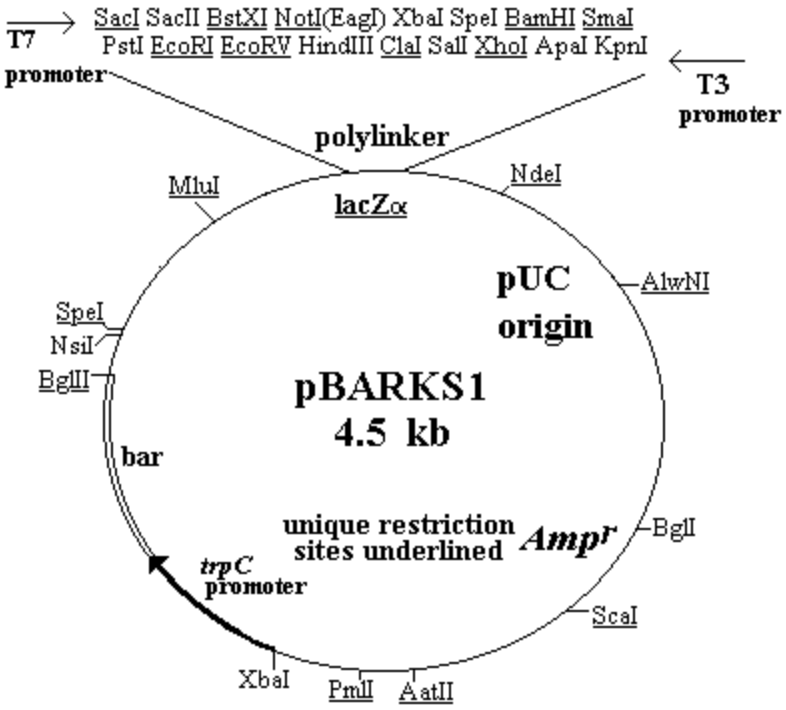
306 (SalI site) of the insert of the pCVN2.9 plasmid described previously by Koo and Stuart (1991, Genome 30:198-203). It was prevously thought that the large open reading frame of the mtr gene started at position 975 (Koo and Stuart, op cit) of this sequence but Davin Dillon and David Stadler (personal 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 libaries. 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.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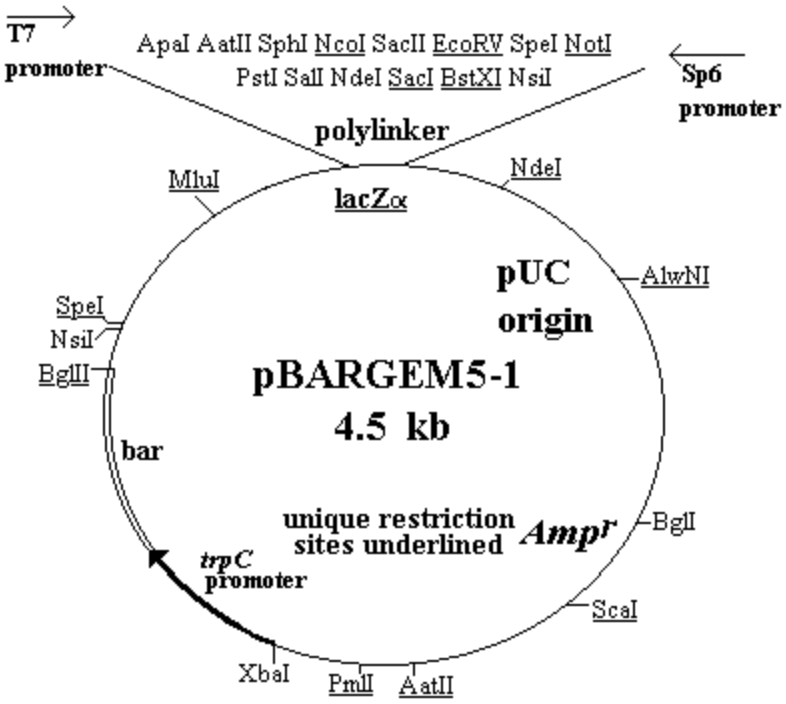
promoter
SacI SacII BstXI NotI(EagI) XbaI SpeI BamHI SmaI
PstI EcoRI EcoRV HindIII ClaI SalI XhoI ApaI KpnI
T3
promoter
polylinker
MluI
lacZα
NdeI
pUC
origin
AlwNI
SpeI
NsiI
BglII
pBARKS1
4.5 kb
bar
unique restriction
sites underlined
Ampr
BglI
trpC
promoter
ScaI
XbaI
PmlI
AatII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promoter
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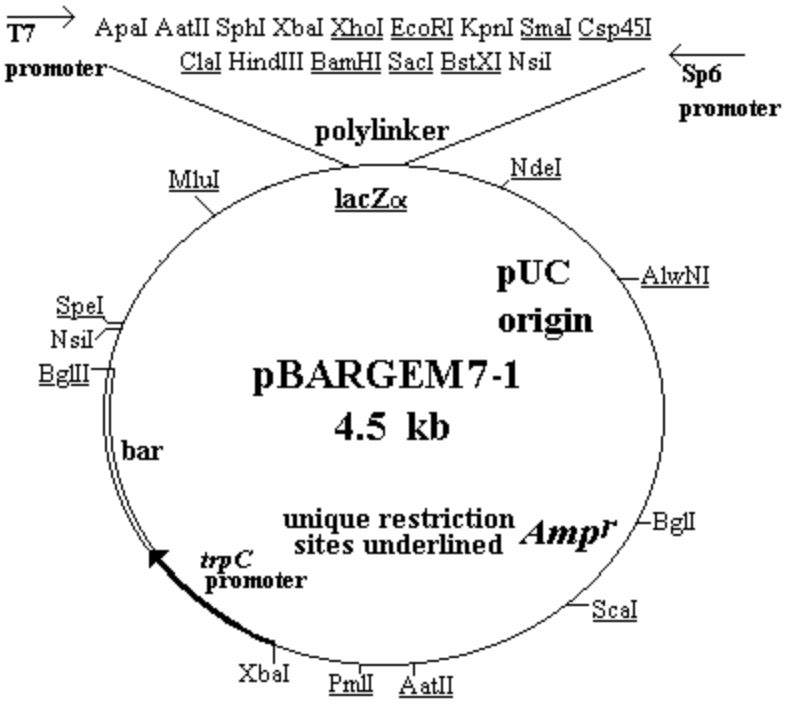
PstI SalI NdeI SacI BstXI NsiI
Sp6
promoter
polylinker
MluI
NdeI
lacZα
pUC
origin
AlwNI
SpeI
NsiI
BglII
pBARGEM5-1
4.5 kb
bar
unique restriction
sites underlined
Ampr
BglI
trpC
promoter
ScaI
XbaI
PmlI
AatII


T7
promoter
ApaI AatII SphI XbaI XhoI EcoRI KpnI SmaI Csp45I
ClaI HindIII BamHI SacI BstXI NsiI
Sp6
promoter
polylinker
MluI
NdeI
lacZα
pUC
origin
AlwNI
SpeI
NsiI
BglII
pBARGEM7-1
4.5 kb
bar
unique restriction
sites underlined
Ampr
BglI
trpC
promoter
ScaI
XbaI
PmlI
AatII

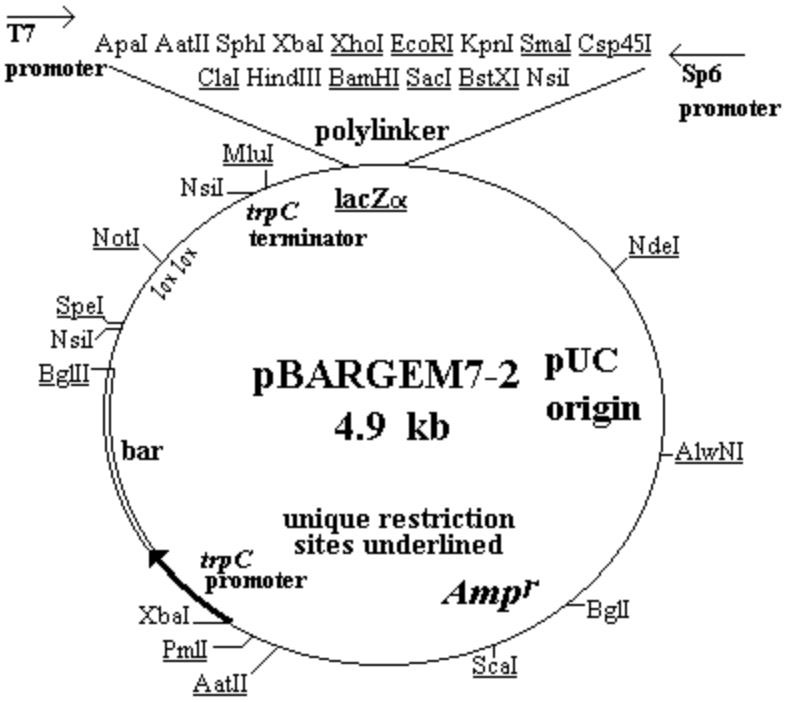
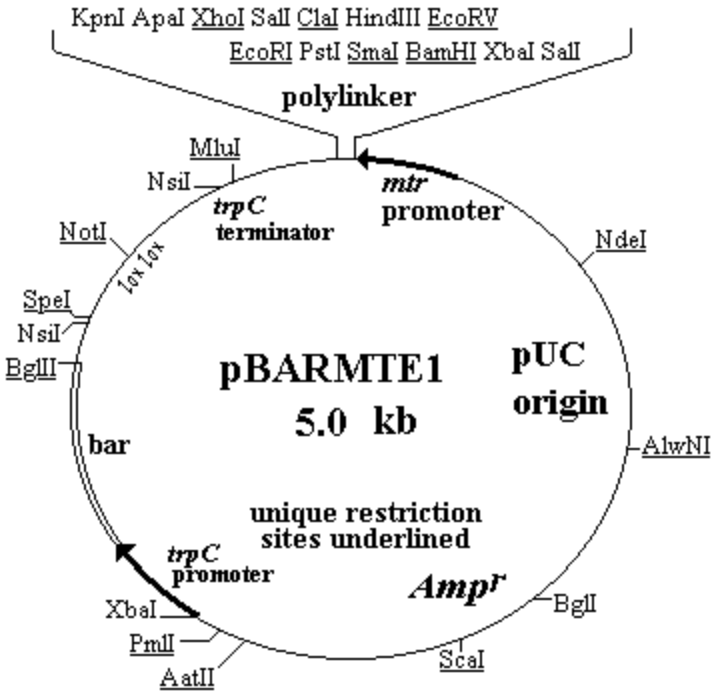


Figure 1. Plasmid maps of four fungal transformation vectors. Each carries the bar gene as a selectable marker (lower left portion of map), a polylinker and lacZalpha sequence from pGEM or pBluescript vectors, including promoters from T3, T7 and/or SP6, and ampicillin-resistance gene and pUC type origin 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.


KpnI ApaI XhoI SalI ClaI HindIII EcoRV
EcoRI PstI SmaI BamHI XbaI SalI
polylinker
MluI
NsiI
trpC
terminator
mtr
promoter
NotI
lox lox
NdeI
SpeI
NsiI
BglII
pBARMTE1
5.0 kb
pUC
origin
bar
AlwNI
unique restriction
sites underlined
trpC
promoter
Ampr
BglI
XbaI
PmlI
AatII
ScaI

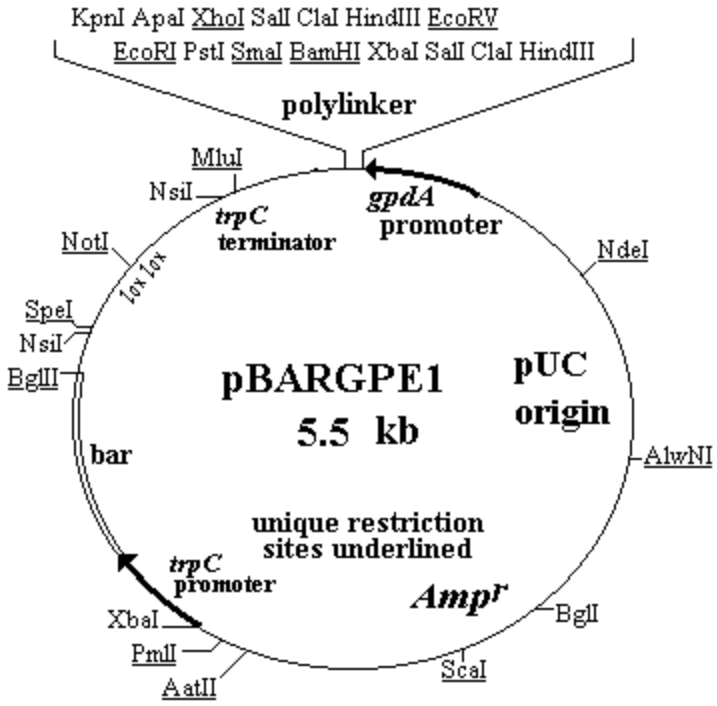


Figure 2. Plasmid maps of two fungal expression vectors. Each plasmid carries a polylinker with 5 or 6 unique restriction sites flanked on the right by a fungal promoter directing transcription into the polylinker and flanked on the left by the trpC terminator. Both plasmids carry a bar selectable marker and a lox-NotI-lox site for insertion into lambda and for site-specific recombination between lox sites.