Vectors for construction of translational fusions to beta-galactosidase

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The use of *lacZ* as a reporter gene requires appropriate plasmid constructions to produce fusions of fungal promoter and translations initiation sequences to the *E. coli lacZ* gene. I have constructed three plasmids which may facilitate construction of *lacZ* fusions.

The three plasmids are identical except for a *Bam*HI site adjacent to a unique *Sma*I site in three different reading frames with respect to *lacZ*. Thus any blunt-ended fragment containing a functional promoter and translation start site can be used to produce an in frame translational fusion by choosing the appropriate plasmid vector.

The vector contains *E. coli lacZ* from pMC1871 (Shapira et al. 1983, Gene 25:71-82) followed by the terminator of *Aspergillus nidulans trpC* obtained from plasmid DH25 (Cullen et al. 1987, Gene 57:21-26). It also contains the truncated *his-3* gene from pH303 (see accompanying paper) which allows targeted integration of the *lacZ* fusions at the *his-3* locus in FGSC strain 462. The vector for these plasmids is pSP72 (available from Promega Corporation, Madison, WI).

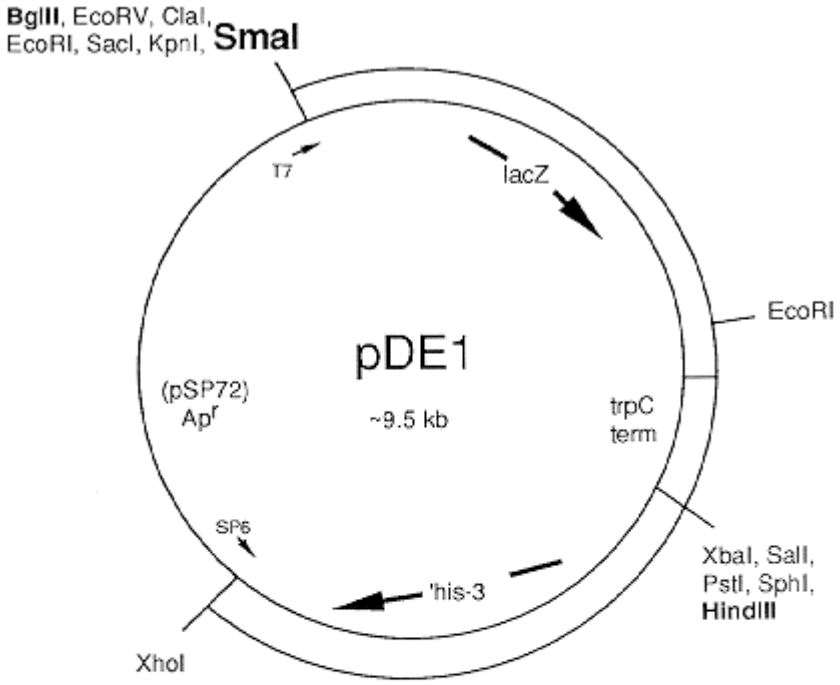
Figure 1 shows a map of pDE1. The plasmid is approximately 9.5 kb in size. The vector sequence of pSP72 runs from the *Xho*I site to the *Sma*I site in all three plasmids. The *Bam*HI site was used to clone the *Bam*HI to *Xba*I fragment from pDH25 containing the *trpC* terminator. The *Hin*dIII to *Sma*I fragment containing the *his-3* gene segment from pH303 was cloned into the pSP72 polylinker at *Hin*dIII to *Pvu*II. Finally the *Bam*HI fragment containing *lacZ* was cloned into the *Bam*HI site adjacent to the *Sma*I site of the pSP72 polylinker. The *Bam*HI site at the *lacZ*/*trpC* terminator junction was destroyed by cleavage with *Bam*HI and filling of the 5' overhangs followed by ligation. Plasmids pDE1, pDE2 and pDE3 differ at the *Bam*H1 site adjacent to the *Sma*I site. The plasmid with this *Bam*H1 site left intact was designated pDE3. pDE3 was digested with *Bam*HI and ligated following i) mung bean nuclease digestion to generate pDE1 or ii) Klenow fragment of DNA polymerase and dNTP's to generate pDE2.

The nucleotide sequence of this region was determined on both strands to verify the sequence shown in Fig. 1 for each plasmid. The arrows show the cleavage point of *Sma*I. The codon triplets of *lacZ* following the *Sma*I site are also shown. The unique sites in the plasmids are shown in bold: *Bgl*II, *Sma*I and *Hin*dIII. pDE3 also contains the unique *Bam*H1 site adjacent to *Sma*I.

The *lacZ*/*trpC* terminator can be removed as a cassette with *Bgl*II (or *Sma*I) and *Hin*dIII for transfer to new plasmids (for example, a plasmid conferring benomyl resistance). Another feature of the plasmid is the T7 and SP6 promoters for synthesizing RNA from each direction for making probes for Southern analysis. The "T7" sequencing primer and *lacZ* "-40" primers can be used for sequencing fragments cloned into the plasmids for verification of constructions.

The *lacZ* gene of pDE3 is in frame with an upstream ATG in the vector sequence and potentially could be used to make transcriptional fusions from promoters cloned into the *Bgl*II site; however, the ATG has a poor sequence context for translation in *N. crassa*.

The possible limitation of using these plasmids in the low transformation frequencies obtained by requiring integration at the *his-3* locus in FGSC strain 462. These plasmids have been deposited with the Fungal Genetics Stock Center. This work was performed in the laboratory of Dr. Charles Yanofsky.



KpnI SmaI

pDE1-- GGTACC CGG GCC GTC GTT TTA--lacZ

pDE2-- GGTAC CCG GGG ATC GAT CCC GTC GTT TTA--lacZ

pDE3-- GGTACCC GGG GAT CCC GTC GTT TTA--lacZ