Small Broad-Host-Range lacZ Operon Fusion Vector with Low Background Activity

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The use of gene fusion technologies for transcriptional analysis of gene expression, especially of those genes whose products are difficult to assay, has been well documented (12–15). Many narrow and broad-host-range plasmid-based systems are available for constructing such gene fusions (6,7,14). To circumvent some of the inherent problems of plasmid-based systems, which have been discussed before (3), systems have been developed for single-copy, chromosomal insertion of gene fusions (4,9,14). For some applications (e.g., deletion mapping of regulatory regions or use in bacteria for which no integration-proficient vectors are known), plasmid-based fusions are still indispensable. The most useful vectors use a promoterless β-galactosidase (β-gal) gene, lacZ, as a reporter. However, most broad-host-range lacZ-based vectors available to date exhibit some potentially serious drawbacks, including (i) high levels of background expression due to transcription from plasmid-encoded promoters and/or efficient translation of low levels of lacZ message due to a strong consensus Shine-Dalgarno (SD) sequence, (ii) a lack of unique cloning sites, and (iii) large size due to the presence of unknown and usually unnecessary nucleotide sequences. To address these issues, a new small broad-host-range transcriptional lacZ operon fusion vector was constructed and tested in Pseudomonas aeruginosa.

The new lacZ vector, pTZ110 (Figure 1A), was obtained in two steps. First, a 1544-bp blunt-ended PstI-StuI fragment from pSF2 (10), containing an origin of transfer, the ori1600 broad-host-range origin of replication and its associated replication protein (16), was ligated into a BalI site located 271 nucleotides downstream of the lacZ gene of pGE593 (2) to obtain pTZ100. This operon fusion vector contains unique EcoRI, SmaI, and BamHI sites upstream of a promoterless lacZ gene. Second, pTZ110 was obtained by ligating a 26-bp oligonucleotide linker with EcoRI and BamHI overhangs between the same sites of pTZ100. The resulting operon fusion vector contains a multiple cloning site with six unique restriction enzyme cleavage sites, stop codons in the three reading frames, and a modified SD sequence, which is close to consensus but not too strong. Other abbreviations: bla, β-lactamase-encoding gene; ori, pBR322 origin of replication; ori1600, pRO1600 origin of replication; oriT, origin of transfer; rep, ori1600 replication protein. The sequence of pTZ110 was submitted to GenBank® and assigned accession no. AF376056. (B) Transcriptional activity of the glpFK-operon promoter present on pPFK1 was assessed by measuring either β-gal activities in strains PAO1 (wild-type) or PAO160 (glpR) containing the indicated plasmids. The strains were grown aerobically at 37°C in LB medium (Invitrogen, Carlsbad, CA, USA) to log phase (absorbance at 540 nm of approximately 0.7). β-gal expression was measured in chloroform/SDS-permeabilized cells, and activity units were calculated as previously described (5). Samples were assayed in triplicate, and the average values are given above the columns.
con, this vector replicates with an intermediate copy number in *E. coli* and using ori1600 with a low copy number in *Pseudomonas* (10–15 copies) (8). These copy numbers allow for easy isolation and manipulation of plasmid DNA from *E. coli* hosts, while they are at the same time desirably low for analysis of gene expression in *Pseudomonas* hosts.

To assess the functionality of pTZ110, a glpF-lacZ transcriptional fusion was constructed and analyzed. The *glpF* gene is part of the *P. aeruginosa* *glpFK* operon, whose expression is negatively regulated by the glycerol-3-phosphate regulon repressor, GlpR (11). The amino-terminal 14 codons of *glpF* and its upstream 219-bp regulatory region containing a single GlpR operator site were PCR amplified from pPS585 (11). The reactions contained the 24-mer universal *lacZ* primer and a *glpF*-specific primer (5′-CTCGGACA-GGCTTTGCGGACAG-3′), which introduced a single base change (lowercase letter) and therefore a *Stu*I site (underlined) at codon 14 of *glpF*. The resulting PCR fragment was digested with EcoRI and *Stu*I, and a 282-bp EcoRI-*Stu*I fragment was gel-purified and ligated between the EcoRI and *Sma*I sites of pTZ110 to form pPFK1. In this construct, translation of an 18-amino-acid GlpF fusion peptide is terminated at the second TAA codon (1) followed by selection on LB media (3) containing 100 µg/mL carbenicillin. Alternatively, plasmids were transferred by electroporation (1) followed by selection on LB medium containing 100 µg/mL carbenicillin. β-gal expression from the *glpF-lacZ* operon fusion was then measured after growth in the appropriate media (Figure 1B). As expected, *glpF-lacZ* was expressed in a GlpR-dependent manner, and very low background β-gal activity was observed in the pTZ110-containing strains. β-gal expression in the wild-type strain is not completely repressed because LB medium contains traces of the inducer glycerol-3-phosphate and *glpFK* operon expression is easily induced by dislodging GlpR from the single operator site (11).

In summary, with the construction of the pTZ110 *lacZ* operon fusion vector, several problems associated with similar broad-host-range fusion vectors have been resolved. First, background β-gal expression levels are minimized because of the incorporation of an SD sequence that is close to consensus but not too strong. Second, cloning of promoter-containing fragments is facilitated by six unique cloning sites, including two blunt-end-generating restriction sites. Third, the complete nucleotide sequence of the new small 7856-bp vector is known, and it contains only fully functional DNA sequences. Although this broad-host-range vector has thus far only been tested in *P. aeruginosa*, it will undoubtedly be useful with the many Pseudomonads and other bacteria in which the *ori1600* replicon is functional (10).

REFERENCES


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