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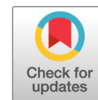

Broad-Host-Range Plasmids for Constitutive and Inducible Gene Expression in the Absence of Antibiotic Selection

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ABSTRACT Plasmid vectors are a valuable research tool for characterizing bacterial gene function, but there is a limited range of plasmids that are functional in nonmodel bacterial species. Described here is a set of broad-host-range plasmids modified for stability in the absence of antibiotic selection and for gene expression manipulation.

For many nonmodel bacterial species, plasmid vectors for gene expression manipulation are unavailable. The broad-host-range pBBR1-MCS series of plasmids was developed for work in Gram-negative bacteria and have been used in a wide range of bacterial species (1). Although they are highly versatile and relatively stable under a variety of conditions (2), these vectors are typically used with antibiotic selection, and control of gene expression level is limited. Addition of a toxin-antitoxin system (*pemI-pemK*) from the slow-growing plant pathogen *Xylella fastidiosa* further increased plasmid maintenance over extended periods of time for bacterial gene complementation during plant infection (plasmid pBBR5pemIK) (3–5). This announcement describes four additional plasmid vectors that were derived from pBBR5pemIK for rapid cloning and control of gene expression in a variety of applications involving nonmodel Gram-negative bacteria.

Plasmid pBBR4pemIK (Fig. 1A) is an alternate version of pBBR5pemIK, derived from the pBBR1-MCS4 backbone (ampicillin selection) rather than from pBBR1-MCS5 (gentamicin selection). This vector has a multiple cloning site for restriction cloning and has been modified to contain the *pemI-pemK* toxin-antitoxin system from *X. fastidiosa* for increased stability in the absence of antibiotic selection. pBBR4pemIK was created by cloning a 700-bp fragment containing the *pemI* and *pemK* open reading frames from *X. fastidiosa* strain Riv11 (5) into the EcoRI restriction site of pBBR1-MCS4. A Gateway cloning-compatible version, pBBR4pemIK-GW, also was developed for use with rapid cloning methods using the Gateway vector conversion kit (Invitrogen; Fig. 1B).

Alternate promoters were added to the Gateway cloning-compatible pBBR5pemIK-GW vector for constitutive overexpression (pBBR5pemIK-pKan; Fig. 1C) or arabinose-inducible expression (pBBR5pemIK-pBAD; Fig. 1D) of a gene of interest. Constitutive expression plasmid pBBR5pemIK-pKan was created using PCR amplifying a 139-bp fragment containing promoter elements from the kanamycin resistance cassette on plasmid pCR2.1 (Invitrogen) with SacI and XbaI restriction sites added to the PCR primers. This promoter fragment was cloned directionally into the SacI and XbaI restriction sites of pBBR5pemIK-GW so that the promoter would be in frame with the Gateway recombination site. Plasmid pBBR5pemIK-pBAD for inducible expression was created by inserting a 1.2-kb fragment containing the pBAD arabinose-inducible promoter and *araC* regulatory protein open reading frame into the SacI and XbaI sites of pBBR5pemIK-GW. Plasmid characteristics, sequencing primers, and GenBank accession numbers for the full sequences are listed in Table 1. Primer sequences are as follows: M13-rev, 5′-GTCATAGCTGTTTCCTG-3′; pBBR4-Seq-Fwd, 5′-GGCTTCTTTCGTTAAGT-3′; and pBBR5-Seq-Fwd, 5′-CAATTTCCATTCGCCATT-3′.

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FIG 1 Plasmid pBBR4pemIK for restriction cloning (A) and pBBR4pemIK-GW for Gateway cloning (Invitrogen) (B) were derived from broad-host-range vector pBBR1-MCS4 (1), which carries ampicillin resistance. Plasmid pBBR5pemIK-pKan for constitutive expression (C) and pBBR5pemIK-pBAD for inducible expression (D) were derived from Gateway cloning-compatible broad-host-range vector pBBR5pemIK-GW (3). All the vectors were modified with toxin-antitoxin system *pemI-pemK* from *Xylella fastidiosa* for stability without antibiotic selection.

TABLE 1 Plasmid characteristics

Plasmid	Characteristics	Propagation strain(s) (*Escherichia coli*)	GenBank accession no.	Sequencing primers
pBBR4pemIK	Amp^r, restriction cloning	DH10β, DH5α, JM101	MN044101	pBBR4-Seq-Fwd, M13-Rev
pBBR4pemIK-GW	Amp^r, Gateway cloning	ccdB survival 2 T1^R	MN044102	pBBR4-Seq-Fwd, M13-Rev
pBBR5pemIK-pBAD	Gm^r, Gateway cloning, arabinose-inducible promoter	ccdB survival 2 T1^R	MN044103	pBBR5-Seq-Fwd, M13-Rev
pBBR5pemIK-pKan	Gm^r, Gateway cloning, constitutive promoter	ccdB survival 2 T1^R	MN044104	pBBR5-Seq-Fwd, M13-Rev

Data availability. Plasmid sequences are available in GenBank with the accession numbers listed in Table 1. Plasmids are available from the Addgene repository (https://www.addgene.org) or by contacting the corresponding author.

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