

A Series of IncQ-Based Reporter Plasmids for Use in a Range of Gram-Negative Genera

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Many studies require expression analysis of the same gene/promoter across a range of bacterial genera. However, there is currently a lack of availability of reporters based on the broad-host-range IncQ replicon, which is compatible with a popular improved IncP transfer system that is self-transfer defective. We report IncQ *lacZ* reporter plasmids with features including (1) compatibility with IncP, IncW, and pBHR/pBBR replicons, (2) a variety of antibiotic markers (Sp-r, Sm-r, Km-r, Cm-r), (3) convenient mobilization via a novel self-transfer-defective IncP conjugation system, and (4) GenBank DNA sequences. Utility is demonstrated using three different promoters in different Gram-negative genera.

Keywords: IncQ plasmid, broad host range, IncP, pUZ8002, pQlacZ

The broad field of microbiology continues to expand to include analysis of different bacterial species with potential applications in environmental, industrial, and medical microbiology. With increasing amounts of *in-silico*-genomic and array-based data, there is continual need to have relatively simple and convenient experimental approaches to assay the expression of the same gene and/or promoter in a wide range of different bacterial backgrounds to help make conclusions about the utility and evolution of that gene/promoter. Broad-host-range reporter vectors continue to be convenient and easy-to-use tools for such approaches. Some examples of potential applications of these vectors include gene testing and design for bacterial metabolic engineering [12, 13, 20], testing of a specific gene expression requirement in mixed populations for applications such as bioremediation or bioenergy production [3, 7, 10, 14, 25], and bacterial evolution studies involving gene transfer [19,

23]. Basic experimental questions in these cases would be (1) is the promoter for “gene X” transcriptionally active in a range of different bacterial backgrounds and growth conditions? or (2) has “gene X” evolved in such a way that its expression is narrowly limited to its strain of origin or closely-related strains? However, the availability of convenient genetic tools to allow analysis of the expression of the same gene or promoter in a broad range of different bacterial genera is limited relative to commonly-used narrow-range molecular reagents. In addition, there is a lack of reporter vectors based on the IncQ replicon, which is active in a broad range of bacteria and compatible with an improved IncP plasmid-based transfer system (see below). Currently, IncQ-based reporters offer many advantages (as detailed in the text) as the vehicle of choice for broad-host-range studies.

During the course of our studies, we required a broad-host-range *lacZ* reporter vector based on the IncQ replicon, but after literature searches, we found one reference to such a vector (from many years ago: 1993) [11]. We were unable to obtain this vector, and moreover, the vector encoded resistance to a single antibiotic (gentamicin) and did not encode resistance to the range of antibiotics that we needed for selection in our studies. To fill this void in availability of these plasmids, we report here a series of plasmids that allow analysis of gene expression in a range of Gram-negative bacterial genera via transcriptional fusion to *lacZ* on an IncQ-based vector. These plasmids, termed pQlacZ1, pQlacZ2, and pQlacZ3, encode resistance to spectinomycin/streptomycin, kanamycin/spectinomycin/streptomycin, and chloramphenicol, respectively (Fig. 1).

These plasmids are able to be mobilized to different genera using a donor strain containing a co-resident IncP plasmid termed pUZ8002 that is defective for self-transfer but can efficiently mobilize other plasmids containing suitable *oriT* and DNA processing functions [1, 6, 17, 23]. The use of pUZ8002 alleviates the need for the frequently-used IncP donor strains *Escherichia coli* SM10 or S-17,

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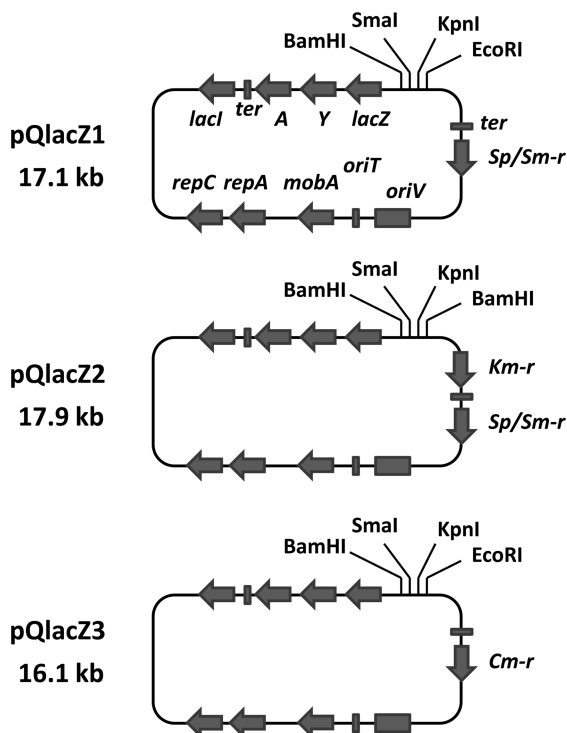


Fig. 1. Plasmid maps.

Circular plasmid maps of reporter vectors pQlacZ1, pQlacZ2, and pQlacZ3 are presented to show major features. Genes are indicated by arrows and labeled with names (the three genes *lacZYA* are indicated with a single *lacZ* designation on the *lacZ* gene). Boxes indicate the vegetative origin of replication (*oriV*), the origin of transfer (*oriT*), and transcriptional terminators (*ter*). Lines indicate the location of unique restriction sites for cloning upstream of the *lacZ* gene, with the exception of the *Bam*HI sites in pQlacZ2 where the unique two sites in the vector are shown. Maps are not drawn to scale. Plasmids pQlacZ1 and pQlacZ3 were constructed by cloning the *Bam*HI–*Sal*I fragment from pRS415 [16] into the *Bam*HI–*Sal*I sites of the IncQ plasmids pJAK13 and pJAK17, respectively [15, 24]. Plasmid pQlacZ2 is a derivative of pQlacZ1 containing a kanamycin resistance gene (obtained from pCR4-TOPO, Invitrogen, Carlsbad, CA, U.S.A.) cloned into the *Eco*RI site of pQlacZ1 (oriented away from the *lacZ* gene). Nucleotide sequences for the plasmids described here are deposited using GenBank accession numbers GU981673 (pQlacZ1), GU981674 (pQlacZ2), and GU981675 (pQlacZ3). The sources of the promoters tested here are the kanamycin resistance gene promoter from pCR4-TOPO (nucleotides 1021–1953), the 1.21 kb of DNA extending upstream of the *S. Typhimurium* STM4318 start codon, and a deletion of 0.64 kb from this fragment from the natural *Eco*RV site to the STM4318 distal end (corresponding to the STM4318 and Δ STM4318 UP fragments, respectively) [8], and the pBAD promoter and *araC* region from the plasmid pBAD18 (nucleotides 4172–1322) [5].

which contain an active Mu phage that can undetectably transfer to and randomly mutate recipient genomes [21]. The use of SM10 or S-17 can cause unwanted and undetectable background mutations in recipient strains that can affect experimental conclusions in downstream studies and applications [21]. Since many broad-host-range plasmids are based on the IncP replicon (which is incompatible with pUZ8002 and other IncP-based donor strains), the IncQ series of vectors presented here provide an option to

researchers using pUZ8002 or other plasmid-based IncP transfer systems. The IncQ plasmids described here are compatible with other broad-host-range replicons of the IncP, IncW, and pBHR–pBBR groups [18].

To demonstrate the utility of these plasmids, we inserted promoters into each vector upstream of the *lacZ* gene and mobilized each resulting construct (and corresponding promoterless vector) to a range of Gram-negative bacterial genera using a donor containing pUZ8002. Strains used as recipients were *Citrobacter freundii* ATCC 8090, *Enterobacter cloacae* ATCC 23355, *Escherichia coli* TOP10 (Invitrogen, Carlsbad, CA, U.S.A.), *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* PAK *pilA* [22], and *Salmonella Typhimurium* χ 3339 [4] (American Type Culture Collection, Manassas, VA, U.S.A.). We assayed construct-containing strains for LacZ activity as previously described [9] and compared each strain to the corresponding vector-only negative control (Table 1). To test the plasmid pQlacZ1, we individually inserted a kanamycin resistance gene promoter and the *S. Typhimurium* STM4318 gene promoter upstream of *lacZ* (see Fig. 1 legend for construct details). Additionally, we constructed another pQlacZ1 derivative termed Δ STM4318 UP (see below). To test pQlacZ2 and pQlacZ3, we inserted the arabinose-inducible pBAD promoter upstream of *lacZ* in these plasmids [5]. For pQlacZ3, the kanamycin resistance gene promoter was also separately cloned upstream of the *lacZ* gene. The data in Table 1 show that significant LacZ expression ranging from 9-fold to 2,887-fold above the vector can be obtained in a range of bacterial genera, with fully active promoters generally giving between 200–800-fold expression over the vector (with a few constructs giving 1,880–2,887-fold in certain genera). To demonstrate that these vectors can be used for molecular analysis of the expression signals in a given promoter fragment, a deletion of DNA sequence upstream of STM4318 that is essential for full expression from the STM4318 promoter was constructed and assayed for LacZ activity in pQlacZ1 (Table 1, Δ STM4318 UP) [23]. As predicted, the Δ STM4318 UP deletion fragment displayed significantly decreased reporter activity in pQlacZ1 (as compared with pQlacZ1+STM4318 promoter) in all genera tested (Table 1).

To further demonstrate that altered expression levels of the same promoter can be analyzed using these plasmids, the pQlacZ2+pBAD promoter construct was assayed for LacZ activity in the absence and presence of arabinose in LB medium. Under these two conditions, a predicted difference in pBAD activity was observed in the tested genera (approximately 10-fold between uninduced and induced) (Table 2). Note that the “uninduced” level of pBAD expression in this case (*i.e.*, absence of added arabinose) is actually 22–39-fold greater than the vector alone, most likely due to leaky pBAD activity caused by the presence of small concentrations of arabinose (about

Table 1. Fold LacZ expression above strain with vector only.

Bacterial genus	pQlacZ1			pQlacZ2		pQlacZ3	
	Promoter			pBAD	Kan	pBAD	
	Kan	STM4318	Δ STM4318				
<i>Escherichia</i>	292	496	12	297	332	272	
<i>Salmonella</i>	302	728	97	262	296	299	
<i>Pseudomonas</i>	475	180	9	468	NT	NT	
<i>Enterobacter</i>	400	553	12	650	779	681	
<i>Citrobacter</i>	961	2,010	56	504	529	538	
<i>Klebsiella</i>	1,821	2,682	103	881	638	2,887	
<i>Proteus</i>	415	463	11	338	259	268	

Values are the mean from three independent cultures with the standard deviation being 15% or less of the mean for all strains.

Strains containing pBAD were grown in the presence of 0.1% arabinose.

Differences in LacZ activity between vector-only and promoter-containing strains were found to be significant at p -value<0.001.

Basal LacZ activity in vector control strains was typically 10–40 Miller Units.

NT=not tested.

0.003%) in LB medium from the yeast extract component [2] (Table 2). Virtually identical data were obtained with the pQlacZ3+pBAD construct in the same bacterial backgrounds (data not shown). These data show that altered levels of activity from the same promoter in different genera can be observed *via* these reporter vectors.

Here we report the availability of a new series of IncQ-based, broad-host-range plasmid reporter vectors that are compatible with an improved IncP plasmid-based conjugative transfer system. Using a combination of three different promoters, the utility of these reporters has been demonstrated in a range of bacterial genera including *Escherichia*, *Salmonella*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Proteus*. We have also used this transfer system to mobilize plasmids to strains of *Agrobacterium* and *Rhizobium* (data not shown). The results indicate that the promoters driving expression of a common kanamycin resistance gene, the *S. Typhimurium* gene STM4318, and the arabinose-inducible pBAD expression cassette are all highly active (between

180- and 2,887-fold activities over the vector-only strains) in the bacterial genera tested. The STM4318 promoter has been previously shown to be detectably expressed in a range of genera both closely and more distantly related to *Salmonella* [23]. The data obtained here expand the list of genera in which this promoter is active and contribute to the evidence that the STM4318 gene has evolved in such a way that it is able to be expressed in a wide array of bacterial backgrounds. This promoter may have use in future applications where a strong constitutive promoter is needed to express genes in many bacterial types (and is not a promoter associated with antibiotic resistance genes). Future experimental directions include further development of IncQ replicons for use in broad-host-range expression studies, use of the vectors described here to analyze other promoters for broad-host-range activity, and addition of other antibiotic resistance and reporter cassettes to expand the experimental options of these plasmids.

Table 2. Fold LacZ expression above strain with vector only: Different levels of pBAD expression.

Bacterial genus	Arabinose	Vector	Promoter
		pQlacZ2	pBAD
<i>Escherichia</i>	none	1	22
	0.1%	1	226
<i>Salmonella</i>	none	1	23
	0.1%	1	261
<i>Pseudomonas</i>	none	1	39
	0.1%	1	412

Values are the mean from three independent cultures with the standard deviation being 15% or less of the mean for all strains.

LB media without added arabinose contains approximately 0.003% arabinose from the yeast extract component.

Differences in LacZ activity between induced and uninduced strains were found to be significant at p -value<0.001.

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