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RESEARCH ARTICLE

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# Construction of plasmid vectors bearing a *Not*I-expression cassette based on the *lac* promoter

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**Summary** We have constructed two plasmid vectors for cloning and expression of DNA fragments controlled by the *lac* promoter as a *Not*I-expression cassette. Whereas plasmid pSJ33 allows mobilization of the expression cassette into a wide variety of Gram-negative bacteria by RP4-mediated conjugation, the low-copy-number plasmid pSJP18Not facilitates cloning and expression in *Escherichia coli* when high gene dosage may be detrimental. In addition to their suitable cloning features (e.g. multiple cloning site, *lacZ*a fragment, compatible with ColE1-derived vectors), these plasmids are particularly useful as auxiliary vectors for cloning of the expression cassettes at the *Not*I site of mini-transposon elements [1, 2] and their eventual stable insertion into the host chromosome.

**Key words** *lac* Promoter · Broad-host-range vectors · Low-copy-number plasmids · Mini-transposons · Gram-negative bacteria

A versatile expression plasmid should allow the expression of cloned genes in as many different bacteria as possible, and therefore should be endowed with a broad host-range promoter element. The well characterized *lac* promoter (*Plac*) of *Escherichia coli* has been shown to drive expression of cloned genes in a wide variety of Proteobacteria. Such an expression can be constitutive or regulated depending on the absence or presence, respectively, of the *lacI*$^{q}$ repressor gene. A number of vectors for cloning and expression of heterologous genes under the control of *Plac* are now available, and although most of them are restricted to *E. coli* and other related enterobacterial species, some possess a wide-host-range among Gram-negative bacteria [6, 9, 12]. However, these vectors lack suitable restriction sites when further subcloning of the *Plac* fusion is required, e.g. for its integration into the bacterial chromosome through transposon delivery systems. Therefore, the aim of the present study was to construct versatile *Plac*-based expression vectors that allow easy excision of the cloned gene together with *Plac* as an independent expression cassette.

Plasmid pNot18, a pUC18 [13] derivative whose *Hae*II *lacZ*a fragment, i.e. the one containing *Plac*, the multiple cloning site (MCS) and the *lacZ*a fragment, has been substituted by an equivalent *Not*I *lacZ*a fragment, was the source of the *Plac*-based expression cassette. We have inserted this cassette as a 0.9-kb DNA fragment into the kanamycin(Km)-resistant broad-host-range plasmid pVLT33 (Fig. 1), a RSF1010 derivative and therefore able to replicate in a wide variety of Gram-negative bacteria and susceptible of efficient mobilization by the RP4 conjugation system [3]. The resultant plasmid, pSJ33, has five unique restriction sites which facilitate the cloning of DNA fragments under the transcriptional control of *Plac* (Fig. 1). Significant features of other broad-host-range *Plac*-based expression plasmids [6, 9, 12] are also present in pSJ33: (i) positive selection of recombinant plasmids on 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (XGal)-containing Luria-Bertani indicator medium [10] can be accomplished in appropriate host strains by inactivation of the a-complementation; (ii) sequencing of inserted DNA using the universal and/or reverse primers; (iii) plasmid pSJ33 is compatible with replicons other than that of RSF1010 and the Km resistance gene is a widely useful marker. However, the most distinguishing feature of plasmid pSJ33 is that any gene cloned into the MCS can be excised together with *Plac* as a *Not*I fragment, provided that there are no additional *Not*I sites in the insert, and transferred afterwards as an expression cassette into the others vectors. Since *Not*I is a rare cutter, generation of *Plac*-based *Not*I-expression cassettes are the result in the majority of cases. Plasmid pSJ33 is particularly useful in combination with the previously described mini-Tn*5* and mini-Tn*10* transposon delivery vectors [1, 2], since the engineered

*Not*I-expression cassettes can be directly inserted at the single *Not*I site of the mini-transposons and subsequently stably integrated into the chromosome of a wide variety of Gram-negative bacteria. This combination also facilitates the comparative study of gene expression in single copy or multicopy using different microorganisms and the same regulatory elements.

We have used plasmid pSJ33 to replace its *Not*I *lacZ*a fragment by a 4.0-kb *Not*I cassette containing a complete *lacZ* gene whose transcription is controlled by the *Pr* promoter-operator from the lambda bacteriophage [5]. When the resulting recombinant plasmid was transformed into *E. coli* MV1190 D*lac* cells, all transformants showed a blue phenotype on media containing the b-galactosidase indicator XGal. To check the performance of this plasmid in a different Gram-negative bacteria, we performed tri-parental filter matings using *E. coli* MV1190 cells containing the recombinant plasmid as donor, *E. coli* HB101 (pRK600) providing the RP4-transfer functions as helper and *Pseudomonas putida* KT2440 as recipient [2]. Exconjugants were selected on M9 minimal medium [10] supplemented with 0.1% sodium citrate (for nutritional counterselection of donor and helper *E. coli* strains) and 50 µg/ml Km (to select for the presence of the recombinant plasmid), and they showed, as in the case of *E. coli*, a blue phenotype on XGal containing medium (data not shown). Levels of b-galactosidase activity were measured with permeabilized *P. putida* KT2440 cells harboring the pSJ33 derivative and were around 200 Miller units [8].

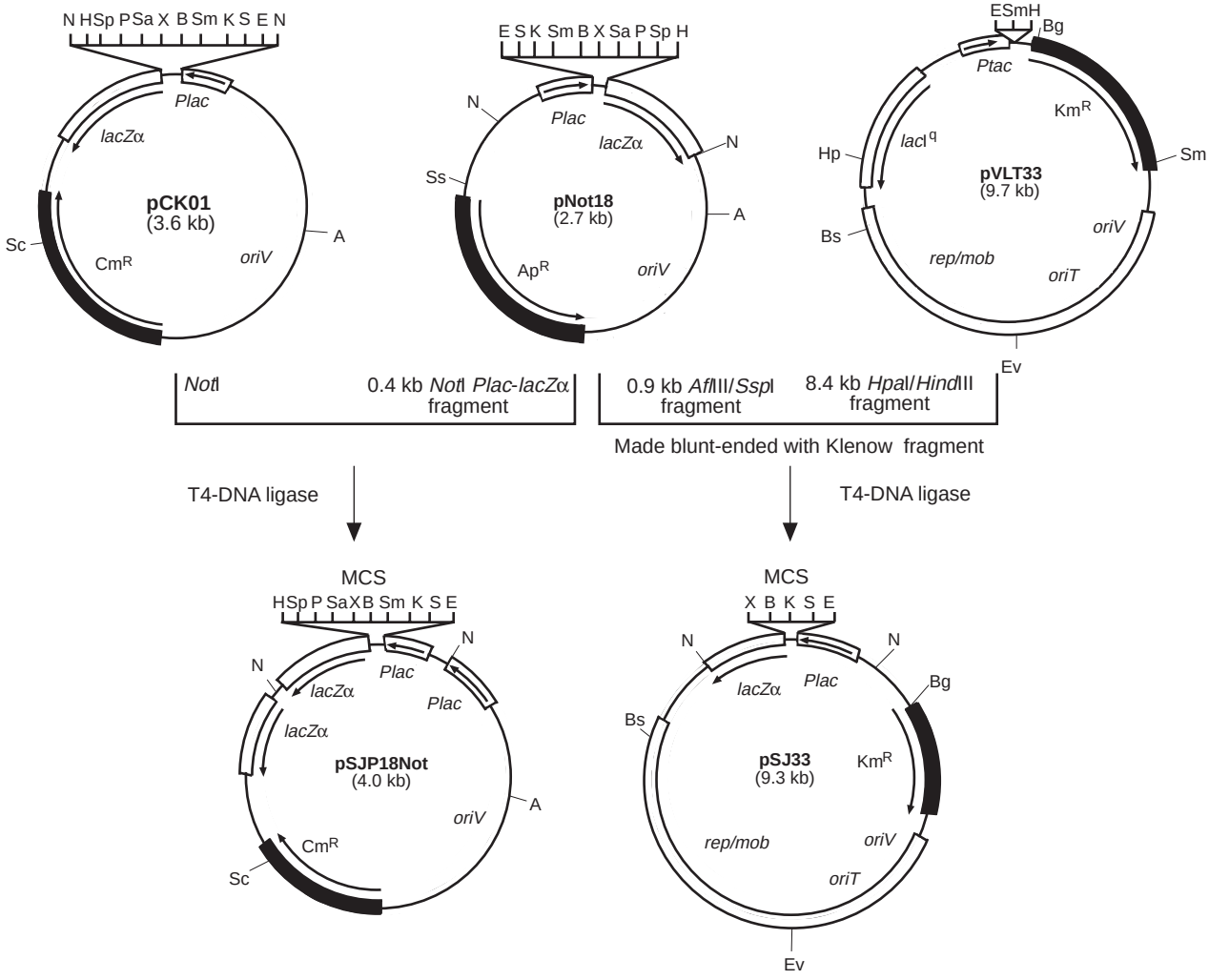


**Fig. 1** Construction of expression plasmids pSJ33 and pSJP18Not. Functional elements of the plasmids such as relevant restriction sites, promoters, selection markers (Ap, ampicillin resistance), MCS (at the top), origen for vegetative (*oriV*) and transfer (*oriT*) replication, and the location of the RSF1010 replication and RP4-mediated mobilization functions (*rep/mob*) are shown. Abbreviations for restriction sites are: A, *Afl*III; B, *Bam*HI; Bg, *Bgl*II; Bs, *Bst*EII; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; N, *Not*I; P, *Pst*I; S, *Sac*I; Sa, *Sal*I; Sc, *Sca*I; Sm, *Sma*I; Sp, *Sph*I; Ss, *Ssp*I; X, *Xba*I. It should be noted that within the *Not*I *lacZ*a fragment of plasmid pSJP18Not, one of the two C at positions 464–465 in the nucleotide sequence of pUC18 ([13]; corresponding to nucleotides 12–13 of the 16-mer reverse sequencing primer) is missing

To facilitate the generation of *Plac*-based *Not*I-expression cassettes in cases of deleterious gene dosage effects to the *E. coli* host cell, we inserted the 0.4-kb *Not*I *lacZ*a fragment from plasmid pNot18 into the *Not*I digested low-copy-number pCK01 vector [4] (Fig. 1). The resultant plasmid, pSJP18Not, contains *Plac*, the MCS of pUC18 and the *lacZ*a fragment bracketed by two *Not*I sites. Cloning of DNA within the MCS can be easily detected by a-complementation in appropriate host strains. Moreover, its chloramphenicol (Cm)-resistance selection marker and pSC101*ori* of replication [11] are compatible with those of the common ampicillin-resistant ColE1-replicon derived vectors of *E. coli*. Plasmid pSJP18Not was of great utility to clone the bacteriophage lambda repressor (*c*I gene). When we tried the cloning of the lambda repressor under control of a synthetic *lac* promoter, $P_{A1/04/03}$, present in plasmid pUHE24-2 [7], the resulting plasmid, pSJ2 [5], showed mutations that abolished expression of the *c*I gene. Sequencing analysis of the pSJ2 mutant plasmids always revealed deletions in the promoter region (data not shown) that might be caused by a 18-nucleotide direct repeat present in the synthetic *lac* promoter. Interestingly, cloning and expression of the *c*I gene in plasmid pSJP18Not under the control of the *Plac* promoter was successful and allowed us to transfer the *c*I-containing *Not*I cassette into a mini-Tn*5* delivery vector for its chromosomal insertion [5].

In conclusion, the plasmids here described behave as effective broad-host-range and low-copy-number compatible expression systems. They have been shown to be particularly useful as auxiliary vectors for the cloning of the expression cassettes at the *Not*I site of mini-transposon elements [1, 2] and for their eventual stable insertion into the host chromosome.

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