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A versatile vector for controlled expression of genes in *Escherichia coli* and *Salmonella typhimurium* *

(ATG vector; translation initiation codon; IPTG induction; *trc* promoter; unfused protein)

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**SUMMARY**

We have constructed two expression vectors based on the pJF118HE vector developed for *Escherichia coli* by Fürste et al. [Gene 48 (1986) 119–131]. The *tac* promoter (*ptac*) was exchanged for the *trc* promoter (*ptrc*) and an NdeI site was created at the appropriate distance from the ribosome-binding site. The *NdeI* site permits cloning of a gene at its translation start point without altering the amino-acid sequence of the synthesized protein, while *ptrc* and the *lacI* gene confer inducible and controllable expression. We have tested these plasmids in *E. coli* and *Salmonella typhimurium*.

**INTRODUCTION**

A versatile expression plasmid for the production of a native, unfused protein should allow the regulated expression of the inserted gene in any desired *Escherichia coli* strain, without changing the aa sequence of the synthesized protein.

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*On request, the authors will supply detailed experimental evidence for the conclusions reached in this Short Communication.*

Abbreviations: aa, amino acid(s); Ap, ampicillin; bp, base pair(s); Δ, deletion; E., Escherichia; HPr, protein of the PEP:carbohydrate phosphotransferase system of Enterobacteriaceae; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; LB, Luria-Bertani (medium); MCS, multiple cloning site(s); nt, nucleotide(s); ORF, open reading frame; ori, origin of DNA replication; p or P, promoter; PA, polyacrylamide; PAGE, PA-gel electrophoresis; PCR, polymerase chain reaction; PolIk, Klenow (large) fragment of *E. coli* DNA polymerase I; PqqB, protein involved in PQQ biosynthesis in Klebsiella pneumoniae; R, resistance/resistant; RBS, ribosome-binding site(s); S., Salmonella; SDS, sodium dodecyl sulfate; *ptac*, *trp*-lac fusion promoter; *ptrc*, derivative of *ptac* in which the spacing between the −10 and −35 sequences is increased from 16 bp to the consensus 17 bp.

The pJFHE/EH series of vectors contain the controllable *ptac* and the *lacI* gene and have the ColEl-type replicon from pBR322 (Fürste et al., 1986). However, when the cloned DNA fragment contains a large fragment upstream from its RBS and/or has a RBS which deviates substantially from the consensus sequence, efficient and controllable expression can be hampered. That problem can be solved by using vectors that permit cloning at the translation start codon. The pKK233-2 vector contains *ptrc* and has a *NcoI* site at the appropriate distance from the RBS to permit this 'ATG cloning' (Amann and Brosius, 1985). A disadvantage of pKK233-2 is that it lacks *lacI*, thus this vector can only be used in LacI repressor-overproducing strains. This difficulty is overcome in vectors pTrc99A, B and C (Amann et al., 1988), containing *ptrc*, the *lacI* gene and a *NcoI* site. However, sometimes the first nt of the second codon will change upon introduction of a *NcoI* (CCATGG) site, resulting in a protein with an altered second aa. This is avoided when an *NdeI* site (CATATG) is created.

We report here the construction of two expression vectors, pBCP367 and pBCP378, which contain the IPTG-inducible *ptrc*, the *lacI* gene and an *NdeI* site at the appropriate distance from RBS for 'ATG cloning'.
 EXPERIMENTAL AND DISCUSSION

(a) Construction of plasmids

The vectors we made are based on pJF118HE (Fürste et al., 1986), in which the promoter region was exchanged for the promoter region of pKK233-2, after converting the Ncol site into an Ndel site (Fig. 1). The vector pBCP378 has a more extended MCS than pBCP367.

MCS pBCP367: Ndel-PstI-HindIII-PstI-Sall-BamHIII-Smal-EcoR1
MCS pBCP378: Ndel-PstI-HindIII-SphI-PstI-Accl-Sall-Xbal-BamHIII-Smal-Apnl-Sacl-EcoR1

(b) Expression studies

We have tested these newly constructed vectors in E. coli and S. typhimurium by studying the controlled synthesis of two different proteins. Fig. 2 shows the overproduction of the PqqB protein of Klebsiella pneumoniae in E. coli when increasing concentrations of IPTG are used. The amount of PqqB varied from less than 1% to 25% of the total cell protein. For this purpose, we cloned the pqqB gene into pBCP367, after introducing an Ndel site at its start codon. The pqqB gene is one of the six genes involved in the biosynthesis of the cofactor pyrroloquinoline quinone (PQQ) in K. pneumoniae (Meulenberg et al., 1990; 1992). The vectors were also used for the controlled production of E. coli HPr. HPr is one of the proteins of the PEP-carbohydrate phosphotransferase system of Enterobacteriaceae, which is involved in carbohydrate transport across the cytoplasmic membrane with concomitant phosphorylation. The E. coli ptsH gene encoding HPr, was cloned in pBCP378 and expressed in a null mutant for HPr production, S. typhimurium PP1744 (ptsH Δfru; Geerse et al., 1986). Upon varying the IPTG concentration, the amount of HPr could be increased from 5% to 125% of the wild-type protein level as measured by assaying HPr activity (J. van der Vlag, R. van 't H. and P.W.P., unpublished results). The ptsH gene was expressed at a much lower level than pqqB. This might be due to the use of another host, combined with a different nt sequence 3' to the start codon. Both factors

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An arrow indicates the position of the PqqB protein (33 kDa).
can affect gene expression (Ringquist et al., 1992; Yarchuk et al., 1992).

REFERENCES


