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Velterop, J.S.; Dijkhuizen, M.A.; van 't Hof, R.; Postma, P.W.

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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)

Joyce S. Velterop, Marjoleine A. Dijkhuizen, Rechien van 't Hof and Pieter W. Postma

E.C. Slater Institute, BioCentrum Amsterdam, University of Amsterdam, 1018 TV Amsterdam, The Netherlands. Tel. (31-20) 525-5111

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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^Q* 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.

Correspondence to: Dr. P.W. Postma, E.C. Slater Institute, BioCentrum Amsterdam, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands. Tel. (31-20) 525-5112; Fax (31-20) 525-5124; e-mail: postma@sara.nl

*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^Q* gene and have the ColE1-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^Q*, 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^Q* 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^Q* 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 *Nco*I site into an *Nde*I site (Fig. 1). The vector pBCP378 has a more extended MCS than pBCP367.

MCS pBCP367: ***Nde*I-PstI-HindIII-PstI-SalI-BamHI-SmaI-EcoRI**

MCS pBCP378: ***Nde*I-PstI-HindIII-SphI-PstI-AccI-SalI-XbaI-BamHI-SmaI-KpnI-SacI-EcoRI**

Fig. 1. Plasmid map and MCS of pBCP367 and pBCP378. Unique sites are shown in bold. To construct pBCP367, the *Nco*I site of pKK233-2 (Amann and Brosius, 1985) was converted into an *Nde*I site by site-directed mutagenesis. Plasmid pKK233-2 was linearized with *Sal*I, and PCR was performed. One primer (5'-GCTTGGCTGCAGC-CATATGCTGTTTCC) was located in MCS of pKK233-2 to create the (indicated in bold) *Nde*I site. The primer for amplification of the other strand (5'-GCAAGGAATGGTGCATGCAAGGAGATGGC) was located upstream from the promoter, around the *Sph*I site of the pBR322 part of pKK233-2. The resulting 0.6-kb PCR product contained *ptrc* and RBS of pKK233-2, and an *Nde*I site instead of a *Nco*I site. This fragment was digested with *Pst*I + *Eco*RI and ligated into the *Pst*I + *Eco*RI-digested vector pKK233-2, giving pKK233-2*. The *Nde*I site of the pBR322 part of pJF118HE (Fürste et al., 1986) was removed by filling in with *Pol*Ik and ligating the blunt ends, resulting in plasmid pBCP366. Plasmid pBCP366 was digested with *Sph*I (in front of *lacI*^Q), and the *Sph*I site was made blunt with T4 DNA polymerase. After a second digestion with *Hind*III, a 4.9-kb blunt-ended *Hind*III fragment which contained *lacI*^Q, *ori*, the Ap^R gene, MCS and the *rrnB* transcription terminators of pBCP366, was isolated. Plasmid pKK233-2* was digested with *Eco*RI (upstream from the promoter region), and the *Eco*RI site was filled in with *Pol*Ik. After a second digestion with *Hind*III the resulting 0.3-kb fragment containing *ptrc*, RBS and *Nde*I site, was ligated into the 4.9-kb blunt-ended *Hind*III fragment of pBCP366. Ligation of the blunt-ended *Sph*I site against the blunt-ended *Eco*RI site created a new *Eco*RI site. The resulting plasmid was pBCP367. Plasmid pBCP378 was made by exchanging the 0.9-kb *Hind*III-*Pvu*I fragment of pBCP367 for the 0.9-kb *Hind*III-*Pvu*I fragment of pJF118HE (Fürste et al., 1986).

(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 *Nde*I 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* Δ*fru*; 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

Fig. 2. Overexpression of *pqqB*. An *Nde*I site was introduced at the start codon of *pqqB*, and the 1.08-kb *Nde*I-*Bam*HI fragment which contained *pqqB* was cloned into pBCP367 digested with *Nde*I + *Bam*HI, resulting in pBCP413. *E. coli* JA221 (*thr leu thi ΔtrpE5 lac gal xyl ml phx hsdR recA supE*; Clarke and Carbon, 1978) was used as host strain. Cultures in 5 ml LB with 100 μg/ml Ap were induced for 16 h with 0, 2.5, 10, 50 or 100 μM IPTG. Total cellular proteins were analyzed by 0.1% SDS-12.5% PAGE followed by Coomassie-blue staining. Lanes: 1, protein standards; 2 and 3, pBCP367, 0 and 100 μM IPTG; 4-8, pBCP413, induced with 0, 2.5, 10, 50 and 100 μM IPTG, respectively. The amount of synthesized PqqB was <1, 9, 16, 22 and 25% of the total cellular proteins, respectively (determined with a BIO-RAD Model 1650 densitometer). The positions of the protein standards are indicated (Pharmacia, LMW electrophoresis calibration kit: 94, 67, 43, 30, 20 and 14 kDa). An arrow indicates the position of the PqqB protein (33 kDa).

can affect gene expression (Ringquist et al., 1992; Yarchuk et al., 1992).

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