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

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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^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.

The pJFHE/EH series of vectors contain the controllable ptac and the lacI^Q^ 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^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 Ndel site (CATATG) is created.

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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'.

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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 NcoI site into an NdeI site (Fig. 1). The vector pBCP378 has a more extended MCS than pBCP367.

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

REFERENCES


