A small, high-copy-number vector suitable for both in vitro and in vivo gene expression

(Cloning: coupled transcription/translation system of Zubay; *Escherichia coli*; phospholipase A₂; adenylate kinase; chicken)

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SUMMARY

A 2433-bp, high-copy-number expression vector, pUK, was constructed from pUC19 and pKK223-3. The genes encoding chicken adenylate kinase (AK) and phospholipase A₂, cloned into pUK, were highly expressed in vitro using the coupled transcription/translation system of Zubay. High-level in vivo expression in *Escherichia coli* was also demonstrated for the AK gene cloned in this vector. The multiple cloning site (MCS) of pKK223-3 was preserved in pUK. In addition, three more sites in the MCS sequence, AccI, BamHI and SalI, were made unique for the convenience of cloning.

INTRODUCTION

In vitro gene expression using the coupled transcription/translation system (Zubay, 1973; Pratt, 1984) requires the preparation of highly supercoiled plasmid DNA in large quantity. However, the widely used expression vectors with a strong promoter, such as the pKK series (Brosius et al., 1984; Amann, 1985) and pTrc99A (Amann, 1988) vectors, are relatively large in size (approx. 5 kb) and low in copy numbers. Large-size plasmid DNAs are less easy to purify in supercoiled form and the low-copy-number feature limits the yield of DNA. Thus, it is desirable to have an expression vector of small size and high copy number for in vitro gene expression.

(a) Construction of pUK

pUK was constructed as a hybrid vector consisting of the high-copy-number replicon and the Ap⁵ derived from pUC19 (Yanisch-Perron et al., 1985), along with the strong tac promoter, the SD sequence, the MCS and the *rnb* transcription terminator derived from pKK223-3 (Brosius et al., 1984). As shown in Fig. 1, the pUC19 component was a 1811-bp *AatII-AfIII* fragment that had been treated with T4 DNA polymerase to create blunt ends while the pKK223-3 segment was a 622-bp *SspI* fragment. *SspI* cuts pKK223-3 16 bp upstream from the -35 region of the tac promoter and at nt 4050, which is downstream from the *rnb* terminator sequence. The two fragments were ligated to yield the plasmid pUK. The relative orientation of the two fragments was confirmed by mapping pUK by digestion with EcoRI and PvuI. In addition to the unique sites at the MCS of pKK223-3, three more sites, AccI, BamHI and SalI, were made unique by eliminating the duplicate sites present in pKK223-3 for these enzymes. This feature makes pUK a more versatile vector for cloning.
(b) In vitro gene expression using the pUK vector

Two genes, encoding the bovine pancreatic phospholipase A$_2$ (PLA$_2$) (Deng et al., 1990) and the chicken muscle adenylate kinase (AK) (Tazinawa et al., 1987), which were previously expressed in vitro using pKK223-3 vector in our lab, (unpublished data), were used to test the pUK. The PLA$_2$ gene was subcloned into pUK between EcoRI and PstI sites and the AK was subcloned into this vector between EcoRI and HindIII sites. In the in vitro expression experiments using the coupled transcription/translation system, both PLA$_2$ and AK genes were expressed when the reactions were primed with pUK containing the corresponding gene (Fig. 2). The expression levels were comparable to those directed by the pKK223-3 vector containing the corresponding genes as shown in Fig. 2. Furthermore, the small-size and high-copy-number features of pUK allow the isolation of highly supercoiled plasmid in very high yield (approx. 2.5 mg DNA/litre of culture). It was estimated that the pUK-based plasmids produce approx. 10-times more plasmid DNA than the corresponding pKK series vectors in the standard large-scale DNA preparation using the CsCl gradient method.

The pUK vector was also shown to direct in vivo gene expression in E. coli. When pUK containing AK gene was introduced into JM105 cells, AK was expressed at a high level shortly after induction by IPTG based on SDS-PAGE analysis (results not shown).

![Diagram of gene expression system](image)

**Fig. 2.** Comparison of in vitro expressions of AK and PLA$_2$ genes cloned into pUK and pKK223-3. For a 30-μl reaction, plasmid DNA was mixed with 8.5 μl E. coli S-30 extract/7.5 μl low-molecular-weight mixture (containing the four NTP/phosphoenolpyruvate/folic acid/E. coli tRNAs/20 amino acids/1 μl [°S]methionine/1.5 μl Mg-acetate under the conditions described by Ellman et al. (1991). The reaction mixture was incubated at 37°C for 1 h on a rotary shaker (approx. 200 rpm). The samples were then centrifuged and the supernatant was analyzed by 0.1% SDS-15% PAGE (Laemmli, 1970). Electrophoresis was carried out at 20 mA for 4 h, and the gel was subjected to autoradiography. (A) In vitro synthesis of PLA$_2$. The in vitro expression reaction was primed with 3 μg plasmid DNA carrying the PLA$_2$ gene cloned into the pKK223-3 vector (lane 1) or the pUK vector (lane 2); 5 μl of the reaction mixture was loaded for each lane. (B) In vitro synthesis of AK. The in vitro expression reaction was primed with 3 μg plasmid pKK223-3 DNA carrying the AK gene (lane 1) or 1.8 μg of plasmid pUK DNA carrying the AK gene (lane 2). 1 μl of the reaction mixture was loaded for each lane. Notice that equal molar amounts of the two vector DNAs were used in lanes 1 and 2.

**Fig. 1.** Construction of pUK, a hybrid vector of pKK223-3 and pUC19. The part containing the tac promoter ($P_{tac}$), the SD sequence, MCS and a transcription terminator ($rnnB$) was from pKK223-3. The other part of pUK containing the Ap-resistance-encoding gene (Ap$^R$) and the replication elements ($ori$) was from pUC19. All the unique sites in MCS are shown.
(c) Conclusions

The pUK vector has been constructed and demonstrated to be especially advantageous for the in vitro expression of genes using the coupled transcription/translation system which works best with high concentrations of highly supercoiled DNA. This vector has also been shown to be suitable for in vivo gene expression in E. coli. The presence of three additional unique sites at the MCS of pUK than that of pKK223-3 should make it more convenient for cloning genes into pUK.

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REFERENCES


