ColE1-compatible vectors for high-level expression of cloned DNAs from the T7 promoter

(Recombinant DNA; cloning; T7 polymerase; pACYC177; M13 ori; P15A ori)

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SUMMARY

A new family of T7-based expression plasmids with unique features is described. The plasmid origin of replication (ori), derived from P15A, is compatible with that of ColE1-derived plasmids, which facilitates the co-production of proteins from these vectors and from ColE1-derived T7 expression vectors in the same cell. The plasmids are medium-copy-number and also carry the M13 ori. Consequently, both double- and single-stranded DNA can be easily obtained. The plasmids encode KmR, thus avoiding the potential for plasmid loss associated with ApR-based systems. One of the plasmids carries the lacI gene, to allow for more stringent regulation of the production of potentially toxic proteins. When the plasmids are introduced into an Escherichia coli strain such as BL21(DE3), which contains the T7 polymerase-encoding gene under control of the lacUV5 promoter, addition of IPTG initiates the production of high levels of the recombinant protein.

INTRODUCTION

An ideal bacterial expression system should have at least the following characteristics: (i) under repressed conditions, minimal basal expression of the gene to be expressed, (ii) fast and straightforward induction of cloned genes to high levels and (iii) simplicity of cloning, DNA manipulation and DNA sequencing. These criteria are well met by the original T7-based pET expression vectors (Studier et al., 1990) and the more recently described pRSET vectors (Schoepfer, 1993). There are, however, certain circumstances in which added flexibility is desired, and to this end we describe a new set of T7-derived vectors that retain the features described above, but incorporate additional ones.

The distinguishing features of the vectors described in this communication are that they are KmR and they have an ori that is compatible with that of ColE1-derived plasmids, such as pBR322 and its derivatives (Bolivar et al., 1977; Vieira and Messing, 1982). The vectors can be used to produce proteins that cannot be conveniently synthesized from plasmids with ColE1-type ori. In addition, and most importantly, the vectors can be used in conjunction with the existing ColE1-based ApR T7 expression vectors to conveniently co-produce two different proteins in the same cell.

EXPERIMENTAL AND DISCUSSION

(a) Overview of the vectors

The parent plasmid that was used in the construction of these vectors is the medium-copy-number plasmid...
pACYC177, which has the P15A ori and Km\textsuperscript{R} gene (Chang and Cohen, 1978; Rose, 1988). The ori from M13 phage was incorporated into this plasmid to facilitate the production of ss DNA for DNA sequencing and mutagenesis. Finally, the T7lac promoter (Studier et al., 1990) (in which a 25-bp lac operator sequence is inserted 2 bp downstream from the T7 promoter) and associated cloning sites were introduced, to generate the expression vector named pMR101 (Fig. 1).

To facilitate the insertion of synthetic genes containing a maximum number of unique restriction sites, pMR101 was further modified to remove two superfluous restriction sites, creating the plasmid pMR102. Finally, to generate a vector in which there is tight repression of transcription from the T7lac promoter under non-inducing conditions, the lacI gene, which encodes the lac repressor, was cloned into pMR102 to create plasmid pMR103 (Fig. 2).

For each of the vectors described above, the gene encoding the protein to be produced is cloned between the NcoI and BamHI sites; the sequence of the NcoI site contains the AUG codon of the start Met. Consequently, it is easy to move genes that have already been cloned in the pET8c or pET11d series of vectors into the pMR vectors.

(b) Generation of ss DNA

To generate ss DNA, the plasmids must be introduced into an F\textsuperscript{−} or F\textsuperscript{+} strain, which is then superinfected with an appropriate helper phage, such as the M13 phage R408 (Russel et al., 1986). Detailed protocols for the production of ss DNA by helper phage superinfection are described elsewhere (Zagursky and Berman, 1984). The orientation of the M13 ori in the pMR vectors is such that the sequence of the gene in the 5′ → 3′ direction (reading the AUG of the start Met directly) is read from the sequencing gel using primers upstream from the NcoI cloning site.

introduce EcoNI and Sall sites at the 5′ and 3′ ends, respectively. The resulting PCR product of 538 nt, and the T7 expression vector, pET8c (Studier et al., 1990), were digested with EcoNI + Sall. The appropriate vector fragment and the PCR product were both gel purified, then ligated together to create pMR99. Next, PCR was used to amplify the T7 promoter together with the M13 ori from pMR99, using the primers pMR99(1) (5′-CGGCCGCGCGGTACGATCATGGGACC- ACCACCGCGTG- CAGGTTAGGAGGCTCTCAAGGCG), which introduce BseIII and PstI sites at the 5′ and 3′ ends, respectively. This PCR product of 1138 nt, and pACYC177 were digested with BseEI + BglII, the appropriate fragments were gel purified and ligated together to create pMR100. To create pMR101, the T7lac region from pET11d (Studier et al., 1990) was removed by digestion with BamHI + BglII and subsequently ligated into BamHI + BglII-digested pMR100. In this figure, the M13 ori is denoted M-ori; the CoE1 ori, C-ori; and the P15A ori, P-ori.
tion of IPTG to a growing culture of BL21(DE3) induces the production of T7 RNA polymerase, which in turn transcribes the target DNA in the plasmid. Proteins that are too toxic to be produced in this straightforward fashion can be expressed by infecting non-lysogenic cells containing the expression plasmid with a bacteriophage, such as CE6, that provides T7 RNA polymerase to the cell. These methods have been described in detail elsewhere (Studier et al., 1990).

All three vectors have been used routinely in our laboratory for protein production. Fig. 3 illustrates protein overproduction using the pMR101 vector. In this example, the protein that is being overproduced is Rop (ROM), a ColE1 protein whose involvement in the ColE1 regulatory mechanism precludes the use of ColE1-based vectors for its overproduction (Polisky, 1988). It is possible to routinely obtain approx. 50 mg of purified Rop per litre of culture, using this system.

(c) Protein production

For protein production, the recombinant plasmid is introduced into an appropriate expression strain, like BL21(DE3). In this strain, transcription of the T7 RNA polymerase-encoding gene is controlled by the lac repressor and lacUV5 promoter/operator; consequently, addi-

![Diagram](image)

**Fig. 2.** Schematic outline of the construction of the plasmids pMR102 and pMR103 from pMR101. The BglII and XhoI sites in pMR101 were removed by sequential digestion with the restriction enzymes, filling-in the ends with PolIk, and finally religating the product. The resulting plasmid, from which both the BglII and XhoI sites have been removed, is called pMR102. To create pMR103, the lacI gene from pET11d was amplified by PCR using the primers: lacI(1) (5'-CGCCCGCGCCGT- CGACCCATCGAATGCCGCAA) and lacI(2) (5'-GCCCGCGCCC- GCGCGTCGACTCGCCCGCTTTCCAGTCC) and was inserted into SalI + PstI-digested pMR102. In this figure, the M13 ori is denoted M-ori; the ColE1 ori, C-ori; and the P15A ori, P-ori.

![Diagram](image)

**Fig. 3.** An example of protein overproduction using vector pMR101. The 0.1% SDS-18% polyacrylamide gel was used to electrophorese total cell lysates from uninduced cells (–IPTG) and cells after (+IPTG) induction. The position of the Rop protein is indicated. DNA encoding the wild-type Rop protein was cloned between the NcoI and BamHII sites of pMR101, and the resulting plasmid transformed into BL21(DE3) cells. Cultures were grown to mid-log phase and a pre-induction sample was removed. IPTG was then added to a final concentration of 1 mM and the cultures were incubated for a further 2.5 h before taking the post-induction sample.
de novo designed four-helix-bundle protein, α₄ (Regan and DeGrado, 1988), has also been successfully overproduced using these vectors.

(d) Conclusions

We have described a set of ColE1-compatible T7 expression vectors. The vectors are easily manipulated and can be used alone or in conjunction with existing ColE1-based vectors. Synthesis of the recombinant protein is tightly controlled, with negligible ‘leakiness’ under uninducing conditions, and results in reproducibly high levels of the overproduced protein under inducing conditions.

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