Multicopy expression vectors carrying the lac repressor gene for regulated high-level expression of genes in *Escherichia coli*

(Recombinant DNA; *tac* promoter; *lacI* gene; *lac* operator; *lacZα*; pUC plasmids; polylinker; gene fusion; luciferase)

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SUMMARY

A series of new expression vectors (the pTTQ series) has been constructed for the regulated expression of genes in *Escherichia coli*. Based on the pUC plasmids, the pTTQ vectors contain a polylinker/*lacZα* region flanked by the strong hybrid *trp-lac* (*tac*) promoter and the *rrnB* transcription terminator. Foreign genes can be inserted into the polylinker region of this expression cassette, to give either transcriptional or translational fusions within the *lacZα* coding region. In most commonly used strains of *E. coli*, multiple copies of the *lac* operator titrate out the *lac* repressor. This phenomenon leads to significant expression from *tac* or *lac* promoters present on multicopy plasmids, even in the absence of inducers such as IPTG. To ensure maximal repression of the *tac* promoter on the pTTQ vectors in any host strain, the *lacIQ* allele of the *lac* repressor gene was added to the vectors. This makes them particularly useful for cloning genes when expression at high level is desired but is detrimental to cell growth.

INTRODUCTION

In recent years, a variety of different vectors have been described for achieving the regulated, high-level expression of cloned genes in *E. coli* (Pouwels et al., 1985). Applications for such vectors include identification of the products of cloned genes, production of large amounts of protein for biochemical studies or raising antisera and studies of gene function in vivo. In the expression vector, the gene of interest is inserted downstream from a strong promoter. In a transcriptional fusion, translation of the inserted gene is dependent on its own translation start signals (RBS, ATG initiation codon) and the vector is used only to generate high levels of transcript. Alternatively, a gene
may be expressed as a translational fusion, where the vector also provides the RBS and ATG initiation codon. In this case, the coding sequence of the gene of interest must be fused in frame with the ATG codon at a suitable downstream restriction site, leading to the production of a hybrid protein.

The ability to regulate expression of a cloned gene can be of great importance if the gene product is detrimental to the host cell. If such a protein is expressed prematurely it may lead to selection against the plasmid, selection of mutant plasmids or even failure to obtain a construct from which the gene can be expressed. Many E. coli expression vectors have therefore utilized either the lac or bacteriophage λ promoters, both of which can be repressed. Vectors using λ promoters need to be propagated in strains that carry the λ d1857 gene (encoding a thermolabile repressor) and induction requires a temperature shift, which inactivates the repressor. Plasmids utilizing the lac promoter must also be propagated in special strains that overproduce the lac repressor (lacI0 mutants), since multiple copies of the lac operator titrate out the levels of repressor in wild-type (lacI+) strains (Backman et al., 1974; Heyneker et al., 1976).

Here the construction and use of a new range of plasmids (the pTTQ vectors) are described. These vectors can be used for the high-level, regulated expression of genes in E. coli using either transcriptional or translational fusion strategies and their complete nucleotide sequences are known. They are based on the pUC plasmids (Vieira and Messing, 1982), but contain the hybrid trp-lac (lacI) promoter instead of the lac promoter. The tac promoter contains the -35 region of the trp promoter fused to the lacUV5 -10 and lac operator sequences (Amann et al., 1983; De Boer et al., 1983). The hybrid promoter is still subject to regulation by the lacI gene product, but is 5 to 10 times stronger than the lacUV5 promoter (De Boer et al., 1983). The pTTQ vectors also carry a strong transcription terminator from the E. coli rrnB operon (Brosius et al., 1981) downstream from the polylinker region, together with the lacI0 allele (Muller-Hill et al., 1968). The latter produces sufficient repressor to prevent expression from the tad promoter, even in a lacI- strain, until addition of the inducer IPTG. When making translational fusions, versions of the pTTQ vectors with different polylinker sequences enable in-frame gene fusions to be made at several restriction sites in either two or all three reading frames.

MATERIALS AND METHODS

(a) Bacterial strains and basic methodology

E. coli strains used were 5K (F thi thr-1 leuB6 lacI Y1 tonA2I supE44 λ r63 m6- and NM522 (Δ lac-proAB thi supE r63 m6- [F’ proAB lacI0 ZΔM15]). All the routine recombinant DNA methods used have been described by Maniatis et al. (1982). DNA fragments were isolated by squash elution following PAGE (Maxam and Gilbert, 1977).

(b) Subcloning the lacI0 gene from plasmid pMC7

The lacI0 gene was subcloned from plasmid pMC7 (Calos, 1978) into plasmid pUC18, as indicated in Fig. 1.

(c) Construction of the pTTQ expression vectors

As shown in Fig. 2, the lac promoter and polylinker region of pUC18 was first replaced by the tac promoter. The latter was isolated as an EcoRI-PvuII fragment from ptac12 (Amann et al., 1983). The EcoRI site was rendered blunt by ‘filling in’ (using PolIk and dNTPs) and then inserted between the PvuII sites of pUC18, replacing the small PvuII fragment of the latter (giving pUCtac).

An EcoRI fragment from pEA302T that carries the rrnB transcription terminator (Amann et al., 1983) was next isolated and inserted into the unique NdeI site of pUCtac after both vector and fragment had been ‘filled in’. The unique EcoRI site generated in the previous step was then removed by cleaving the plasmid with EcoRI, followed by ‘filling in’ and religation (yielding pUCtacT).

Polylinker/lacZα fragments from pUC8, 9, 18, and 19 were next inserted into pUCtacT to generate pTT8, 9, 18, and 19, respectively. The tac promoter fragment of pUCtacT carries the
Fig. 1. Subcloning the lacI\(^Q\) gene. Plasmid pMC7 (Calos, 1978) was first cut with HpaII and a 369-bp fragment encoding the 3’ portion of lacI\(^Q\) (fragment B) was isolated. This fragment was inserted into the AccI site of pUC18 as shown (giving pMJR1550). The 5’ portion of lacI\(^Q\) was next isolated as a 935-bp HinclII fragment (fragment A) and inserted between the SmaI site and HincII site of pMJR1550. This reconstructed an intact lacI\(^Q\) gene, which may be excised from the vector (pMJR1560) by a variety of restriction endonucleases.

The inserted DNA contains only the lacI\(^Q\) gene and not the downstream lacZpo region, so sub-cloning of the insert in other plasmids will not lead to transcription of adjacent DNA. Restriction sites are denoted as follows: AccI (Acc), HincII (\(\wedge\)), HincIII (H3), HpaII (\(\wedge\)), EcoRI (RI), KpnI (Kpn), PstI (Pst), SmaI (Sma), SphI (Sph), SstI (Sst). \(\wedge\) denotes loss of a site or sites during the preceding cloning event. Tc denotes tetracycline resistance gene. I\(^Q\) denotes lacI\(^Q\) gene. Fragt. refers to DNA fragment.

lac RBS and requires the addition at the PvuII site of an ATG start codon preceded by 2 bp to generate a functional translation start point. The following stratagem was therefore adopted (shown for pTT18 only in Fig. 2). First, each pUC plasmid was linearized with either EcoRI (pUC8, 18) or HindIII (pUC9, 19) and the ends ‘filled in’. A single non-phosphorylated ClaI linker (CATCGATG) was next inserted to introduce a unique ClaI site at the extreme 5’ end of each polylinker region, adding an in-frame ATG codon (underlined) in each case. Each polylinker/lacZ\(\alpha\) region was then isolated as a ClaI-PvuII fragment, the ClaI site ‘filled in’ and the fragment cloned into the PvuII site of pUCtacT. Screening for \(\alpha\)-complementation of lacZ\(\Delta\)M15 using strain NM522 allowed identification of the desired clones (the pTT plasmids).

Finally, the lacI\(^Q\) gene was excised from pMJR1560 as a KpnI-PstI fragment and introduced into the unique AatII site of each pTT vector by the use of synthetic oligodeoxyribonucleotide linkers. These adapted the AatII ends of the vectors to the KpnI and PstI ends of the fragment such that all three sites were lost. Plasmids with the insert orientation shown (pTTQ vectors) were identified and retained. Each polylinker/promoter region and each new junction created was verified by direct sequencing of plasmid DNA using synthetic oligodeoxyribonucleotide 17-mer primers (Haltiner et al., 1985). These data, together with the published sequences of the lacI\(^Q\) gene (Calos,
Fig. 3. Nucleotide sequences of the promoter and polylinker regions of the pTTQ vectors and pUC18. Sequence extending from the -35 region of the lac or tac promoter to the distal region of the polylinker is given for pTTQ8, 9, 18, 19 and 181. The comparable region of pUC18 is also shown. Unique cloning sites in the polylinker, the -35 and -10 regions of the promoter and the RBS are shown.
1978; Farabaugh, 1978) and the pUC vectors (Yanisch-Perron et al., 1985) enabled deduction of the entire nucleotide sequence of each vector.

Fig. 3 shows the nucleotide sequences of the promoter/polylinker regions of the pTTQ vectors, indicating the unique cloning sites. It should be noted that the unique Sall site is no longer either a unique AccI or HindIII site and that pTTQ9 and pTTQ19 lack the HindIII sites of pUC9 and pUC19.

Since the pTTQ vectors encode a lacZα fragment, they produce blue colonies on plates containing IPTG and XGal in a lacZAM15 strain by α-complementation (Vieira and Messing, 1982). This colony color screen can therefore be used with the pTTQ vectors (but see RESULTS AND DISCUSSION, section c). The chromogenic reaction is weaker than with pUC vectors.

(d) Changing the polylinker sequence of pMJR1700 and construction of pTTQ181

Plasmid pMJR1700 was linearized at its unique EcoRI site (immediately following the lacZ ATG start codon) and an oligodeoxynucleotide adapter (made from the two oligonucleotides AATTTGATTACG and AATTCGTAATCA) inserted. The desired orientation of insert (ATGAATTTGATTACGAATTC, with oligodeoxynucleotide sequence in lower case) retained the correct reading frame and restored the EcoRI site (underlined) distal to the ATG initiation codon (underlined). The new plasmid was pMJR1750.

This insertion was transferred to pTTQ18 as follows. Plasmid pMJR1750 was digested with EcoRV + EcoRI and a fragment extending from within the lacI region to the polylinker was isolated. An EcoRI-EcoRV fragment of pTTQ18 extending from the polylinker to the lacI region was also isolated. The two fragments were combined to generate pTTQ181, identical to pTTQ18 except for the additional nucleotides derived from pMJR1750 in the polylinker (Fig. 3).

(e) Analysis of proteins expressed from genes cloned in expression vectors

Cultures of cells were grown overnight at 37°C in LB medium containing 0.1 mg Ap/ml and 0.5% glucose (to ensure that the lac or tac promoters of constructs not encoding their own lac repressor remained fully repressed). Cells were then subcultured into fresh medium (20 ml) and grown to an A600 of 0.5. After centrifugation (3 min at 5000 rev./min) the cells were resuspended at 0.5 A600 in pre-warmed LB medium containing 0.1 mg Ap/ml. Portions (4 ml) were distributed to 30-ml culture tubes, some of which contained 20 μl 0.1 M IPTG (0.5 mM final concentration). Cultures were shaken at 37°C for 2.5 h.

For analysis of total cellular protein, culture A600 was measured (using a 40-fold dilution) and then cells from 1.5 ml of culture were pelleted, resuspended in 200 μl of SDS gel sample buffer (Laemmli, 1970) and boiled for 2.5 min. Volumes equivalent to 0.3 A600 of cells were analyzed by SDS-PAGE (Laemmli, 1970), using Coomassie Brilliant Blue R stain. The proportion of total protein represented by specific bands was estimated using an LKB Ultrascan XL scanning densitometer.

Plasmid DNA present in cultures was also examined to ensure that differences in expression of plasmid-coded proteins were not caused by gross changes in plasmid structure or copy number. Plasmid DNA was prepared by boiling lysis and analyzed by agarose gel electrophoresis after restriction with suitable enzymes. No evidence for such problems was ever found.

RESULTS AND DISCUSSION

(a) Expression of transcriptional and translational gene fusions in pTTQ vectors

 Constructs were made to test the utility of the pTTQ vectors for the regulated expression of genes as either translational or transcriptional gene fusions. A transcriptional fusion was made by insertion of a Sall-BamHI fragment from pAG120 (A.J. Mileham, unpublished), which encodes the Vibrio harveyi luxA and luxB genes, (Belas et al., 1982) into the Sall-BamHI region of the pTTQ19 polylinker. This permitted expression of the luxA and luxB gene products (the α and β...
Fig. 4. Summary of plasmid constructions used. For each construct, the plasmid from which the inserted DNA was derived is shown with important restriction sites marked. The vector used is indicated and the name of the resulting construction shown together with a map of its expression cassette, where Δ denotes loss of a restriction site. Boxes indicate coding regions (lacZα sequence is shaded). In-frame fusions of coding sequences are indicated by . Plasmids pC77/2 and pC86/2 were kindly provided by Dr. G.S. Plastow. Key to restriction sites: A, AosI; B, BamHI; D, DraI; P, PstI; Pv, PvuII; S, SalI; Sm, SmaI; X, XhoI.
subunits of bacterial luciferase, respectively) from the tac promoter in the resulting plasmid pC86/2 (Fig. 4). High levels of the α and β subunits of luciferase were made from pC86/2 (Fig. 5A, lane 2) in the presence of the inducer IPTG, each protein corresponding to between 10 and 18% of the total. Low levels of protein were detected in the absence of inducer (compare Fig. 5A, lanes 1 and 3). Since no other constructs made using the pTTQ vectors showed any detectable levels of synthesis in the absence of inducer, this may indicate the presence of a second promoter within the inserted DNA of pC86/2 rather than failure to repress completely the tac promoter.

Fig. 5B shows expression of the Tn9 cat gene as a translational fusion in pTTQ18. The CAT-coding sequence was isolated from pCM4 (Close and Rodriguez, 1982) as a PvuII-BamHI fragment and inserted into the Smal-BamHI region of the pTTQ18 polylinker, producing plasmid pMJR1820. This generated an in-frame fusion between the lacZα coding region and the 39th codon of CAT (Fig. 4). Fig. 5B that, while no fusion polypeptide was detectable in the absence of IPTG (lane 1), in the presence of the inducer a prominent band with the expected Mr ~22000 appeared (lane 2). This band was entirely dependent on pMJR1820 (not shown) and was estimated to represent around 10% of the total protein.

(b) Regulated expression from the pTTQ vectors

Initially, expression vectors were constructed without the lacIQ gene (the pTT vectors: see Fig. 2). Expression of genes from

Fig. 5. Expression of genes using the pTTQ vectors. (Panel A) Expression of the luxA and luxB genes of V. harveyi from pC86/2 as a transcriptional fusion. Proteins from uninduced (lane 1) or induced (lane 2) cultures were analyzed by PAGE using a 12.5% polyacrylamide gel, with proteins from an induced culture carrying pTTQ18 shown for comparison (lane 3). The positions of the α (A) and β (B) subunits of luciferase (the luxA and luxB gene products, respectively) are indicated. The α subunit migrated more slowly than noted by Belas et al. (1982) but could be identified since band A was missing from cultures expressing the luxB gene alone (not shown). (Panel B) Expression of the fusion protein (C) encoded by the lacZα/cat fusion gene of pMJR1820. Protein from uninduced (lane 1) and induced (lane 2) cultures was analyzed by PAGE using a 17.5% polyacrylamide gel. In both panels the positions of protein Mr standards β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa) and soybean trypsin inhibitor (20.5 kDa) are indicated.
pTT vectors was never fully repressed in the absence of inducer, even in lacI\textsuperscript{Q} strains, which overproduce the lac repressor about tenfold (Muller-Hill, 1975). Addition of the lacI\textsuperscript{Q} allele to the vectors did, however, allow proper control of transcription. This was shown using constructs carrying the Km\textsuperscript{'} gene from pUC4K (Vieira and Messing, 1982).

The Tn903 Km\textsuperscript{R} gene was isolated from pUC4K as a XhoI-PstI fragment and inserted into the SalI-PstI region of pUC18, pTT18 or pTTQ18 to give pMJR1610, pMJR1500 and pMJR1600, respectively (Fig. 4). In each case, an in-frame fusion between lacZ\textalpha{} and the tenth codon of the Km\textsuperscript{R} gene was formed which led to the production in induced cultures of a fusion protein with M\textsubscript{r} 31000 (e.g., Fig. 6, lanes 4, 6 and 8). This protein was not present in induced cells containing the vector pTTQ18 (Fig. 6, lane 2). However, Fig. 6 (lanes 3 and 5) shows that this polypeptide was synthesised at high levels in non-induced cells containing either pMJR1610 (pUC construct) or pMJR1500 (pTT construct), corresponding to about 30\% and 55\% of the respective induced levels. By comparison, synthesis of the fusion protein

Fig. 6. Regulation of expression from plasmids carrying the lac operator. Protein from uninduced (−; lanes 1, 3, 5 and 7) or induced (+; lanes 2, 4, 6 and 8) cultures of cells containing different plasmids was examined by PAGE using a 12.5\% gel. Cells contained pTTQ18 (lanes 1 and 2), pMJR1610 (lanes 3 and 4), pMJR1500 (lanes 5 and 6) or pMJR1600 (lanes 7 and 8). In the latter three plasmids, a lacZ\textalpha{}/Km\textsuperscript{R} gene encodes a fusion protein (K); these plasmids are based, respectively, on vectors pUC18, pTT18 and pTTQ18 (Fig. 4). The positions of protein standards (see Fig. 5) are indicated (M\textsubscript{r} × 10\textsuperscript{3}).
from plasmid pMJR1600 (pTTQ construct, carrying lacI was undetectable without induction (Fig. 6, lane 7), showing that sufficient lac repressor was produced. For purposes of comparison, this experiment was performed in an F’ lacI Q- carrying strain (NM522) since plasmids pMJR1500 and pMJR1610 gave aberrant colony morphology when transformed into a lacI + strain (5K), presumably due to synthesis of excessively high levels of fusion protein in the absence of inducer. However, plasmid pMJR1600 gave normal colony morphology in strain 5K and synthesis of fusion protein was again undetectable in the absence of IPTG (not shown).

Fig. 6 also shows that the induced level of protein synthesized from pMJR1600 (pTTQ construct) was around 30% less than that obtained from pMJR1500 (pTT construct). This indicates that use of lacI Q in cis may produce so much repressor that maximal induced levels of expression are slightly reduced. The lacI Q gene was deliberately added to the pTTQ vectors such that it could not be transcribed from the upstream regions due to the rrnB transcription terminators. In other experiments, readthrough transcription of a plasmid-encoded lacI Q was shown to render both host and plasmid lac promoters uninducible by IPTG (not shown).

Plasmid pC77/2 contains the luxA and luxB genes of V. harveyi isolated as a SalI-BamHI fragment and inserted into the SalI-BamHI region of pUC19 (Fig. 4). It is therefore analogous to pC86/2 (above). Plasmid pC77/2 could be introduced into an F’ lacI Q strain (NM522), although expression of the inserted genes occurred at a very high level even without induction (not shown). Table I shows that a lacI’ strain (5K) could not be transformed with pC77/2, even in the absence of the lac inducer IPTG or when selective plates were supplemented with 0.5% glucose, indicating that plasmid pC77/2 is lethal to a lacI’ cell. Glucose acts to inhibit expression of the lacI promoter via catabolite repression (see, e.g., De Crombrugghe and Pastan, 1978) and addition of glucose to growing NM522 cells reduced expression of the lux genes to very low levels (not shown). Even growth of strain 5K in the presence of glucose prior to competent cell preparation did not permit transformation with pC77/2 under these conditions. By comparison, pC86/2 (pTTQ version) could readily be transformed into strain 5K provided selection was performed in the absence of the inducer.

(c) Genes whose expression at high level is lethal can be cloned in pTTQ vectors

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Cells grown without glucose</th>
<th>Cells grown with glucose</th>
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<tr>
<td>5K</td>
<td>pUC8 b</td>
<td>845</td>
<td>892</td>
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<tr>
<td>(lacI’)</td>
<td>pC77/2 c</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>pC86/2</td>
<td>395</td>
<td>0</td>
</tr>
<tr>
<td>NM522</td>
<td>pUC8 b</td>
<td>579</td>
<td>909</td>
</tr>
<tr>
<td>(F’ lacI Q)</td>
<td>pC77/2</td>
<td>543</td>
<td>530</td>
</tr>
<tr>
<td></td>
<td>pC86/2</td>
<td>389</td>
<td>0</td>
</tr>
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</table>

a Competent cells of E. coli strains NM522 and 5K were prepared from cultures grown in the presence or absence of 0.5% glucose. Plasmid DNA (0.2 pmol) was used to transform 0.2 ml competent cells. Transformants were selected on LB agar containing 0.1 mg Ap/ml and in some cases 0.25 mM IPTG or 0.5% glucose (Glu). When selective plates contained glucose, phenotypic recovery was also in the presence of 0.5% glucose. After 20 h at 37°C the number of colonies was scored.

b Included as a control to demonstrate the transformation competency of the cells.

c While pC77/2 could not transform 5K, its ability to transform NM522 indicated that there was nothing inhibitory to transformation in the DNA preparation.

d not determined.
IPTG. This shows that the presence of lacI\(^0\) in cis can protect a lacI\(^+\) strain from the product of a plasmid-encoded gene whose expression during transformation is lethal. Table I shows that pC86/2 can transform neither a lacI\(^+\) nor an F' lacI\(^0\) strain under conditions of induction. This highlights a potential problem when using vectors such as pUC and pTTQ. Inducing conditions are necessary when using the blue/white colony color screen afforded by the lacZ \(\alpha\)-complementation system, employed to differentiate recombinant and non-recombinant clones. Since plasmids that express a desired gene at high levels may be impossible to isolate under conditions of induction, it may be essential to select transformants using non-inducing conditions. If so desired, transformants could then be screened in the presence of inducer for loss of \(\alpha\)-complementation or inviability.

(d) Expression of lacZ from pUC18 is tightly regulated without lacI\(^0\) in cis

The entire lacZ gene was reconstructed in pTTQ18 by insertion of a BamHI-DraI fragment from pMLB1034 (Shultz et al., 1982) into the BamHI-AosI segment of the vector (yielding pMJR1700). The same construct was also made in pUC18 for comparison (termed pMJR1720). When pMJR1720 was transformed into NM522 (F' lacI\(^0\)), \(\beta\)-galactosidase was estimated to comprise 50% of total cell protein after induction (Fig. 7, lane 4). Surprisingly, \(\beta\)-galactosidase synthesis was tightly regulated and little was detected in the absence of inducer (lane 3), suggesting that repressor level in the F' lacI\(^0\) strain is sufficient to repress expression of the homologous lacZ gene. This is in marked contrast to the results obtained above with heterologous genes, implying that lac repressor acts more effectively when downstream regions of lacZ are present. Previous work (Reznikoff et al., 1974) has identified within these downstream regions a sequence (codons 121-127) that strongly resembles the lac operator. This secondary lac operator binds repressor in vitro approximately one-fifth as well as the primary lacZ\(\alpha\) sequence (Winter and von Hippel, 1981) but its role in vivo is unclear. The present data therefore support the notion that the secondary operator does play a role in lac regulation in vivo and may be consistent with the observation (Besse et al., 1986) that lac repressor can interact with two operators concurrently.

(e) Some translational fusions in the pTTQ vectors are poorly expressed

By comparison with pMJR1720 (pUC construct), the maximal induced level of \(\beta\)-galactosidase synthesis from pMJR1700 (pTTQ construct) was much reduced (Fig. 7, lanes 1 and 2). This is consistent with the observation that \(\alpha\)-complementation of lacZ\(\Delta\)M15 by pTTQ vectors was weaker than with pUC vectors (not shown). In constructing the pTTQ vectors, four codons immediately following the initiator ATG of lacZ were removed (see Fig. 3). To determine if this was causing the reduced \(\beta\)-galactosidase expression from pMJR1700, four codons were added at this position using a synthetic oligodeoxynucleotide linker. In the new plasmid (pMJR1750) the length of the lacZ\(\alpha\) region was restored but differed in three nucleotide positions from the same region of pMJR1720. The induced levels of \(\beta\)-galactosidase synthesis from pMJR1750 (Fig. 7, lane 6) and pMJR1720 were comparable. These results suggest that the construction of the pTTQ vectors has brought sequences that are inhibitory to translation initiation closer to the ATG initiation codon. Since high-level expression has always been obtained using transcriptional fusions (see RESULTS AND DISCUSSION, section a), effects on transcription would be highly unlikely. Such inhibitory sequences might promote formation of local secondary structure occluding the RBS or ATG initiation codon, although comparison of predicted secondary structures in the relevant regions of the pMJR1700 and pMJR1750 transcripts do not support this particular idea. Translational fusions to the Km\(^R\) and cat genes using the pTTQ vectors were clearly expressed at high levels. This shows that sequences which are required for efficient translation of gene fusions in pTTQ vectors have not been lost and
Fig. 7. Expression of the lacZ gene from pTTQ18, pUC18 and pTTQ181. Protein from uninduced (−, lanes 1, 3, 5, 7) and induced (+, lanes 2, 4, 6, 8) cultures of cells containing different plasmid constructs was examined by PAGE using a 12.5% gel. Cells contained pMJR1700 (lanes 1 and 2), pMJR1720 (lanes 3 and 4), pMJR1750 (lanes 5 and 6) or pTTQ18 (lanes 7 and 8), as indicated. The first three plasmids encode the entire E. coli lacZ gene and are based on vectors pTTQ18, pUC18 and pTTQ181, respectively (Fig. 4). The positions of β-galactosidase (B) and of protein standards (M, × 10³) are indicated (see Fig. 5)

that the introduced DNA could restore a favorable context to the initiation codon.

The altered poly linker was transferred from pMJR1750 into pTTQ18 to give pTTQ181 (Fig. 3). The KmR gene fusion described above was expressed only marginally better from pTTQ181 as compared with pTTQ18 (not shown), suggesting that the extra sequence could not boost significantly expression that was already good.

(f) Conclusions

The pTTQ vectors described in this work constitute a series of plasmids for the high level expression of genes in E. coli. However, the most important feature of the plasmids is their ability to show tight regulation of the inserted gene regardless of the host strain used, by virtue of encoding their own lac repressor. Similar use of the lacI gene on multicopy vectors has also been described by Bagdasarian
et al. (1983) and Masui et al. (1984). Both these sets of vectors differ somewhat from the pTTQ plasmids. Plasmids pMMB22 and pMMB24 (Bagdasarian et al., 1983) are large, broad host-range plasmids which permit transcriptional fusions at a single restriction site, while the pINIII vectors (Masui et al., 1984) permit expression of genes from the E. coli lpp promoter under the control of lacZo as either transcriptional or translational fusions within the lpp gene at one of three possible restriction sites. Complete repression of the lac operator on multicopy plasmids can also be achieved by using lacIq strains (Amann et al., 1983), which overproduce the lac repressor 100-fold (Muller-Hill, 1975). However, most strains commonly used with vectors such as pUC are lacIq and overproduce lac repressor just 10-fold (Muller-Hill, 1975). Here and elsewhere (Amann et al., 1983) it can be clearly seen that this level of repressor is inadequate to cope with lacZo in multicopy. The pTTQ vectors permit the use of strains which are not lacIq but which have other desirable features (e.g., very high transformation efficiency). The pTTQ plasmids should be useful for all the applications described earlier (see INTRODUCTION). For example, a restriction fragment encoding the C-terminal 23 kDa of E. coli haemolysin 2001 has been cloned and expressed in pTTQ18, an application that had proved impossible using pUC vectors (Nicaud et al., 1986). The S. typhimurium hisP gene has also been cloned in pTTQ18 to obtain high level, regulated expression in E. coli, both in normal cells and in minicells (Y. Gibson-Harris, personal communication).

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The vectors pTTQ8, pTTQ9, pTTQ18, pTTQ19 and pMJR1560 will shortly be available from Amersham International plc.

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