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Strict regulation of gene expression from a high-copy plasmid utilizing a dual vector system

David F. Gruber [a,*], Vincent A. Pieribone [b], Barbara Porton [a], Hung-Teh Kao [a]

[a] Brown University, Department of Psychiatry and Human Behavior, Division of Biology and Medicine, 171 Meeting Street, Room 187, Box G-B187, Providence, RI 02912, USA
[b] The John B. Pierce Laboratory, Cellular and Molecular Physiology, Yale University, New Haven, CT 06519, USA

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ABSTRACT

High-copy plasmids are useful for producing large quantities of plasmid DNA, but are generally inadequate for tightly regulating gene expression. Attempts to suppress expression of genes on high-copy plasmids often results in residual or "leaky" production of protein. For stringent regulation of gene expression, it is often necessary to excise the gene of interest and subclone it into a low-copy plasmid. Here, we report a dual plasmid technique that enables tight regulation of gene expression driven by the lac promoter in a high-copy vector. A series of plasmids with varying copies of the *lacI*^{q} gene have been constructed to permit titration of the LacI protein. When a high-copy plasmid is transformed along with the appropriate *lacI*^{q}-containing plasmid, tight gene regulation is achieved, thus eliminating the need to subclone genes into low-copy plasmids. In addition, we show that this dual plasmid technique enables high-copy gene expression of a protein lethal to *Escherichia coli*, the *ccdB* protein. In principle, this technique can be applied to any high-copy plasmid containing the popular pUC replication of origin and provides an easier means of obtaining rigid control over gene expression.

To facilitate the manipulation of DNA, cloned genes are routinely propagated in high-copy number plasmids. Cloned genes are most commonly propagated in the gamma proteobacterium, *Escherichia coli*, due to its ability to grow rapidly and densely on numerous inexpensive substrates, its well-characterized genetics and cellular properties, and the availability of increasingly numerous cloning vectors and mutant strains [1,2]. However, when recombinant proteins are required, genes are typically subcloned and expressed from medium- or low-copy plasmids to avoid residual gene expression or "leakiness" that occurs in high-copy plasmids. Low-copy plasmids provide a lower gene dosage, which minimizes background levels and maintains tight regulation of expression. Typically, a plasmid that carries a gene of interest often requires a trans-acting regulatory protein that is expressed from the genome, an episome, or a second plasmid in order to suppress its expression [3,4]. It is especially difficult to avoid basal gene expression when using the popular lac repressor system, because four repressor molecules (forming a tetramer) are needed to bind to a single operator sequence in each cell in order to suppress gene expression.

Here, we describe a new, flexible strategy for controlled regulation of gene expression from a high-copy plasmid in *E. coli*. To test this strategy, we chose a high-copy plasmid that is commonly used to clone and sequence PCR fragments, pCR4-TOPO-Blunt (Invitrogen), which contains a pUC origin of replication that allows copy numbers of greater than 400 per cell [5]. This vector is popular because it exerts negative selection on vectors that do not contain an insert in its cloning site. The cloning site lies 5' proximal to the cytotoxic *ccdB* gene, which is driven by the lac promoter. In the absence of an insert, the *ccdB* protein is expressed, poisons DNA gyrase complexes [6,7], and kills the cell. In the presence of an insert, *ccdB* expression is disrupted (unless there is an open reading frame that continues expression of *ccdB*), permitting the cell to survive. If a gene is ligated into the cloning site, such that it is translated in the proper reading frame, the lac promoter may be utilized for the expression of that gene. This type of gene expression is constitutive, since there are insufficient repressor molecules available to suppress gene expression from a high-copy number plasmid. We have engineered a series of compatible plasmids that permit titration of the LacI repressor protein in *E. coli*, thereby allowing the investigator to choose a level of regulation that optimizes inducible expression from the lac promoter. This technique could be applied to expression from any high-copy plasmid with a compatible origin of replication.

Materials and methods

EGFP-containing plasmids to monitor gene expression

The Enhanced Green Fluorescent Protein (EGFP)[1] gene was used as a visual indicator to monitor expression. The EGFP gene was

* Corresponding author.
E-mail address: David_Gruber@Brown.edu (D.F. Gruber).

[1] *Abbreviations used:* EGFP, enhanced green fluorescent protein; IPTG, isopropyl-beta-D-1-thiogalactopyranoside.

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amplified from the vector pEGFP-1 (Clontech) by PCR, and cloned via topoisomerase I into pCR4-TOPO-Blunt (Invitrogen) to create the plasmid pCR4-TOPO-Blunt-EGFP, using the manufacturer's directions. Similarly, the amplified EGFP gene was subcloned into pET30 (Novagen) to create the plasmid pET30-EGFP.

Construction of a series of compatible plasmids containing different copies of the lac repressor gene

We selected a plasmid containing the RSF origin of replication for co-expression of the lac repressor, because the RSF replication

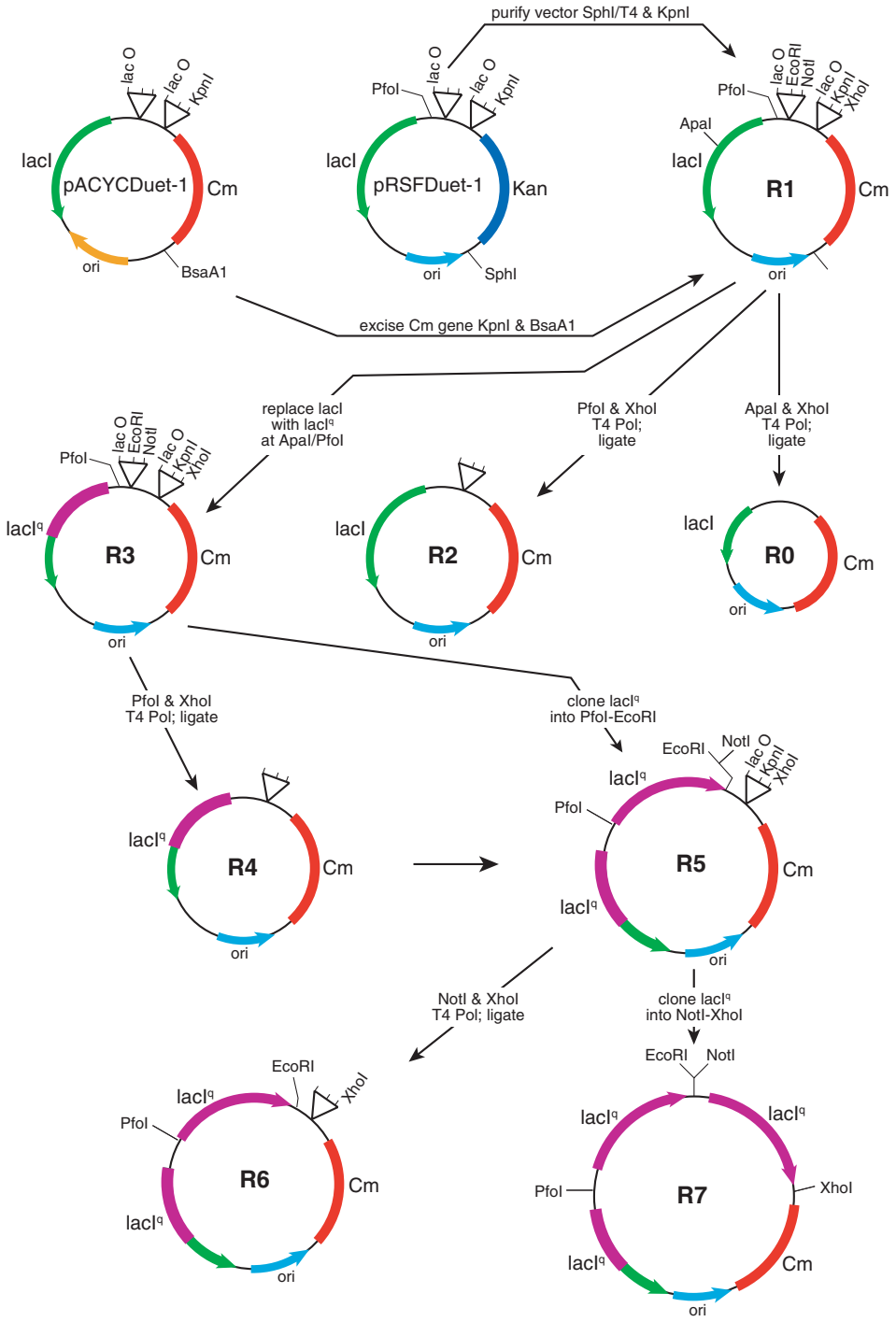

Fig. 1. Construction of a series of plasmids containing the RSF replication of origin and varying number of *lacI* or *lacI*^{q} genes. The parental vector for this series is R1, which was constructed by replacing the kanamycin cassette (Kan) of pRSFDuet-1 (excised with SphI and KpnI) with the chloramphenicol cassette (Cm) of pACYCDuet-1 (excised with KpnI and BsaA1). The SphI site was made compatible with the blunt-ended BsaA1 site by treatment with T4 DNA polymerase. The replication of origin (ori), location of *lac* operators (lacO), deleted sequences (Δ), and various restriction enzyme sites, are schematically shown. R0, which contains a truncated *lacI* gene and has no lac operator sequences, was created by digesting R1 with ApaI and XhoI, and recircularizing the vector. R2 possesses no lac operator sequences, which were deleted from R1 by PfoI-XhoI digestion and recircularization. In R3, the *lacI* gene is replaced with the *lacI*^{q} gene. A portion of the *lacI*^{q} gene (from pGex6P-1) was amplified using gene-specific primers, digested with ApaI and PfoI, and inserted into the ApaI-PfoI region of R1, thereby converting the *lacI* gene into *lacI*^{q}. R4 was constructed from R3, and is similar except that the two lac operators were deleted by PfoI-XhoI digestion and recircularization. In R5, a second *lacI*^{q} gene was inserted into the first cloning site of R3 (using PfoI and EcoRI, which deletes one of the two lac operators). R6 was constructed from R5, and is similar except that the lac operators were deleted by NotI–XhoI digestion and recircularization. In R7, a third *lacI*^{q} gene was inserted into the second cloning site of R5 (using NotI and XhoI, which deletes the remaining lac operator).