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F′-Coded, Temperature-Sensitive λ *c*I857 Repressor Gene for Easy Construction and Regulation of λ Promoter-Dependent Expression Systems

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We describe the construction and properties of an F′ factor which carries the temperature-sensitive *c*I857 allele of the repressor gene of coliphage λ and which lacks the λ *cro* function. This episome can easily be transferred to any F⁻ and F′ *Escherichia coli* strain, thus facilitating the construction and regulation of λ promoter-dependent expression systems without the use of defective prophages.

As excessive production of proteins is detrimental to the growth of microorganisms (2, 11), it is essential to regulate the expression of genes which are cloned downstream of strong promoters. Otherwise there would be a counterselection, and cells which have lost their plasmids would rapidly overgrow the culture (4).

Because of their high efficiency and the ease of their regulation, the promoters p_L and p_R of coliphage λ are widely used as promoters for expression vectors (14, 15, 19). λ expression vectors can be regulated in combination with a λ *c*I857 repressor gene which codes for a temperature-sensitive repressor. At 28°C, this repressor is active and represses the transcription from λ promoters. At 42°C, the temperature-sensitive repressor protein is inactive, and transcription is derepressed (22).

A λ prophage can be used as a carrier for the λ *c*I857 repressor gene only if the regulation unit lacks lytic functions resident on prophages. Otherwise cells would be killed after heat induction, leaving not enough time to produce large amounts of the gene product of interest. In addition, the regulation unit must be devoid of λ *cro* gene activity, because the Cro protein is a secondary repressor for the right as well as the left λ promoter. Thus, the presence of the functional Cro protein reduces the expression of genes cloned downstream of λ promoters p_R and p_L (5). Moreover, the C_1o protein blocks the synthesis of the λ repressor protein after derepression. Therefore, cells which are lysogenic for a temperature-sensitive defective prophage stay anti-immune in the presence of the Cro protein after growth at 40°C even at low temperature (3, 12). Given these constraints, there are different possibilities to achieve regulation of λ p_L-directed gene expression. (i) The *c*I gene can be placed on a defective prophage which lacks *cro* gene activity and lytic functions (1). However, the use of defective phages is restricted by difficulties in constructing and propagating defective phages. (ii) The regulation unit can be placed on an additional compatible plasmid which must be kept in the cells by selection (24). (iii) The *c*Its gene can be carried on the expression vector itself (23). However, in high-copy-number plasmids, the copy number of the *c*Its gene is also elevated, which leads to prolonged times before maximal induction is accomplished.

TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Relevant characteristics (genotype)	Source or reference
W620	F⁻ *thi-1 pyrD36 gltA6 galK30 rpsL129*	8
CSH50	F⁻Δ(*lac pro*) *ara thi rpsL*	13
DP90cNal	F⁻Δ(*lac pro*) *thi gyrA*	D. Gho
MM82-1	F⁻Δ(*lac pro*) *ara thi rpsL hsdR recA* Val^r (φ80 d*lacZ*ΔM15)	M. Mieschendahl (unpublished data)
F′*lac22 A4*	F′*cro*⁺*c*I ts857 *rex*⁺ Φ(*N′-lacZ*⁺)*22 A4* (Hyb) *proA*⁺*B*⁺	12
F′*lac22 A4 c*I⁺	F′*cro*⁺*c*I⁺ *rex*⁺ Φ(*N′-lacZ*⁺)*22 A4* (Hyb) *proA*⁺*B*⁺	This work
F′*lacZ*⁺*c*I857	F′*cro c*I ts857 *rex*⁺ Φ(*N′-lacZ*⁺)*22 A4* (Hyb) *proA*⁺*B*⁺	This work
pBR322-*lac22*	pBR322 derivative carrying the λ *N′-lacZ*⁺ fusion including genes *cro* and *c*I ts857	12
pEM1	pBR322-*lac22* derivative carrying a deletion including the N-terminal part of the *cro* gene	This work
pRM1	λ p_L expression vector	M. Mieschendahl (unpublished data)
pRM12	pRM1 carrying the citrate synthase gene *gltA*	This work

To avoid the difficulties described above, we constructed an episome, F′*lacZ*⁺*c*I857 (*cro*⁻ *c*Its) (Table 1), which expresses the temperature-sensitive λ repressor gene *c*I857 and the λ *rex* gene but lacks lytic functions as well as λ *cro* gene activity (Fig. 1). In addition, the episome carries a λ *N*⁻-*lacZ*⁺ gene fusion (12) and the genes *proAB* for easy

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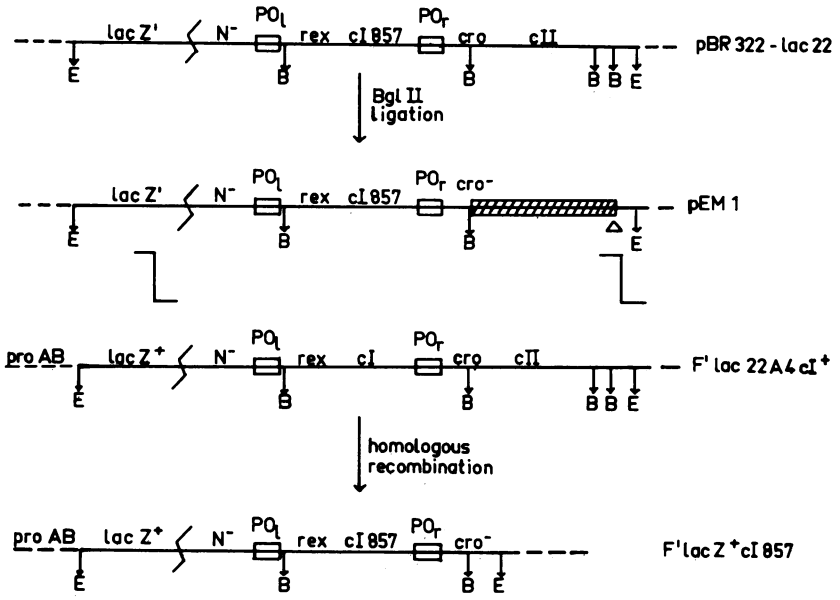

FIG. 1. Construction of F'*lacZ*$^+$*c*I857. *Eco*RI DNA fragments of plasmid pBR322-*lac22*, pEM1, and episomes F'*lac22 A4* cI^+ and F'*lacZ*$^+$*c*I857. Open boxes represent λ promoters and operators (PO), the hatched box represents the deletion of two *Bgl*II fragments (△), ⟆ indicates the λ N^--*lacZ*$^+$ gene fusion, ⌝ indicates the crossover points of the homologous recombination, and B and E indicate *Bgl*II and *Eco*RI restriction sites, respectively (see the text for details; not drawn to scale).

detection and selection. We used this episome for temperature-dependent control of λ p_L promoter plasmids.

To construct F'*lacZ*$^+$*c*I857, the *c*Its gene of F'*lac22 A4* (*cro*$^+$ *c*Its) (Table 1) was first replaced by the λ *c*I wild-type allele to allow selection for the deletion of the λ *cro* gene. The cI^+ allele was introduced by homologous recombination (13) with a wild-type prophage. After recombination, the episomes were transferred into *Escherichia coli* CSH50 (Table 1) which was lysogenic for the temperature-sensitive prophage λ *c*I857 *S7*. Cells which carried the recombinant episome F' *lac22 A4* cI^+ (*cro*$^+$cI^+) were selected at 42°C, because the repressor of the wild-type *c*I gene of F'*lac22 A4* cI^+ represses the temperature-sensitive prophage at high temperature.

To obtain the *cro*$^-$*c*Its derivative of F'*lac22 A4* cI^+ (*cro*$^+$cI^+), plasmid pEM1 (Table 1) had to be constructed and recombined with the episome (Fig. 1). Plasmid pBR322-*lac22* (12) is a derivative of pBR322 which carries an *Eco*RI fragment of the episome F'*lac22 A4* (*cro*$^+$*c*Its) including the λ genes *c*II, *c*I857, *rex*, and the λ N^--*lacZ*$^+$ gene fusion *lac22* (λ map 39169-35300 [18]) under the control of the λ p_L (Fig. 1). Plasmid DNA was digested with *Bgl*II to delete the two *Bgl*II fragments between λ map 38814 and 38103 which carry most of the *cro* gene. The religated DNA was transformed into the nonlysogenic *E. coli* strain, MM82-1 (Table 1), and incubated at 30°C. Ampicillin-resistant survivors were suspected of carrying the 2392-base-pair *Bgl*II fragment (λ map 38103-35711) which codes for the λ *c*I857 repressor, because derepression of the left λ promoter p_L on the plasmid is lethal to *E. coli* (6, 14, 21). This plasmid was called pEM1.

After the recombination of pEM1 with F'*lac22 A4* cI^+ (*cro*$^+$cI^+) to obtain F'*lacZ*$^+$*c*I857 (*cro*$^-$*c*Its) (Fig. 1), the episomes were crossed into strain DP90cNal (Table 1) which carries a *lac pro* deletion. The replacement of the cI^+ wild-type allele of F'*lac22 A4* cI^+ by the temperature-sensitive *c*I857 allele of pEM1 allows at high temperature the λ p_L-dependent transcription of the λ N^--*lacZ*$^+$ gene fusion carried by both episomes. Therefore, cells which carry

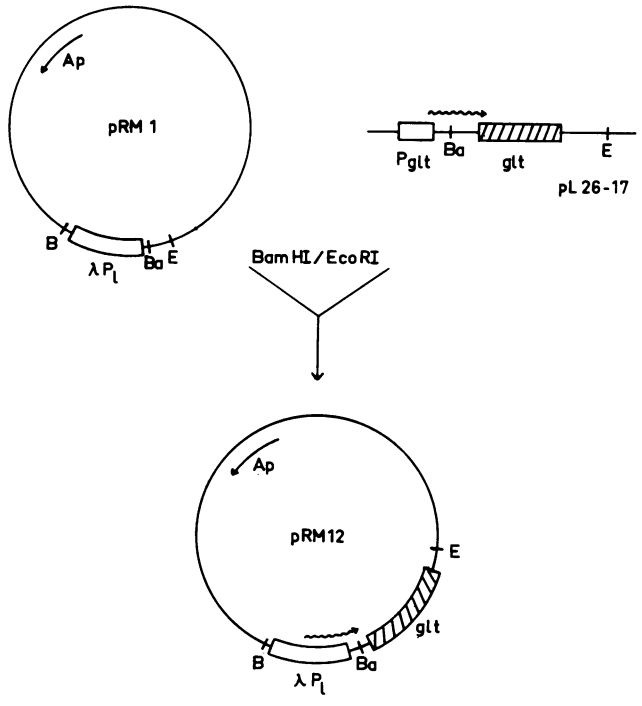

FIG. 2. Construction of plasmid pRM12. A *Bam*HI-*Eco*RI fragment of ColE1 plasmid pL26-17 (8) carrying the citrate synthase gene (*glt*) but not the citrate synthase promoter (9) was cloned into the *Bam*HI–*Eco*RI-restricted pRM1. Plasmid pRM1 carries the left λ promoter p_L on a *Bgl*II-*Alu*I fragment including the first of two possible Shine-Dalgarno sequences of the λ *N* gene but lacks a start codon (λ map 35711-35444 [18]). Open boxes indicate promoters, and the hatched box indicates the citrate synthase gene; B, Ba, and E represent *Bgl*II, *Bam*HI, and *Eco*I restriction sites, respectively. The waved line indicates the direction of transcription.

F'*lacZ*$^+$*c*I857 are Lac$^+$ at high temperature and could be selected on minimal salt lactose agar plates after prolonged incubation at 42°C. Cells which carry F'*lacZ*$^+$*c*I857 became only partially Lac$^+$, presumably because protein fusions are often unstable in *E. coli* at high temperature (7, 10, 25). The amount of β-galactosidase produced in this strain by the episome F'*lacZ*$^+$*c*I857 is sufficient for the selection but does not achieve the concentration which is produced in Lac$^+$ wild-type strains.

The episome F'*lacZ*$^+$*c*I857 (*cro*$^-$*c*Its) is LacZ$^-$ at 30°C and LacZ$^+$ at 40°C. In contrast to episome F'*lac22 A4* (*cro*$^+$*c*Its) which stays LacZ$^+$ at 30°C after growth at 40°C due to λ *cro* gene activity, F'*lacZ*$^+$*c*I857 (*cro*$^-$*c*Its) becomes immune again and thereby LacZ$^-$ at 30°C after growth at 40°C. This immunity-phase shift (3, 12) can be seen on glucose X-gal indicator plates (13) by the formation of blue (LacZ$^+$) or white (LacZ$^-$) colonies, respectively.

The usefulness of the regulation properties of episome F'*lacZ*$^+$*c*I857 for the regulation of λ p_L plasmids was examined by assaying the expression of the citrate synthase gene cloned into the λ p_L expression vector pRM1 (Table 1). The construction of this expression system is shown in Fig. 2. The plasmid was transformed into the citrate synthase auxotrophic strain W620 (Table 1) carrying the episome F'*lacZ*$^+$*c*I857. Cells were diluted into rich medium at 28 and 42°C, and the proteins were analyzed after 8 h of growth (Fig. 3). The proteins were quantitated by scanning the polyacrylamide-sodium dodecyl sulfate gel with a densitometer. Of soluble cell protein, 24% consisted of citrate synthase of a subunit molecular weight of 46,000 to 47,000 (9). The enzymatic activity of citrate synthase was assayed spectrophotometrically at 412 nm by using 5,5'-dithiobis(2-nitrobenzoate) to record the reduction of acetyl coenzyme A (20). Less than 7 mU/mg of protein and 1 U/mg of protein was found in ultrasonic extracts of cells grown at 28 and 42°C, respectively.

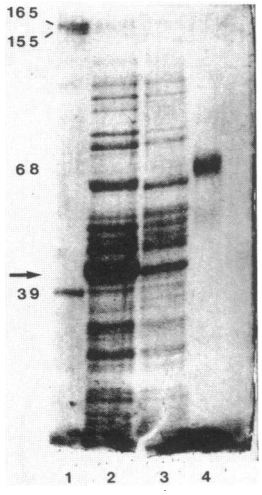

FIG. 3. Polyacrylamide-sodium dodecyl sulfate gel of W620/F'*lacZ*$^+$*c*I857 (pRM12) extracts. Cells grown overnight were diluted into fresh media and incubated for 6 h at 28 or 42°C. Extracts were prepared by suspending cells in a 1/20 volume of buffered sodium dodecyl sulfate and boiling for 3 min. The extracts were electrophoresed on a 7.5% polyacrylamide-sodium dodecyl sulfate gel. Lanes: 1, RNA polymerase marker protein (165, 155, and 39 kilodaltons); 2, extracts of cells grown at 42°C; 3, extracts of cells grown at 28°C; 4, bovine serum albumin marker protein (68 kilodaltons). The position of citrate synthase is indicated by the arrow. The strong band in lane 3 represents an *E. coli* protein of a molecular weight similar to that of citrate synthase. In an enzyme assay of citrate synthase (20), 1 U/mg of protein and less than 7 mU/mg of protein were found in extracts of sonicated cells grown at 42 and 28°C, respectively.

F'*lacZ*$^+$*c*I857 lacks all lytic functions and changes its immunity state from immune to anti-immune and vice versa, depending on the incubation temperature, because there is no *cro* gene activity. The episome can easily be transferred to any F$^-$ *E. coli* strain and even to F$^+$ strains by phenocopy crossing (13), because it carries selectable markers. This versatility, which cannot be matched by defective prophages, is of special interest, for higher level of expression of cloned genes or the selection of clones often is restricted to strains which carry specific mutations. In addition, the efficiency of temperature induction of λ lysogens sometimes varies with the host cells (16). Because the copy number of *E. coli* F' factors is only 1.5 to 2 times that of the chromosome (17), the λ *c*I857 repressor concentration in strains carrying F'*lacZ*$^+$*c*I857 is low compared with situations in which the repressor gene is cloned on multicopy plasmids. This should lead to reduced induction times. Therefore, F'*lacZ*$^+$*c*I857 should be a valuable tool for temperature-controlled expression of λ promoter-carrying plasmids.

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