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BL21-SI™ Competent Cells for Protein Expression in E. coli

E. coli are widely used for overproduction of recombinant proteins quickly, economically, and on a large scale. *E. coli* strains have been constructed specifically for expression of recombinant proteins *(table 1).* The most popular strain, BL21 and its derivatives (1,2), are descended from *E. coli* B and thus are deficient in the Lon protease. Additionally, the BL21 background lacks the OmpT outer membrane protease. Cells deficient in these proteases accumulate recombinant proteins at a high rate and are less likely to degrade some proteins during purification.

Overexpression of recombinant proteins in *E. coli* can have a toxic effect. One way to minimize the toxic effects is to clone the gene in a vector with a controllable promoter. A controllable expression system allows the culture to grow to a high density before inducing expression of the recombinant protein. Classically, *lac/tac* promoters that are induced with IPTG have been used in BL21 cells. Now, the most popular systems use the T7 RNA promoter (1,2) to induce high levels of recombinant protein expression. T7 RNA polymerase not only allows high levels of expression, but also is very specific and expresses only gene(s) cloned downstream of a T7 promoter. A derivative of BL21, BL21(DE3), has been modified to carry the T7 RNA polymerase gene under the control of a *lac*UV5 promoter. The T7 RNA polymerase is expressed after induction with IPTG and transcribes the recombinant DNA sequence.

In this paper, we describe a new host for inducible T7 promoter-based *E. coli* protein expression. The BL21-SI strain uses osmolarity to regulate expression. The BL21-SI strain is a derivative of the GJ1158 (3,4) salt-inducible strain. BL21-SI cells contain an integrated T7 RNA polymerase gene (1,2) under control of the salt-inducible *proU* promoter (5). High osmolarity (*e.g.,* 0.3 M NaCl) induces expression of T7 RNA polymerase from the *proU* promoter. The gene of interest is cloned in plasmid vectors that allow overexpression from a T7 promoter. In addition, this strain allows controllable expression of genes cloned into *lac/tac* promoter vectors using IPTG.

METHODS

Transformations were carried out following the manufacturers' instructions. BL21-SI Competent Cells (Cat. No. 11665) (>1 × 10^7 transformants/µg pUC19 DNA) should always be cultured in medium without salts until recombinant protein production is desired. Cells were plated on medium containing the appropriate antibiotics and incubated for 12 h to 16 h at 37°C before scoring. Cells containing recombinant clones were inoculated as small-scale cultures for overnight growth at 25°C in LBON medium [LB medium

Strain	*lac/tac* Promoter Expression	Inducer	T7 Promoter Expression	Inducer	*endA*
BL21-SI	Yes	IPTG	Yes	NaCl	Yes
BL21	Yes	IPTG	No	—	No
BL21(DE3)	No	—	Yes	IPTG	No

TABLE 1. Strain attributes. Note: DNA from cells without the *endA* mutation requires phenol extraction before further manipulations to prevent degradation.

The Help Box from Your Technical Support & Training Team

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Recombinant Protein Expression in Bacteria

Q. What *E. coli* strains are best for protein expression?

A. The strain you choose will depend on the plasmid and promoter being used. BL21-SI has the ability to express proteins under the control of either a T7 RNA polymerase promoter or *lac* and *trc* promoters. In addition, its genotype is lon⁻ and ompT⁻ to minimize degradation of expressed proteins. Also, proteins under the control of the *lac* and *trc* promoter have been expressed in DH5α and DH10B strains. Unique to BL21-SI is that genes expressed under the T7 RNA polymerase promoter can be induced with 0.3 M NaCl instead of IPTG.

Q. How can I improve protein solubility?

A.
- Lowering the induction temperature to 30°C may help increase solubility and reduce the formation of inclusion bodies.
- Use a low copy number plasmid, such as the pProEX™ HT Protein Expression Vectors.
- Use a less rich medium, such as M9 minimal medium.
- If the protein requires a cofactor, such as a metal, add the cofactor to the medium.

Q. How can I improve recombinant protein yield?

A.
- Inoculate from fresh bacterial cultures, since higher protein yields are generally obtained from a fresh bacterial colony.
- Check the codons usage in the recombinant protein sequence for infrequently used codons. Replacing the rare codons with more commonly used codons can significantly increase expression levels (1). For example, the arginine codons AGG and AGA are used infrequently by *E. coli*, so the level of tRNAs for these codons is low (1).
- Add protease inhibitors, such as PMSF, to buffers during protein purification. Use freshly made PMSF, since PMSF loses effectiveness within 30 min of dilution into an aqueous solution.
- Change the *E. coli* strain used for expression.

Reference

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BL21-SI Competent Cells continued

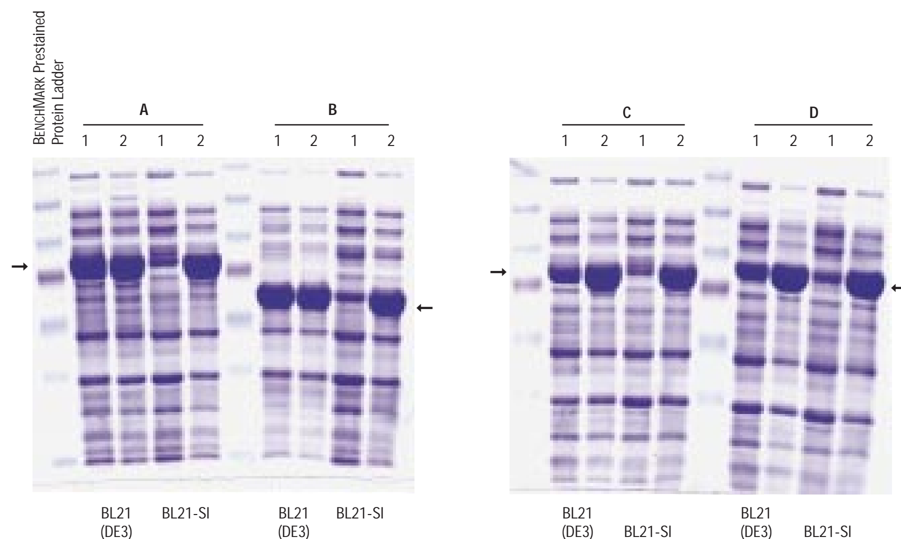

FIGURE 1. Regulation of T7-based expression. The cultures containing different proteins (dead box protein, RNA helicase, Klenow fragment, and β-glucuronidase, *panels A–D*, respectively) were induced with IPTG for BL21(DE3) cells or 0.3 M NaCl for BL21-SI cells. Extracts of uninduced *(lane 1)* and induced *(lane 2)* cultures were examined by electrophoresis.

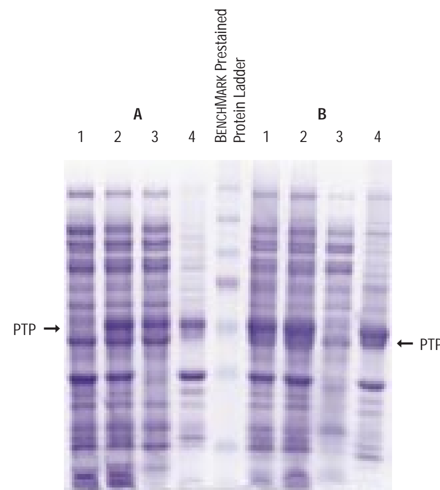

FIGURE 2. Recombinant protein solubility. The PTP gene was expressed in T7-based systems in BL21-SI cells *(panel A)* and BL21(DE3) cells *(panel B)*. *Lane 1.* Uninduced sample. *Lane 2.* Lysate of induced sample. *Lane 3.* Soluble fraction. *Lane 4.* Pellet.

without NaCl (3)] containing 100 µg/ml ampicillin for BL21-SI cells or in LB medium containing 100 µg/ml ampicillin for BL21 and BL21(DE3) cells. The overnight growth for each clone was diluted into two 250-ml baffled shake flasks containing 50 ml of fresh medium to an initial OD_{600} = 0.1. The cultures were incubated until OD_{600} = 0.5, and one of the flasks was induced. BL21-SI cells containing plasmids with T7 promoters were induced by adding NaCl to a final concentration of 0.3 M. BL21-SI cells containing pPROEX-HT™ (6) plasmids with a *trc* promoter (*trp/lac* hybrid), as well as BL21 and BL21(DE3) cells, were induced by adding IPTG to 1 mM. The cultures were induced for 2 h before harvesting. Cell pellets were lysed (7), and a portion of the induced sample was centrifuged to separate soluble and insoluble fractions.

Proteins (20 µg each) were electrophoresed on a 10% Tris-glycine polyacrylamide gel in GIBCO BRL 0.5X TGS at 150 V and at 150 mA (8). The gel was stained with COOMASSIE BRILLIANT BLUE R®-250.

RESULTS AND DISCUSSION

Various BL21 cells were examined for their ability to regulate recombinant protein expression from T7 or *lac/tac* promoters. Only BL21-SI cells can be used with both promoters.

For expression from T7 promoters, BL21-SI cells had high levels of protein produced with much tighter regulation than BL21(DE3) cells *(figure 1)*. Significant amounts of recombinant protein were seen in uninduced cultures of BL21(DE3) cells, but little or no protein was seen in uninduced BL21-SI cultures. Production of proteins requiring tight regulation of expression (*e.g.,* toxic genes, cell cycle proteins) would be difficult in cells with high levels of uninduced, leaky protein expression. Other BL21(DE3) derivatives [BL21(DE3)pLysS and BL21(DE3)pLysE] are less leaky, but these strains tend to produce less recombinant protein after induction (2).

It has been proposed that the osmotic shock used for induction of BL21-SI cells may increase protein solubility (3). This was seen for a rat tyrosine phosphatase protein (PTP). At least half of the PTP expressed in BL21-SI cells was soluble, while virtually all of the PTP produced in BL21(DE3) cells was in the pellet *(figure 2)*. Thus, at least in some cases, the BL21-SI strain may express more protein in a soluble form.

The effect of salt concentration used for induction was examined *(figure 3)*. Since expression was titratable (75 mM to 200 mM), it may help in soluble production of some proteins, especially those that tend to aggregate (9).

BL21-SI cells also can be used to express proteins from *lac/tac* promoters, as has been done with BL21 cells. Both BL21 and BL21-SI strains produced high levels of protein when induced with IPTG, with no discernible expression when uninduced *(figure 4A and B)*. In addition, other proteins cloned in *trp/lac* promoter vectors expressed high levels of protein with IPTG induction, but little protein in the absence of induction *(figure 4,C–F)*.

In summary, BL21-SI cells provide advantages over the existing BL21 strains. BL21-SI cells are highly competent; expression is tightly regulated; expression can use T7 or *lac/tac* promoters for salt or

IPTG induction, respectively; and the concentration used in salt induction can control the amount or rate of protein produced. In addition, BL21-SI cells contain an *endA* mutation that allows for quick recovery of intact plasmid DNA without the need for phenol extraction.

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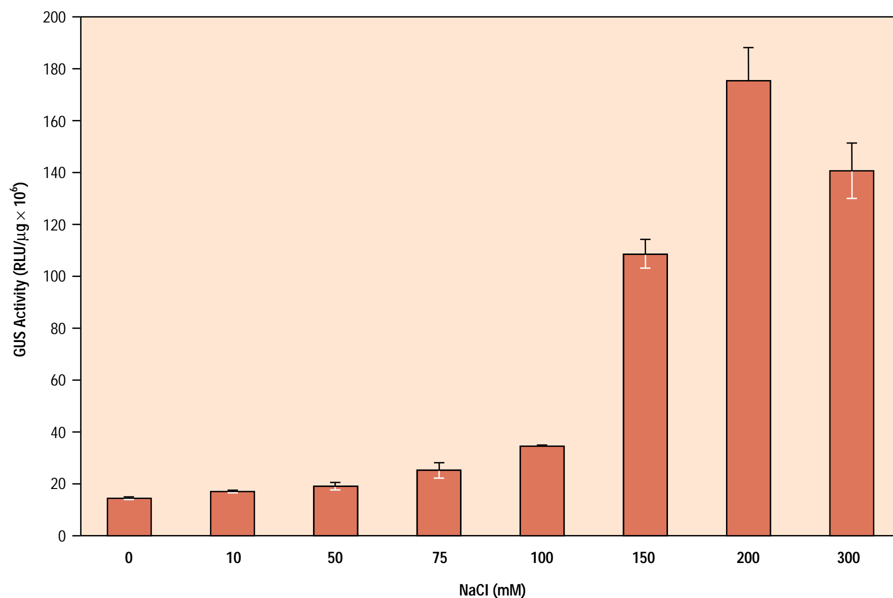

FIGURE 3. Titration of salt concentration. β-glucuronidase (GUS) cloned downstream of a T7 promoter was transformed into BL21-SI cells and expression was induced with various concentrations of NaCl. GUS activity was measured with a luminescent assay using 2 µl of extract diluted 1,000 times (10). Results are the mean ± SE for N = 3.

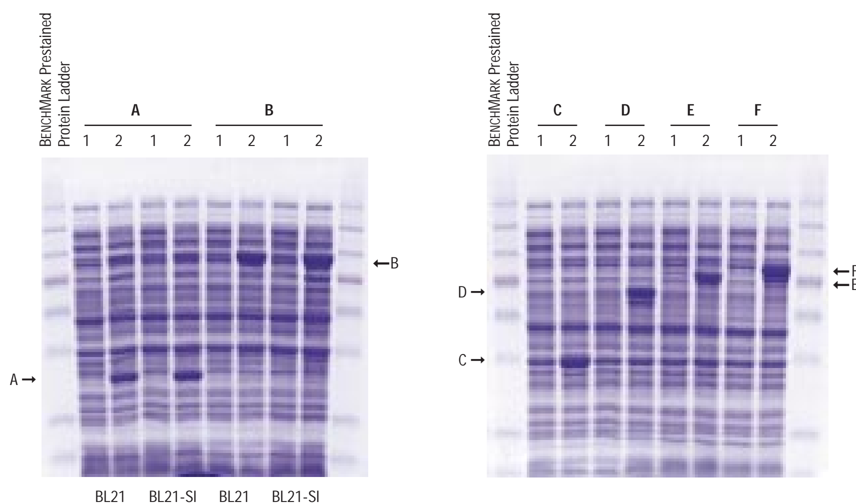

FIGURE 4. Expression from *trp/lac* promoters. Cultures containing different proteins (green fluorescent protein, GUS, galactose binding protein, RNA helicase, luciferase, and dead box protein, *panels A–F,* respectively) were induced *(lane 2)* with IPTG for *trp/lac*-based expression in BL21-SI cells. For A and B, expression in BL21-SI cells was compared with expression in BL21 cells.