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Communication

A freeze-thaw method for disintegration of *Escherichia coli* cells producing T7 lysozyme used in pBAD expression systems

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The pLysN plasmid containing the T7 lysozyme gene under control of the *lac* promoter was constructed to facilitate cell disintegration after expression of recombinant proteins in arabinose-induced expression systems. The usefulness of this plasmid was tested in *Escherichia coli* TOP10 and *E. coli* LMG194 cells carrying pBADMHA*Dge*SSB plasmid containing *Deinococcus geothermalis* SSB protein gene under control of the *araBAD* promoter. The results showed that low-level expression of T7 lysozyme did not interfere with the target SSB protein production, and that the freezing-thawing treatment was sufficient for disruption of the *E. coli* cells producing low amounts of T7 lysozyme.

Keywords: T7 lysozyme, disintegration, pBAD expression systems

Escherichia coli is the most frequently used prokaryotic expression system for production of heterologous proteins. However, efficient expression of different genes in *E. coli* is not a routine matter and requires a set of experiments for optimization of cultivation conditions such as induction time, inducer concentration and incubation time for maximum expression. Often, the samples must subsequently be analyzed by SDS/PAGE after lysis of the *E. coli* cells. Nowadays, several cell disruption methods are used (Bollag *et al.*, 1996), but sonication or incubation with lysozyme from chicken egg play the main role in laboratory practice. All these methods are time consuming, and an easier and faster method is desired. The commercially available *E. coli* strains, like BL21(DE3)pLysS or Rosetta(DE3)pLysS, used in Novagen's pET System or T7 Expression Systems (Invitrogen) enable high-level expression of recombinant proteins and elimination of basal expression level. They are also designed for easier cell disruption after expression as they produce low amounts of T7 lysozyme (Studier, 1991). Unfortunately, there is no such option available for the pBAD expression systems. Therefore, we have developed self-lysing *E. coli* strains for the arabinose-induced expression systems. First, we constructed a pLysN plasmid (Fig. 1) that allowed expression of lysozyme T7 at low levels in bacterial cells. This plasmid was obtained using the pACYC184 cloning vector. The DNA fragment containing *lac* promoter and *lac* operator was amplified by PCR using the DNA of plasmid pUC19 as the template. The primers used were: PromS1 5′-GAGGATCGGTCGACGCGCAACGCAATTAATGTGA-3′ and PromClys 5′-GTACACGAGCCATAGCTGTTTCCTGTGTGTGAAATTG-3′. For PCR amplification of the lysozyme T7 gene, the LysNprom 5′-GAAACAGCTATGGCTCGTGTACAGTTTAAACAACG-3′ and LysCB1S1 5′-CAGTCGACGGATCCTTATCCACGGTCAGAAGTGACCAG-3′ primers were used with the pLysS plasmid (Novagen) as the template. Both PCR products, diluted 250 times, were then mixed with primers PromS1 containing *Sal*I recognition site (underlined) and LysCB1S1 containing *Sal*I and *Bam*HI recogni-

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Abbreviations: SSB, single-stranded DNA-binding; *Dge*SSB, single-stranded DNA-binding protein from *Deinococcus geothermalis*; LB medium, Luria-Bertani medium; SDS/PAGE, SDS/polyacrylamide gel electrophoresis.

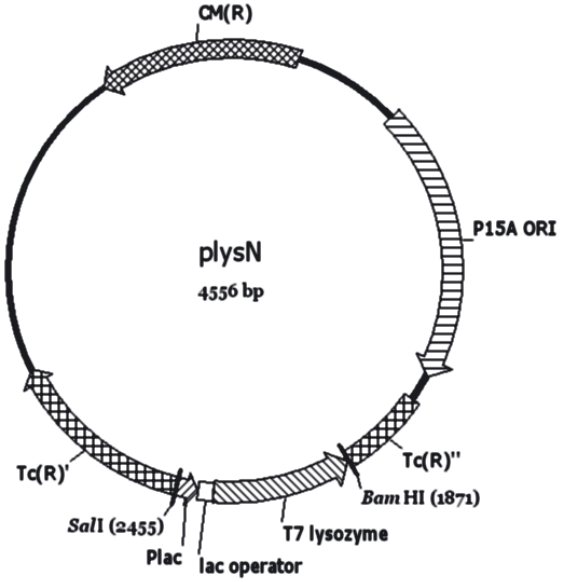

Figure 1. Map of the pLysN plasmid.

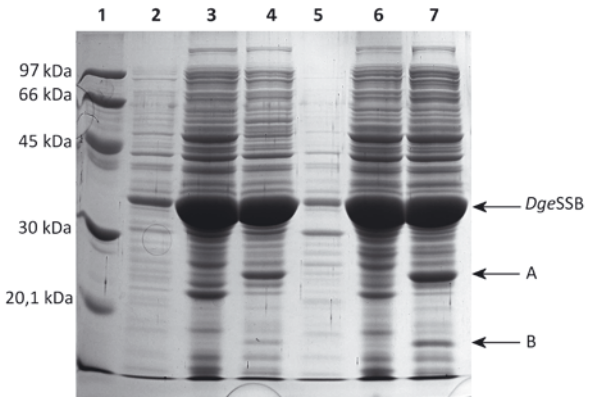

Figure 2. SDS/PAGE analysis of soluble fractions of cell lysates obtained after expression of the recombinant *Dge*SSB protein.
Electrophoresis in 15% polyacrylamide gel by the method of Laemmli. 1. LMW SDS marker (Amersham Biosciences AB, Uppsala, Sweden); 2. *E. coli* TOP10 + pBADMHA*Dge*SSB frozen and thawed; 3. *E. coli* TOP10 + pBADMHA*Dge*SSB sonicated; 4. *E. coli* TOP10 + pLysN+ pBADMHA*Dge*SSB frozen and thawed; 5. *E. coli* LMG194 + pBADMHA*Dge*SSB frozen and thawed; 6. *E. coli* LMG194 + pBADMHA*Dge*SSB sonicated; 7. *E. coli* LMG194 + pLysN + pBADMHA*Dge*SSB frozen and thawed. A. Chloramphenicol acetyl transferase; B. T7 lysozyme.

tion sites (underlined) and used in a PCR reaction to obtain the DNA fragment for cloning. Following *Sal*I and *Bam*HI digestion this DNA fragment, containing the T7 lysozyme gene under control of the *lac* promoter, was cloned into the pACYC184 vector digested with the same restriction enzymes. Next, competent cells of *E. coli* TOP10 (Invitrogen) or LMG194 (Invitrogen) strains (the most frequently used hosts for protein expression in arabinose-induced expression systems) were transformed with the constructed pLysN plasmid. These strains displayed a low-level production of T7 lysozyme when cultivated in LB medium (Fig. 2). The lysozyme was expressed constitutively since *E. coli* TOP10 and *E. coli* LMG194 strains do not contain the entire *lac* operon or the *lac*I gene encoding the LacI repressor (Δ*lacX*74 strains).

To test the usefulness of the constructed strains in an arabinose-induced expression system, competent cells of *E. coli* TOP10 or LMG194 strains containing the pLysN plasmid were transformed with the pBADMHA*Dge*SSB plasmid (Filipkowski *et al.*, 2006), carrying *Deinococcus geothermalis* SSB protein gene under control of the *araBAD* promoter. The same bacterial strains without the pLysN plasmid were used as expression controls. Fifty milliliters of the bacterial cultures were grown to mid-log phase (OD_{600} 0.5) and the expression was induced with 0.2% of L-arabinose for 12 h. Two milliliters of each cell suspension was centrifuged, and the pellets were resuspended in 100 μl of 20 mM Tris/HCl, pH 7.5 and placed at –20°C for 15 min. After thawing at room temperature, the samples were centrifuged and 20 μl samples of the supernatants were separated by SDS/PAGE (Fig. 2, lanes 2, 4, 5 and 7). In addition, control samples were sonicated four times for 30 s at 0°C (Fig. 2, lanes 3 and 6). The results showed that the presence of T7 lysozyme did not interfere with the target SSB protein expression, and that the cell disruption was facilitated by the freezing-thawing treatment.

In summary, the developed freeze-thaw method for disintegration of *E. coli* cells producing T7 lysozyme used in pBAD expression systems is very simple and rapid and its efficiency for small volume samples is comparable to the efficiency of sonication.

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