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Expression and Preparation of Fusion Proteins from Recombinant λgt11 Phages

Sheng-He Huang and Ambrose Jong

1. Introduction

The phage λgt11 system has become increasingly popular for expression of cDNAs or genomic DNAs either in phage plaques or in bacteria lysogenized with recombinant phages *(1,2)*. It offers the advantages of high cloning efficiency, high-level expression, the relative stability of β-galactosidase fusion proteins, and simple approaches to purify the fusion proteins. After the desired clone is detected and purified, it is often necessary to obtain preparative amounts of recombinant protein specified by the fusion of the foreign sequence to the carboxyl-terminus of β-galactosidase in λgt11 expression system. The conventional method for preparing fusion proteins from the recombinant λgt11 clones involves production of phage lysogens in *Escherichia coli* strain Y1089 followed by inducing *lacZ*-directed fusion protein expression with isopropyl-β-D-thiogalactopyranoside (IPTG) *(1)*. This method has two limitations: it is time-consuming, and phage lysogeny occurs at a low frequency. We have previously described a method for making fusion proteins from LB agar plates containing *E. coli* Y1090 infected with a high concentration of recombinant λgt11 phages (up to 5×10^6 PFU/150 × 15-mm plate) *(3)*. A liquid culture method for preparing fusion proteins from *E. coli* Y1090 infected with the λgt11 clones has previously been described *(4)*. More recently, some improvements have been made on the plate method by repeating induction and elution *(5)*. Although the liquid culture method allows the recovery of only 0.2–1% of total proteins *(6,7)*, this method generally yields 5–10% of expressed protein in solution, that is, most lysed cells are trapped in the agar and the expressed proteins are recovered in a small volume of inducing solution, resulting in a higher final concentration of protein. More than 200 μg of fusion protein can be obtained from one plate.

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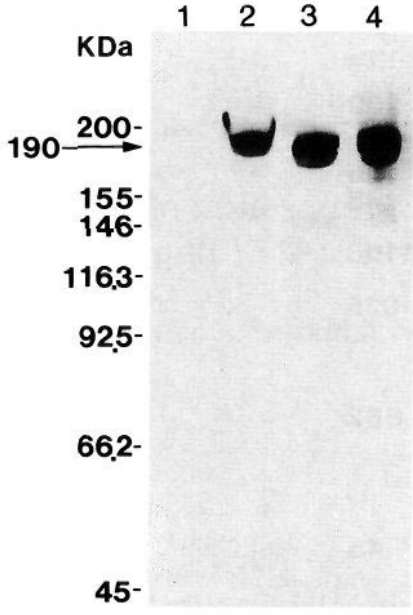

Fig. 1. Induction of the 190-kDa ERP72 fusion protein with various concentrations of IPTG (lane 1, no IPTG; lane 2, 2.5 m*M*; lane 3, 5 m*M*; lane 4, 10 m*M*). Fusion protein was detected by Western blot analysis with anti-ERP72 antibody *(8)*.

Currently, the plate method appears to be a simple and efficient way to express and prepare fusion proteins from recombinant λgt11 phages (Figs. 1 and 2). This chapter describes how the β-galactosidase fusion proteins can be made and isolated from the recombinant λgt11 phages with the plate method.

2. Materials

2.1. Plating Bacteria

1. Bacterial strain: Y1090 strain is deficient in the *lon*$^-$ protease. In *lon*$^-$ cells, β-galactosidase fusion proteins can accumulate at much higher levels than in wild-type cells.
2. Phage: λgt11.
3. LB/ampicillin medium: 10 g bacto-tryptone, 5 g yeast extract, 5 g NaCl/L. After autoclaving and cooling, add 100 mg/L ampicillin from a 100 mg/mL stock solution prior to use.
4. LB plates: 1.5% agar in LB containing 10 m*M* $MgCl_2$ and 100 μg/mL ampicillin.
5. LB top agar: 0.7% agar in LB containing 10 m*M* $MgCl_2$ and 100 μg/mL ampicillin.
6. 1*M* $MgSO_4$ (autoclave).
7. Phage buffer (SM): 50 m*M* Tris-HCl, pH 7.5, 100 m*M* NaCl, and 10 m*M* $MgCl_2$ or $MgSO_4$.

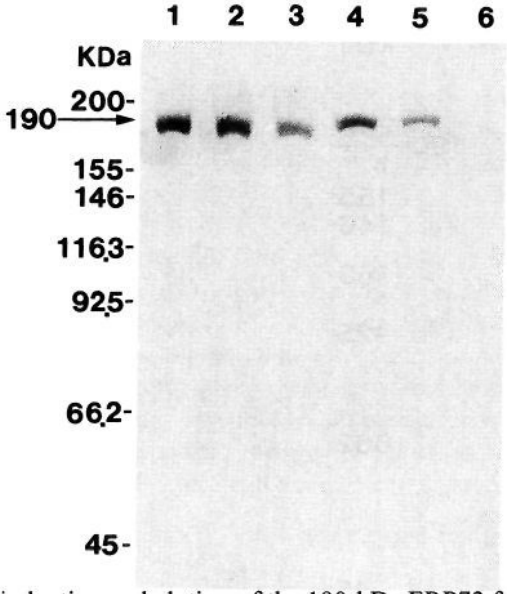

Fig. 2. Repeated induction and elution of the 190-kDa ERP72 fusion protein from agar plates. *E. coli* Y1090 cells were infected and plated by the procedure described in Section 3. Plates were incubated for 3 h at 42°C, and 5 mL of 5 m*M* IPTG in 0.5X LB containing 10 m*M* $MgSO_4$ added to each plate. Incubation was carried out at 37°C for 3 h (lane 1), and the supernatant saved. Induction and elution were repeated five times with 1-h intervals between inductions (lanes 2–6). Fusion proteins recovered after each induction was analyzed by Western blotting.

2.2. Fusion Protein Expression

1. 1*M* IPTG: 240 mg in 1 mL H_2O. Store frozen.
2. 0.5X LB containing 10 m*M* $MgSO_4$.

2.3. Detection of Fusion Proteins

1. 5X Polyacrylamide gel electrophoresis (PAGE) sample buffer: 15% β-mercaptoethanol, 15% sodium dodecyl sulfate (SDS), 1.5% bromophenol blue, and 50% glycerol.
2. 10% Polyacrylamide gels containing 0.2% SDS.

2.4. Preparing Crude Fusion Proteins

1. TEP buffer: 0.1*M* Tris-HCl, pH 7.5, 10 m*M* EDTA, and 1 m*M* phenylmethylsulfonyl fluoride (PMSF).
2. Saturated or solid $(NH_4)_2SO_4$.

2.5. Immunoaffinity Purification of Fusion Proteins

1. Immunoadsorbent: ProtoSorb *lacZ* Adsorbent (Promega, Madison, WI).
2. Necessary buffers *(6)*.

3. Methods

3.1. Plating Bacteria

1. Grow host cells: Streak the Y1090 on LB agar plates containing 100 μg/mL ampicillin. After growing this plate overnight at 37°C, store it at 4°C. Inoculate the plating cells from a single colony in LB containing 100 μg/mL ampicillin for 8–18 h at 37°C.
2. Infect the cells with phages: Up to 5×10^6 recombinant phages/plate (150 × 15 mm) can be used. Mix 700 μL of the Y1090 culture with up to 200 μL of the phage in SM. Allow the phage to adsorb the cells for 15–30 min at room temperature.
3. Plate cells and phages: Add 7.5 mL of LB top agar (at 45–50°C) to the infected cells, pour onto an LB agar + ampicillin plate (at room temperature), and plate evenly over the surface of the plate. Use slightly dry plates (2 d old) so that better adhesion of the top agar to the bottom agar will be obtained
4. Grow plaques: Allow the top agar of the plates to solidify for 30 min at room temperature. Incubate the plates at 42°C for 3–3.5 h.

3.2. Fusion Protein Induction

1. Add 5–10 mL of 5 m*M* IPTG in 0.5X LB containing 10 m*M* $MgSO_4$ to each plate
2. Incubate at 37°C for 3 h. Recover the supernatant.
3. Repeat steps 1 and 2, except that the incubation time is reduced to 1–2 h Pool the eluate from each induction.

3.3. Detection of Fusion Proteins

1. Transfer 24 μL of the eluate to a microcentrifuge tube. Spin at 12,000*g* for 5 min to remove the cellular debris and agar, and then transfer the supernatant to a fresh tube.
2. Add 6 μL of 5X PAGE sample buffer to the tube, and boil for 5 min. Analyze samples on a 10% polyacrylamide gel and visualize proteins with Coomassie blue, or detect the fusion protein on Western blot with specific antibodies.

3.4. Preparing Crude Fusion Proteins

1. Centrifuge the pooled eluates at 15,000*g* for 10 min to get rid of the debris and save the supernatant.
2. To the supernatant, add either solid ammonium sulfate or 3 vol of saturated ammonium sulfate to 75% saturation, and stir at 4°C for 20 min.
3. Centrifuge at 15,000*g* for 20 min, discard the supernatant, and redissolve the pellet in cold TEP buffer at approx 20 mg/mL

3.5. Immunoaffinity Purification of Fusion Proteins (6)

After diluting the crude fusion proteins with 50 m*M* Tris-HCl buffer, pH 7.3, to about 4 mg/mL total protein, it is convenient to purify the fusion protein by the prepared immunoaffinity column (ProtoSorb *lacZ* Immmunoaffinity Adsorbent) available from Promega.

4. Notes

1. In some cases, allowing expression at 30°C rather than at 37°C could help stabilize expressed fusion proteins.
2. Low amount of IPTG and phages may be used if expression is too high. The expression level of fusion proteins can be estimated by immunoblotting analysis on a plaque lift along with dot blotting a series of known amounts of the antigen on a membrane disk, which is also used to make a plaque lift The lower detection limits of alkaline phosphatase- and peroxidase-conjugated second antibodies are 20–50 and 200–500 pg of antigens, respectively *(1)*.
3. In certain cases where the fusion protein recovery is extremely low (i.e., <5 μg fusion protein/plate), the yield may be improved by a couple of ways. Some fusion proteins trapped in the top agar can be extracted with TEP buffer and precipitated with ammonium sulfate as above. The recovery may be increased by including a fractionation step (such as gel filtration) prior to immunoaffinity purification.
4 Fusion proteins may be purified by alternative ways. If the immunoaffinity column is not available, the large size of the fusion protein is suitable for preoperative SDS-PAGE and gel-filtration chromatography

Acknowledgments

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