Rosetta DE3 pRare pLysS

Centro Andaluz de Biología del Desarrollo

Expresión 08
Resistencia Cloramfenicol 34 µg/mL

Referencias:

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Rosetta™ host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. (1–5). The original Rosetta strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid, pRARE (6). The designation (DE3) means that the strin is lysogenic for a l prophage that contains an IPTG-inducible T7 RNA polymerase. lDE3 lysogens are designed for protein expression from pET, pETcoco™, pETBlue™, pTriEx™, pCDF, pRSF, and Duet vectors. By supplying rare codons, the Rosetta strains provide for "universal" translation, where translation would otherwise be limited by the codon usage of *E. coli*. (4, 6). The tRNA genes are driven by their native promoters (6). In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. Having the pLysS designation carry a pACYC184-derived plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase that serves to repress basal expression of target genes under the control of the T7 promoter.
The tRNA genes are driven by their native promoters (18). In the pLysS derivative of this strain, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme.

Genotype of *E coli* strain Rosetta DL3 pRare: F^- *ompT hsdS*B(rB^- mB^-) *gal dcm* (DE3) pLysSRARE2 (CamR)

TSS transformation:
1. Inoculate one colony in 3 mL LB plus Cm. Incubate o/n 37ºC with shaking

2. Dilute 100 times in 10 mL LB plus Cm. Incubate 37ºC with shaking until reach OD_{600}

0.35. Take 1 ml alicuot and cold on ice.

3. Centrifugue 30 sec. Discard supernatant.

4. Add 75 µL cold LB and mix. Keep on ice 5 min

5. Add 75 µL cold TSS2X, mix softly. Keep on ice 5 min.

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6. Add DNA (5 to 10 µL), mix softly. Keep on ice 30 min.

7. Incubate 42ºC durante 40 seg. Transfer to ice .

8. Add 1 mL LB and incubate 37ºC 90 min.

9. Plate 100 µL on LB with Cm and the specific antibiotic for the plasmid selection.

Centrifugue the rest of cells and plate them in another plaque.Incubeta o/n at 37ºC.

Medium TSS2x, 100 mL
Tryptone 0.8g
Yeast extract 0.5g
NaCl 0.5g
PEG 8000 20g
DMSO 10 mL
$MgSO_4$ $7H_2O$ 1M 10 mL
H_2O 70 mL
Adjust pH 6.5
Add water until 100 mL
Autoclave and make alicuots

Induction of target protein using IPTG:
The following induction protocol is a general guide for expression of genes under the control of IPTG-inducible promoters on an analytical scale (1 ml of induced culture). Most commonly, this protocol is used to analyze protein expression of individual transformants when using BL21(DE3) host strains in combination with plasmids containing T7 promoter constructs (e.g. pET vectors). Expression cassettes under the control of the trp/lachybrid promoter, tac, can be also induced using this protocol. In the case of tacpromoter constructs, non-DE3 lysogen strains can be employed as hosts.

1. Following transformation, pick a colony and inoculate it into 3 ml of LB medium containing the appropriate antibiotic with shaking at 37°C, overnight. For strains with the pLysS, it is preferable to add chloramphenicol at a final concentration of 34 µg/ml in the overnight culture to maintain pLysS.

2. The next morning, transfer 0.5 ml of the overnight culture to a new 10 ml of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD_{600} reaches 0.5 (approximately 2 hrs but this depended on the expression plasmids).
When using BL21(DE3)pLys, chloramphenicol is not usually required in the short-period culture.

3. When the OD_{600} reaches 0.5, transfer an aliquot (e.g., 1 ml) of the culture to a new centrifuge tube and centrifuge it to harvest cells. Store the cells at -80°C until analysis.
Add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours.
The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.
4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 ml) and centrifuge it to precipitate the cells.

Analysis by SDS-PAGE electrophoresis
1. Suspend the precipitated cells (from the 1 ml culture) in 200 µl of 1× PBS buffer.
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.
3. Heat the mixture at 85°C for 5 min, then centrifuge at 10,000 g for 10 min. Subject the supernatant (e.g., 5-25 µl) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

• 2 × SDS sample buffer : 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 20 % glycerol, 0.02% BPB, 62.5 mM Tris-HCl, pH6.8
• 1× PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

● Notes for expression:
1. When expressing proteins in BL21(DE3) cells, if it takes longer time (5 hrs or more) to reach 0.5 at OD_{600} after inoculating the overnight culture (0.5 ml) to a new LB medium (10 ml), the expressed protein is likely toxic to *E. coli* cells. See a, b, and c in the section “otes for transformation 1”
2. When BL21(DE3) cells lyse after induction with IPTG, the expressed protein is likely toxic to *E. coli* cells.