tech NOTE

Preparation of chemo-competent KL740 cells for amplification of P_{70a} vectors

INTRODUCTION

In vitro protein synthesis using the **myTXTL®** system utilizes the primary transcription(TX)-translation(TL)-machinery of *E. coli,* and consequently relies on sigma factor 70 and the endogenous core RNA polymerase for transcription. myTXTL® P_{70a} vectors allow expression of a target gene under transcriptional control of the strong, constitutively active lambda phage promoter P_L resulting in high protein yield. Amplification of such plasmids requires the presence of functional lambda phage repressor protein cI (or its mutant cI857) to prevent the RNA polymerase from binding to P_L and to ensure high plasmid integrity (Figure 1). To this end, an engineered *E. coli* strain, KL740 cI857+, which over-expresses the repressor gene *cI857* from its genome, is recommended for amplification of P_{70a} vectors.

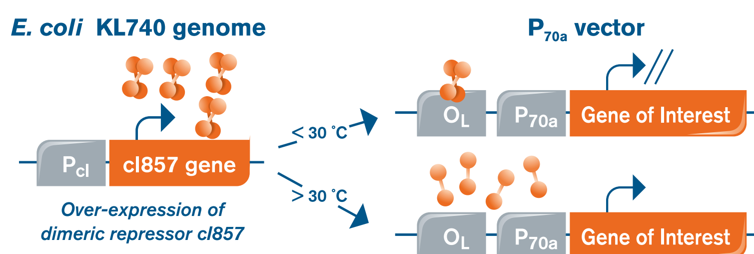

Figure 1. Control of gene expression in *E. coli* KL740 cI857+. This strain over-expresses the temperature sensitive mutant of the lambda phage repressor cI857. A constant growth temperature of 29 °C is crucial to ensure a maximum level of active cI857 repressor, which allows efficient gene repression upon transformation. At higher temperatures, the cI857 repressor protein is inactivated and falls off the operators thus allowing gene transcription.

This Technical Note describes a standard procedure for preparation of chemo-competent KL740 cI857+ cells for amplification of myTXTL® P_{70a} vectors.

MATERIALS AND METHODS

Bacterial Strains, Reagents and Solutions

- *E. coli* KL740 cI857+ (*E. coli* Genetic Stock Center, #14222)
- LB agar plates (with and without selection marker)
- LB medium
- $MgCl_2$
- $CaCl_2$ dihydrate
- Glycerol

Prepare the following solutions at least one day prior to the preparation of chemo-competent cells. All solutions should be sterilized by autoclaving and cooled down to 4 °C before use.

- Wash Buffer 1 (100 mM $MgCl_2$ solution)
- Wash Buffer 2 (50 mM $CaCl_2$ solution)
- Storage Buffer (50 mM $CaCl_2$, 15 % v/v glycerol)

Consumables

- 13 mL culture tube
- 100 mL culture flask
- 50 mL Falcon
- 1.5 mL reaction tubes
- Pipettors and sterile tips

Instruments

- Autoclave
- Incubator
- Shaker incubator
- Refrigerable centrifuge with rotor for 50 mL Falcons
- Heat block set to 37 °C
- - 80 °C freezer

Methods

The following procedure is adapted from the calcium chloride method described in Sambrook *et al*. 1989 (Molecular Cloning: A Laboratory Manual). Other methods might also produce efficient chemo-competent cells but have not been validated by Arbor Biosciences.

A. Preparation of chemo-competent cells

Important: All steps should be carried out aseptically.

A1. Streak *E. coli* KL740 cI857+ cells from a glycerol stock onto a LB agar plate and incubate for ~16 h at 29 °C. [Note: A constant growth temperature of 29°C is crucial to ensure a maximum level of active cI857 repressor, which allows efficient gene repression upon transformation.]

A2. Inoculate 2 mL LB medium in a 13 mL culture tube with a single colony of KL740 cI857+ and grow cells for 16-24 h at 29 °C and 200 rpm.

A3. Next day, dilute stationary KL740 cI857+ culture 1:100 in 50 mL fresh LB medium in a 100 mL culture flask and incubate at 29 °C and 200 rpm till $OD_{550} = 0.5$.

A4. Transfer entire culture into a pre-chilled, sterile 50 mL Falcon. For optimum transformation efficiency, keep cells cold from here on and work as fast as possible under sterile conditions.

A5. Centrifuge cells at 4400 x g, 4 °C for 10 min and discard supernatant.

A6. Resuspend cells in 40 mL ice-cold **Wash Buffer 1**. It is recommended to resuspend cells initially in 1-2 mL of buffer using a pipettor. Then fill up Falcon with buffer to the recommended volume, and mix by inverting.

A7. Centrifuge cells at 4400 x g, 4 °C for 10 min and discard supernatant.

A8. Resuspend cells in 20 mL ice-cold **Wash Buffer 2**. It is recommended to resuspend cells initially in 1-2 mL of buffer using a pipettor. Then fill up Falcon with buffer to the recommended volume, and mix by inverting. Incubate cell suspension for 30 min on ice. Then centrifuge as before.

A9. After removing the supernatant completely, resuspend cells in 2 mL ice-cold **Storage Buffer**.

A10. Aliquot the cell suspension as 150 µL per 1.5 mL tube on ice, and immediately store at -80 °C. Sufficient competency should last at least 6 months.

B. Setting up a transformation reaction

B1. Thaw an aliquot of chemo-competent cells on ice.

B2. Add 10-50 ng plasmid to a 150 µL aliquot of chemo-competent KL740 cI857+ cells.

B3. Incubate for 25 min on ice.

B4. Incubate cells for 5 min at 37 °C using a heat block or water bath.

B5. Transfer cell suspension into 2 mL LB medium in a 13 mL culture tube and incubate for 30 min at 29 °C and 200 rpm.

B6. Centrifuge cells for 1 min at 3000 x g at room temperature, remove 1.9 mL of the supernatant, and resuspend cell sediment in the remaining medium.

B7. Plate at least 30 µL of that cell suspension on a pre-warmed LB agar plate containing the appropriate selection marker (and the entire remaining cell suspension on a separate plate as a back-up) and incubate the plate for 16-24 h at 29 °C. [Note: This protocol may be shortened by omitting Step B6 and plating an aliquot of the DNA-cell mix directly onto antibiotic-containing LB agar plates.]

REFERENCES

Valdez-Cruz N.A. *et al.* (2010) Production of recombinant proteins in *E. coli* by the heat inducible expression system based on the phage lambda pL and/or pR promoters. Microbial Cell Factories. 19;9:18

Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual. Fourth Edition.