TECHNICAL NOTE

Preparation of Chemo-Competent KL740 Cells for Propagation of P70a Vectors

INTRODUCTION

The **myTXTL®** Cell-Free Expression system is compatible with many promoter systems and among those, the strong lambda phage promoter pL encoded on myTXTL P70a vectors constitutes an excellent choice for high-target protein yield. To facilitate maximum plasmid integrity during propagation and cloning procedures involving myTXTL P70a vectors, Daicel Arbor Biosciences highly recommends utilizing the *E. coli* strain KL740 cI857+ which over-expresses the lambda phage repressor variant cI857 from its genome. The repressor protein cI857 mediates tight gene expression control for the pL promoter in a temperature-sensitive manner, thus preventing unwanted target protein synthesis (Figure 1). The results of not following the recommendation will be poor plasmid quality, transformation inefficiency/failure or target gene alteration.

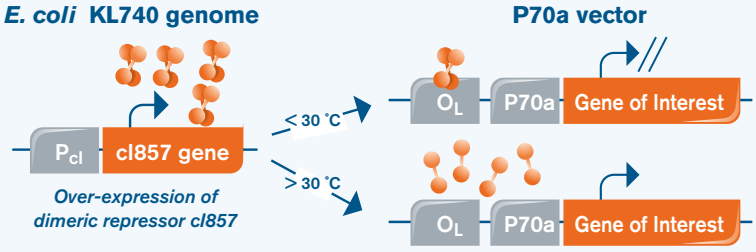

Figure 1. Expression control in *E. coli* KL740 cI857+. *KL740 cI857+ over-expresses a temperature-sensitive variant of the lambda phage repressor protein cI857. Below 30°C, cI857 forms a dimer and binds to the operator region of the P70a vector preventing transcription of the gene downstream. At higher temperatures, cI857 becomes monomeric and falls off the operator thus turning gene transcription ON.*

This Technical Note describes a standard procedure for preparation of chemo-competent KL740 cI857+ cells for propagation of myTXTL P70a vectors. It can also be applied to procedure involving other myTXTL plasmid vectors.

MATERIALS AND METHODS

Bacterial Strains, Reagents and Solutions

- *E. coli* KL740 cI857+ (Daicel Arbor Biosciences, # 502000)
- LB agar plates (with and without selection marker)
- LB medium
- $MgCl_2$
- $CaCl_2$ dihydrate
- Glycerol

Autoclave the following solutions and cool down to 4°C prior to process start.

- 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

- 14 mL culture tube
- 250 mL culture flask
- 50 mL Falcon tube
- 1.5 mL reaction tubes

Instruments

- Autoclave
- Refrigerated centrifuge set to 4°C
- Incubator set to 29°C
- Shaker incubator set to 29°C
- Refrigerable centrifuge with rotor for 50 mL Falcon tubes
- Thermo block set to 37°C
- -80°C freezer

Methods

The following procedure has been validated to generate chemo-competent KL740 cI857+ with high transformation efficiency. Alternatively, the *Mix & Go! E. coli* Transformation Kit (Zymo Research) yields chemo-competent KL740 cI857+ cells suitable for propagation of myTXTL P70a vectors as well. Other methods have not been validated by Daicel Arbor Biosciences.

A. Preparation of chemo-competent cells

Important: All steps should be carried out aseptically.

A1. Streak *E. coli* KL740 cI857+ cells onto a LB agar plate and incubate for about 16 hrs at 29°C. *[Note: A growth temperature of 29°C is crucial for a maximum level of active cI857 repressor protein.]*

A2. Inoculate 2 mL LB medium in a 14 mL culture tube with a single colony of KL740 cI857+ cells and grow for 16-24 hrs at 29°C and 200 rpm.

A3. The next day, dilute the cell culture in stationary phase 1:100 in 50 mL fresh LB medium in a 250 mL culture flask and incubate at 29°C and 200 rpm till $OD_{600} = 0.5$.

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

A5. Centrifuge cell suspension at 4400 x g, 4°C for 10 min. Immediately discard supernatant by pouring.

A6. Wash cells with 40 mL ice-cold **Wash Buffer 1**. For this, resuspend cells initially in 1-2 mL of Wash Buffer 1 using a pipette with a 1 mL tip. Then, fill the 50 mL Falcon tube to 40 mL with Wash Buffer 1 and mix by inverting.

A7. Centrifuge cell suspension at 4400 x g, 4°C for 10 min. Immediately discard supernatant by pouring.

A8. Wash cells with 20 mL ice-cold **Wash Buffer 2**. For this, resuspend cells initially in 1-2 mL of Wash Buffer 2 using a pipette with a 1 mL tip. Then, fill 50 mL Falcon tube to 20 mL with Wash Buffer 2 and mix by inverting.

A9. Incubate the cell suspension for 30 min on ice (no mixing required). Then, centrifuge as previously performed.

A10. After completely removing the supernatant, resuspend cells in 2 mL ice-cold **Storage Buffer** using a pipette with a 1 mL tip.

A11. Split the cell suspension into 100 µL aliquots per 1.5 mL reaction tube on ice, and immediately store at -80°C. [*Note: Competency lasts at least 6 months.]*

B. Setting up a transformation reaction

B1. Thaw an aliquot of competent KL740 cI857+ cells on ice.

B2. Add 1-50 ng plasmid (for example, pTXTL-P70a(2)-deGFP HP, Daicel Arbor Biosciences, # 502138). Mix gently by flicking the tube 4-5 times. Do not vortex.

B3. Incubate for 25 min on ice.

B4. Place the 1.5 mL reaction tube for 5 min in a thermo block at 37°C.

B5. Transfer cells into 2 mL LB medium in a 14 mL culture tube. Agitate for 30 min at 29°C in a shaker incubator.

B6. Centrifuge for 1 min at 3000 x g at room temperature, remove 1.9 mL of the supernatant, and resuspend cells in the remaining volume using a pipette. *[Note: This step is to increase the concentration of cells and may be skipped. If skipped, increase volume in Step B7 to 50-150 µL.]*

B7. Spread 30-50 µL of the cells onto a pre-warmed LB agar selection plate and incubate the plate for 16-24 hrs at 29°C. *[Note: The procedure may be shortened by omitting Steps B5-B6 and plating an aliquot of the DNA-cell mix directly onto a LB agar selection plate.]*

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