

## DH10B-Pro™ Electrocompetent *E. coli* Cells Transformation Protocol

### Introduction

GoldBio's DH10B-Pro™ Electrocompetent *E. coli* cells are especially designed for the most demanding cloning applications. DH10-Pro™ cells will provide the greatest number of transformants for when your research requires it, including assembling large and multi-DNA fragments, cloning large ( $\geq 10$  kb up to 350 kb) or difficult construct transformations, working with synthetic bio-applications, and even BAC cloning. Here, we present a detailed protocol for electroporation using DH10B-Pro™ Electrocompetent *E. coli* cells.

### Materials

- DH10B-Pro™ Electrocompetent *E. coli* cells (GoldBio Catalog # CC-201)
- pUC19 Control DNA, 10  $\mu\text{g}/\mu\text{l}$
- Recovery medium (GoldBio Catalog # CC-300)
- Ampicillin (GoldBio Catalog # A-301)
- LB agar selection plates
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator
- Shaker incubator

### Storage and Handling

- This product may be shipped on dry ice. DH10B-Pro™ Electrocompetent *E. coli* cells should be stored at  $-80^{\circ}\text{C}$ , pUC19 Control DNA should be stored at  $-20^{\circ}\text{C}$  and recovery medium should be stored at  $4^{\circ}\text{C}$  immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.
- Thaw DH10B-Pro™ Electrocompetent *E. coli* cells and pUC19 Control DNA ice and mix by gentle vortexing. After thawing, these products should be kept on ice before use. These products can be refrozen for storage.

**Note:** Efficiency with electroporation is  $\geq 1 \times 10^9$  cfu/ $\mu\text{g}$ .

**Note:** The genotype of DH10B-Pro™ Electrocompetent *E. coli* Cells is  $F^-$  *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*) *endA1 recA1*  $\phi$ 80*dlacZ* $\Delta$ M15  $\Delta$ *lacX74 araD139  $\Delta$ (*ara, leu*)7697 *galU galK rpsL* (*Str*<sup>R</sup>) *nupG*  $\lambda^-$ .*

**Note: Transformation efficiency is tested by using the pUC19 control DNA, ~50kb, and >100kb plasmids. The pUC19 control DNA is supplied with the kit and can be used as instructed in the protocol given below. Transformation efficiency should be  $\geq 1 \times 10^9$  cfu/ $\mu$ g pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.**

## Method

### Transformation protocol

Use this procedure to transform DH10B-Pro™ Electrocompetent *E. coli* cells. Do not use these cells for chemical transformation.

**Note: Handle the competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.**

**Note: Thaw competent cells on ice and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.**

1. Place sterile cuvettes and microcentrifuge tubes on ice.
2. Remove competent cells from the -80°C freezer and thaw completely on ice (10-15 minutes).
3. Aliquot 1  $\mu$ l (1 pg-10 ng) of DNA to the chilled microcentrifuge tubes on ice.
4. When the cells are thawed, add 20  $\mu$ l of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 1  $\mu$ l of (10 pg/ $\mu$ l) DNA to the 25  $\mu$ l of cells on ice. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
5. Pipette 26  $\mu$ l of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well then electroporate.

**Note: A high-voltage electroporation apparatus, capable of generating field strengths of 16 kV/cm is required.**

6. Immediately add 975  $\mu$ l of Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to resuspend the cells.
7. Transfer the cells and Recovery Medium to a culture tube.
8. Incubate tubes at 37°C for 1 hour at 210 rpm in a shaking incubator.

- Dilute the cells as appropriate then spread 20-200  $\mu\text{l}$  cells onto a pre-warmed selective plate. For the pUC19 control, plate 50  $\mu\text{l}$  of diluted transformants onto an LB plate containing 100  $\mu\text{g}/\text{ml}$  ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.
- Incubate the plates overnight at 37°C.

## Calculations

Transformation efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1  $\mu\text{g}$  of plasmid into a given volume of competent cells.

$$\text{TE} = \text{Colonies}/\mu\text{g}/\text{Dilution}$$

Where:

Colonies = the number of colonies counted

$\mu\text{g}$  = amount of DNA transformed in  $\mu\text{g}$

Dilution = total dilution of the DNA before plating

### **Example:**

Transform 1  $\mu\text{l}$  of (10  $\text{pg}/\mu\text{l}$ ) pUC19 control plasmid into 50  $\mu\text{l}$  of cells, add 950  $\mu\text{l}$  of Recovery Medium. Dilute 10  $\mu\text{l}$  of this in 990  $\mu\text{l}$  of Recovery Medium and plate 50  $\mu\text{l}$ . Count the colonies on the plate the next day. If you count 250 colonies, the TE is calculated as follows:

$$\text{Colonies} = 250$$

$$\mu\text{g of DNA in } 10 \text{ pg} = 0.00001$$

$$\text{Dilution} = 10 \mu\text{l}/1000 \times 50 \mu\text{l}/1000 = 0.0005$$

$$\text{TE} = 250/0.00001/0.0005 = 5.0 \times 10^{10}$$

## Associated Products

- DH10B Electrocompetent *E. coli* Cells (GoldBio Catalog # CC-200)
- DH5-alpha Electrocompetent *E. coli* Cells (GoldBio Catalog # CC-203)
- BL21 (DE3) Electrocompetent *E. coli* Cells (GoldBio Catalog # CC-204)
- Competent Cell Recovery Medium (GoldBio Catalog # CC-300)
- Ampicillin (GoldBio Catalog # A-301)