

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

### Introduction

GoldBio's DH10B-Pro™ Electrocompetent *E. coli* cells are suitable for demanding cloning applications requiring the greatest number of transformants, including synthetic bio-applications, BAC cloning, assembling large and multi-DNA fragments, and large construct transformation or cloning difficult targets. Utilizing proprietary manufacturing methods, these cells allow for effective transformation of all large DNA molecules ( $\geq 10$  kb up to 350 kb!). 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 pg/ $\mu$ l
- Recovery medium (GoldBio Catalog # [CC-300](#))
- Ampicillin (GoldBio Catalog # [A-301<sup>ES</sup>](#))
- 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  $\geq 5 \times 10^{10}$  cfu/ $\mu\text{g}$  and  $5-10 \times 10^7$  for 100-150 kb large DNA.

**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*  $\lambda^{-}$ .

**ES:** EZ-Pak and Solution Available

**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  $\geq 5 \times 10^{10}$  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.
9. Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pUC19 control, plate 50 µl of diluted transformants onto an LB plate containing 100 µg/ml ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.
10. 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 µg of plasmid into a given volume of competent cells.

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

Where:

Colonies = the number of colonies counted

µg = amount of DNA transformed in µg

Dilution = total dilution of the DNA before plating

### **Example:**

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

$$\text{Colonies} = 250$$

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

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

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

## Associated Products

- [DH10B Electrocompetent \*E. coli\* Cells \(GoldBio Catalog # CC-200\)](#)
- [DH10B BAC Library Electrocompetent \*E. coli\* Cells \(GoldBio Catalog # CC-202\)](#)
- [DH5-alpha Electrocompetent \*E. coli\* Cells \(GoldBio Catalog # CC-203\)](#)
- [BL21 \(DE3\) Electrocompetent \*E. coli\* Cells \(GoldBio Catalog # CC-204\)](#)

- [TG1 Phage Display Electrocompetent Cells \(GoldBio Catalog # CC-205\)](#)
- [SS320 Phage Display Electrocompetent Cells \(GoldBio Catalog # CC-206\)](#)
- [GV3101 Agrobacterium Electrocompetent Cells \(GoldBio Catalog # CC-207\)](#)
- [AGL-1 Agrobacterium Electrocompetent Cells \(GoldBio Catalog # CC-208\)](#)
- [Competent Cell Recovery Medium \(GoldBio Catalog # CC-300\)](#)
- [Ampicillin \(GoldBio Catalog # A-301\)](#)