Protocol

TD-P Revision 3.0

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GB10B™ Chemically Competent E. coli Cells
Transformation Protocol

Introduction

GoldBio's GB10B™ Chemically Competent E. coli cells are high efficiency cells that are equivalent to DH10B competent cells. GB10B™ competent cells are suitable for a wide variety of applications such as cloning and sub-cloning. GB10B™ Chemically Competent *E. coli* cells have multiple features including the ϕ80lacZΔM15 marker, which provides α-complementation of the β-galactosidase gene with blue/white screening protocol. These cells also have the *mcrA* genotypic marker and the *mcrBC*, *mrr* deletion, which allows for cloning of DNA that contains methylcytosine and methyladenine. Here, we present a detailed protocol for transformation using GB10B™ Chemically Competent *E. coli* cells.

Materials

- GB10B™ Chemically Competent *E. coli* cells (GoldBio Catalog # CC-100)
- pUC19 Control DNA, 500 pg/µL
- Recovery medium (GoldBio Catalog # CC-300)
- Ampicillin (GoldBio Catalog # A-301)
- LB agar selection plates
- Microcentrifuge tubes
- Shaker incubator

Storage and Handling

- This product may be shipped on dry ice. GB10B™ Chemically Competent *E. coli* cells should be stored at -80°C, pUC19 Control DNA should be stored at -20°C and recovery medium should be stored at 4°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 GB10B™ Chemically Competent *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: The genotype of GB10B™ Chemically Competent *E. coli* cells is *F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) endA1 recA1 ϕ80dlacZΔM15 ΔlacX74 araD139 Δ(ara, leu)7697 galU galK rpsL (Str^R) nupG λ⁻*.

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Note: Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using given below. Transformation efficiency should be ≥8.2 x 10^6 cfu/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform GB10B™ Chemically Competent *E. coli* cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

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. Remove competent cells from the -80°C freezer and thaw completely on ice (10-15 minutes).
2. Aliquot 1-5 µL (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.
3. When the cells are thawed, add 50 µL of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 2 µL of (500 pg/µL) DNA to a chilled microcentrifuge tube, prior to adding 50 µL of cells. Mix well by tapping. **Do not** pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
4. Incubate the cells with DNA on ice for 30 minutes.
5. After a 30-minute incubation on ice, heat shock the cells at 42°C for 45 seconds.
6. Transfer the tubes to ice for 2 minutes.
7. Add 950 µL of Recovery Medium or any other medium of choice to each tube.
8. Incubate tubes at 37°C for 1 hour at 210 rpm in a shaker incubator.
9. Spread 50 µL to 200 µL from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have

well-spaced colonies. For the pUC19 control, plate 50 µL on 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.

5 Minute Transformation Protocol
The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

1. Remove competent cells from the -80°C freezer and thaw in your hand.

2. Aliquot 1-5 µL (1 pg-100 ng) of DNA to the microcentrifuge tubes. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.

3. Incubate the cells with DNA on ice for 2 minutes.

4. After the 2-minute ice incubation, heat shock the cells at 42°C for 45 seconds.

5. Transfer the tubes to ice for 2 minutes.

6. Add 950 µL of Recovery Medium at room temperature or any other medium of choice to each tube. Immediate spread 50 µL to 200 µL from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least once plate will have well-spaced colonies. For the pUC19 control, plate 50 µL on al LB plate containing 100 µg/mL ampicillin. Use a sterilized spreader or autoclaved plating beads to spread evenly.

7. 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 = Colonies/µg/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:

Colonies = 250
µg of DNA in 10 pg = 0.00001
Dilution = 10 µL/1000 x 50 µL/1000 = 0.0005

TE = 250/0.00001/0.0005 = 5.0 x 10^{10}

Associated Products

- GB5-alpha™ Chemically Competent *E. coli* Cells (GoldBio Catalog # CC-101)
- BL21 (DE3) Chemically Competent *E. coli* Cells (GoldBio Catalog # CC-103)
- DL39 (DE3) Chemically Competent *E. coli* Cells (GoldBio Catalog # CC-104)
- Competent Cell Recovery Medium (GoldBio Catalog # CC-300)