

CJ236 Electrocompetent *E. coli* Cells Transformation Protocol

Introduction

GoldBio CJ236 Electrocompetent *E. coli* cells are ideally suited for use in the Kunkel method of mutagenesis. CJ236 cells have part of their Thymine replaced with deoxyuracil in the DNA due to dut- and ung- mutations which prevent the cells from removing uracil from DNA caused by misincorporation or dCTP deamination. CJ236 contains the F' gene which confers Chloramphenicol resistance.

Materials

- CJ236 Electrocompetent *E. coli* cells (GoldBio Catalog # CC-251)
- pUC control DNA, 500 pg/μL
- *E. coli* Recovery Medium (GoldBio Catalog # CC-302)
- Chloramphenicol (GoldBio Catalog # C-105)
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator
- Shaker incubator

Storage and Handling

- This product may be shipped on dry ice. CJ236 Electrocompetent *E. coli* cells should be stored at -80°C, pUC 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 CJ236 Electrocompetent *E. coli* cells and pUC 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: Genotype is dut1,ung1,thi-1,recA1 / pCJ105 (F' cam^r).

Note: Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using given below. Transformation efficiency should be $\geq 1 \times 10^9$ cfu/μg pUC DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform CJ236 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 $1\ \mu\text{L}$ (1 pg-10 ng) of DNA to the chilled microcentrifuge tubes on ice.
4. When the cells are thawed, add $25\ \mu\text{L}$ of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC control, add $0.2\ \mu\text{L}$ of ($500\ \text{pg}/\mu\text{L}$) DNA to the $25\ \mu\text{L}$ 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 $26\ \mu\text{L}$ 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 $16\ \text{kV}/\text{cm}$ is required.

6. Immediately add $974\ \mu\text{L}$ 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 $20\text{-}200\ \mu\text{L}$ cells onto a pre-warmed selective plate. For the pUC control, plate $50\ \mu\text{L}$ of diluted transformants onto an LB plate

containing 100 µg/mL ampicillin. Use 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 = 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 10¹⁰

Associated Products

- CJ236 Chemically Competent *E. coli* Cells (GoldBio Catalog # CC-151)
- GB10B™ Electrocompetent *E. coli* Cells (GoldBio Catalog # CC-200)
- GB10B-Pro™ Electrocompetent *E. coli* Cells (GoldBio Catalog # CC-201)
- *E. coli* Competent Cell Recovery Medium (GoldBio Catalog # CC-302)
- Chloramphenicol (GoldBio Catalog # C-105)