Electrocomp™
E. coli Cells

Version A
150405

28-0078

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Contents

5 or 10 x 80 µl electrocompetent *E. coli*
100 ng of pUC18 supercoiled vector, lyophilized
SOC medium

Description

Electrocompetent *E. coli* have a transformation efficiency of 10^9 cfu/µg using control DNA as directed.

Genotypes:

TOP10F′: F'{*lac*I^q*Tn*10(Tet^R)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

TOP10: F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 ΔlacX74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

MC1061/P3: F$^-$ *hsd*R (r_k^-, m_k^+) *ara*D139 Δ(*ara*ABC-*leu*)7679 *gal*U *gal*K Δ*lac*X74 *rps*L *thi* *mcr*B {P3: Kan^R Amp^R (am) Tet^R (am)}

NM522: F′ {*pro*AB$^+$, *lac*I^q *lac*ZΔM15} *sup*E *thi*-1 Δ(*lac-pro*AB) Δ*hsd*5 (r_k^-, m_k^-)

HB101: F$^-$ *hsd*S20 (r_B^-, m_B^+) *thi*-1 *sup*E44 *ara*14 *gal*k2 *lac*Y1 *pro*A2 *rps*L20 (Str^R) *xyl*-5 *mtl*-1 *rec*A13 *mcr*B

1. Introduction

It is crucial for high efficiency electroporation that the cells and cuvettes remain cold until after the pulse is applied. Furthermore, after the pulse is applied, SOC must be added to the cells immediately to ensure good results. Please follow the steps below to prepare for electroporation.

Note: This protocol has been developed for the Electroporator II from Invitrogen (Catalog no. S1670-01) using Invitrogen's 1 mm wide cuvettes, the BioRad Gene Pulser™ using BioRad's 2 mm wide cuvettes, and BRL's Cell-Porator™ using 2 mm wide cuvettes. All three electroporation devices can be used with Invitrogen's ElectroComp™ *E. coli* cells, however, transformation efficiencies may vary. Please call for information on available strains.

1. Pre-chill the cuvettes and the sliding cuvette holder (if applicable) on ice or at -20°C.
2. Set the electroporation device as follows (it is strongly recommended that the manual provided with each device by the vendor be followed for further technical information):

Unit	Capacitance	Resistance	DC Volts	Voltage
Electroporator II (Invitrogen) with power supply	50 µF	150 Ω	-----	1500 V
Gene-Pulser™ with Pulse Controller (BioRad)	25 µF	200 Ω	-----	2.5 kV
Cell-Porator™ with Voltage Booster (BRL)	330 µF	4 kΩ	low ohm	2.5 kV

3. Prepare an ice bucket for the cells and the DNA.
4. Prepare SOC medium to add to the cells after the pulse. Keep the SOC at room temperature.
5. Prepare sterile Pasteur pipettes and 15 ml sterile tubes (Falcon 2059 or similar) for use after the pulse has been delivered.

2. Electroporation

1. Place 1-5 µl of DNA in a sterile microcentrifuge tube and chill on ice.
   CAUTION: The DNA must be resuspended in water (rather than TE buffer) to keep the ionic strength to a minimum. Otherwise, arcing might occur during the pulse which can damage the machine and will result in no transformation and loss of the cells. DNA in ligation or restriction buffer must be precipitated or desalted before electroporation.
   Note: For control DNA, resuspend in 100 µl, dilute 1:100, and use 1 µl (10 pg).
2. Remove the cells (80 µg/vial) from storage at -70°C and gently thaw them on ice. Use these cells immediately, do not leave them on ice for an extended period of time. Unused cells can be re-frozen for later use, but they will suffer a significant loss of efficiency.
3. Transfer the desired amount of cells to the pre-chilled microcentrifuge tube containing the DNA. It is recommended that 20-40 µl be used for a small-scale electroporation (< 100 ng of DNA), and 160 µl for a large-scale or library poration. The volume of DNA added should not exceed 5% of the total cell/DNA mixture. Mix the cells with DNA by pipetting up and down once. Leave the cells on ice for 1 minute.
4. Place the cuvette holder into the pulse chamber (Gene Pulser™ only). Pre-set a pipette to 480 µl (small-scale) or 960 µl (large-scale) for the pipetting of SOC in Step 7.
5. After 1 minute, transfer the cells to a chilled cuvette and gently shake them to the bottom of the cuvette. Check the cuvette by looking at the contents from both sides to make sure the cells make contact all the way across the bottom of the chamber without any air bubbles. It is important to do this as quickly as possible, do not warm up the cuvette and cells. Remove the condensation from the outside surfaces of the cuvette with a Kimwipe.
6. Place the cuvette into the cuvette slot (for the Electroporator II) or the cuvette holder (for the Cell-Porator™) or the sliding cuvette holder (for the Gene Pulser™) and align it with the electrode contacts. Apply a pulse at the settings prescribed in Section I, Step 2.
7. Slide the cuvette holder back (if applicable) and **immediately** add 480 µl (for small-scale) or 900 µl (for large-scale) of SOC.
   Note: A delay of only 1 minute before adding the SOC can result in a 10- to 100-fold reduction in transformation efficiency.
8. Gently mix the cell suspension in the cuvette by pipetting it up and down with a sterile Pasteur pipette. Transfer the suspension to a 15 ml tube (Falcon 2059 or similar). Incubate it at 37°C in a rotary shaking incubator at 225 rpm for 1 hour to allow expression of the antibiotic resistance.
   Note: Cell viability can be increased by adding more SOC in Step 8 and/or by diluting the electroporated cell mixture with SOC just before plating.
9. Plate the cells on LB agar with the appropriate antibiotic.
   Note: From the control DNA, make a 1:10 dilution in SOC medium and plate 25 µl and 100 µl of cells on LB agar with 50 µg/ml of ampicillin.

Electrocompetent cells:

- pellet cells 1 liter LB O.D 550 0.6
- Wash twice in H_2O
- wash twice in 10% glycerol in H_2O
- resuspend in 2ml 10% glycerol in H_2O