

Electroporation Protocol (C2989)

Overview

Protocol

1. Prepare 17 mm x 100 mm round-bottom culture tubes (e.g. VWR #60818-667) at room temperature. Place SOC recovery medium in a 37°C water bath. Pre-warm selective plates at 37°C for 1 hour.
2. Place electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. As a positive control for transformation, dilute the control pUC19 by 1:5 to a final concentration of 10 pg/μl using sterile water. Heat-denatured ligation reactions can be used for electroporation directly; however, column purification is recommended.
4. Thaw NEB 5-alpha Electrocompetent cells on ice (about 10 min) and mix cells by flicking gently. Transfer 25 μl of the cells (or the amount specified for the cuvettes) to a chilled microcentrifuge tube. Add 1 μl of the DNA solution.
5. Carefully transfer the cell/DNA mix into a chilled cuvette without introducing bubbles and make sure that the cells deposit across the bottom of the cuvette. Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators: 1.7 kV, 200 Omega, and 25 μF. The typical time constant is 4.8 to 5.1 milliseconds.
6. Immediately add 975 μl of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to the 17 mm x 100 mm round-bottom culture tube.
7. Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.
8. Dilute the cells as appropriate then spread 100-200 μl cells onto a pre-warmed selective plate.
9. Incubate plates overnight at 37°C.