Electroporation Protocol (C2986)

Protocol

- Prepare 17mm x 100mm 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.
- Place electroporation cuvettes (1mm) and microcentrifuge tubes on ice.
- 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.
- Thaw NEB Turbo 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.
- 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: 2.1 kV,
  100 Ω, and 25 µF. The typical time constant is ~2.6 milliseconds.
- Immediately add 975 µl of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to the 17mm x 100mm round-bottom culture tube.
- Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.
- Dilute the cells as appropriate then spread 100-200 µl cells onto a pre-warmed selective plate.
- Incubate plates 8 hours to overnight at 37°C.