Protocol 1-8: Electro Transformation of Recombinant DNA

General Protocol

1) Thaw electrocompetent cells on ice, keep on ice once thawed
2) Mix DNA(< 2 ul with low salt content to prevent arcing) with 40 ul cells in an eppendorf tube, keep on ice or alternatively, or add DNA to the electro-cuvette (pre-chilled) and then add the electrocompetent cells making sure that the DNA gets mixed well with the cells.
3) Transfer the cell/DNA mix into an electroporation cuvette
4) Set the pulser properly(See Tip 1)
5) Pulse the cells once; the voltage display blinks, and the gene pulser beeps
6) Quickly transfer 37 C SOC to cuvette, mix by gently pipetting up and down, and transfer SOC/cells back to culture tube; replace in 37 C H2O bath
7) Incubate 30 min. @ 37 C, followed by 30 min. @ 37 C/shaking
8) Plate cells on appropriate antibiotic

Tips

1) the gene pulser should be set properly
   - time constant = 4.5 - 5.0 ms
   - resistance = 200 W
   - capacitance = 25 mFD

   for 0.1 cm gap cuvettes, set the volts to
   1.7 kV
   for 0.2 cm gap cuvettes, set the volts to
   2.5 kV

Reference

1) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.
2) Robert F. Weaver. Molecular Biology, McGrawHill, 4th edition, 2007