Section 5
High Efficiency Electrotransformation of *E. coli*

Electroporation provides a method of transforming *E. coli* at efficiencies as high as 10^9 to 10^{10} transformants/µg, which is greater than is possible with the best chemical methods. The following protocol describes a method for for preparing and electrotransforming *E. coli* to high efficiencies. We are interested in hearing of additional strains transformed by electroporation and including this information in subsequent versions in our **Electroprotocols manual**. Please contact your local Bio-Rad representative, access our web site at **www.bio-rad.com**, or, in the U.S., call our Technical Services at (800) 424-6723 with any comments or questions.

5.1 Preparation of Electrocompetent Cells

See Ausubel *et al.* (1987) and Miller and Nickoloff (1995) for additional information.

1. Inoculate 500 ml of L-broth with 1/100 volume of a fresh overnight *E. coli* culture.
2. Grow the cells at 37 °C shaking at 300 rpm to an OD_{600} of approximately 0.5–0.7 (the best results are obtained with cells that are harvested at early- to mid-log phase; the appropriate cell density therefore depends on the strain and growth conditions).
3. Chill cells on ice for ~20 min. For all subsequent steps, keep the cells as close to 0 °C as possible (in an ice/water bath) and chill all containers in ice before adding cells. To harvest, transfer the cells to a cold centrifuge bottle and spin at 4000 x g for 15 minutes at 4 °C.
4. Carefully pour off and discard the supernatant. It is better to sacrifice the yield by pouring off a few cells than to leave any supernatant behind.
5. Gently resuspend the pellet in 500 ml of ice-cold 10% glycerol. Centrifuge at 4000 x g for 15 minutes at 4 °C; carefully pour off and discard the supernatant.
6. Resuspend the pellet in 250 ml of ice-cold 10% glycerol. Centrifuge at 4000 x g for 15 minutes at 4 °C; carefully pour off and discard the supernatant.
7. Resuspend the pellet in ~20 ml of ice-cold 10% glycerol. Transfer to a 30 ml sterile Oakridge tube. Centrifuge at 4000 x g for 15 minutes at 4 °C; carefully pour off and discard the supernatant.
8. Resuspend the cell pellet in a final volume of 1–2 ml of ice-cold 10% glycerol. The cell concentration should be about 1–3 x 1010 cells/ml.

   This suspension may be frozen in aliquots on dry ice and stored at -70 °C. The cells are stable for at least 6 months under these conditions.

5.2 Electroporation

1. Thaw the cells on ice. For each sample to be electroporated, place a 1.5 ml microfuge tube and either a 0.1 or 0.2 cm electroporation cuvette on ice.
2. In a cold, 1.5 ml polypropylene microfuge tube, mix 40 µl of the cell suspension with 1 to 2 µl of DNA (DNA should be in a low ionic strength buffer such as TE). Mix well and incubate on ice for ~1 minute. (Note: it is best to mix the plasmids and cells in a microfuge tube since the narrow gap of the cuvettes prevents uniform mixing.)
3. Set the MicroPulser to “Ec1” when using the 0.1 cm cuvettes. Set it to "Ec2" or "Ec3" when using the 0.2 cm cuvettes. See Section 4 for operating instructions.

4. Transfer the mixture of cells and DNA to a cold electroporation cuvette and tap the suspension to the bottom. Place the cuvette in the chamber slide. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber. Pulse once.

5. Remove the cuvette from the chamber and immediately add 1 ml of SOC medium to the cuvette. Quickly but gently resuspend the cells with a Pasteur pipette. (The period between applying the pulse and transferring the cells to outgrowth medium is crucial for recovering *E. coli* transformants (Dower *et al.*, 1988). Delaying this transfer by even 1 minute causes a 3-fold drop in transformation. This decline continues to a 20-fold drop by 10 minutes.

6. Transfer the cell suspension to a 17 x 100 mm polypropylene tube and incubate at 37 °C for 1 hour, shaking at 225 rpm.

7. Check and record the pulse parameters. The time constant should be close to 5 milliseconds. The field strength can be calculated as actual volts (kV) / cuvette gap (cm).

8. Plate on selective medium.

5.3 Solutions and Reagents For Electroporation

1. L-Broth: 10 g Bacto tryptone, 5 g Bacto yeast extract, 5 g NaCl; dissolve in 1.0 L water. Autoclave.

2. 10% (v/v) Glycerol: 12.6 g glycerol (density = 1.26 g/cc) in 90 ml of water. Autoclave or filter sterilize.

3. TE: 10 mM Tris-HCl pH 8.0, 1 mM EDTA.

4. SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

Section 6
Electroporation of *Staphylococcus aureus*

6.1 Preparation of Electrocompetent Cells

See Lee (1995) for additional information.

1. Inoculate 3 ml of B2 broth in a 17 x 100 mm tube with a colony from a fresh *S. aureus* plate.

2. Incubate at 37 °C overnight, shaking at 250 rpm.

3. Inoculate 1.5 ml of the overnight culture into 150 ml of fresh B2 broth in a 1 liter flask. Incubate at 37 °C, shaking at 250 rpm, to ~2 x 10^8 cells/ml. The doubling time of *S. aureus* is about 30 min at 37 °C.

4. Chill the cells in an ice water bath for 15 min to stop growth. Decant the cells into a sterile 500 ml centrifuge bottle. Harvest the cells by centrifugation at 12,000 x g for 15 min at 4 °C.

5. Carefully pipette off the supernatant, keeping the cell pellet on ice.

6. Resuspend the cell pellet in 500 ml of sterile, ice-cold water. Pellet the cells by centrifugation at 12,000 x g for 15 min at 4 °C; carefully remove the supernatant. Wash the cells 2 more times in 500 ml of sterile, ice-cold water.