*Adapted from protocol for Biorad Gene Pulser*
*http://www.bio-rad.com*

**All steps done on ice in cold room with pre-cooled equipment (tips, pipettes, tubes...)**

# High Efficiency Electro-transformation of *E. coli*

Electroporation provides a method of transforming *E. coli* to efficiencies greater than are possible with the best chemical methods. By subjecting mixtures of cells and DNA to exponentially decaying fields of very high initial amplitude, we routinely obtain $10^9$ to $10^{10}$ transformants/µg of DNA with various strains and several plasmids. The survival and transformation of cells is related to the intensity of the field (field strength = voltage/distance between electrodes) and to the length of the pulse (RC time constant).

Protocols for preparing and electro-transforming *E. coli* to high efficiencies are described in Table 1.

## Table 1. Procedure for High Efficiency Electro-transformation of *E. coli*

### A. Preparation of Cells

1. Inoculate 400ml of 2TY in a 2 liters flask with 1/100 volume of a fresh overnight culture.
2. Grow cells at 37 °C with vigorous shaking to an $ABS_{600}$ of approximately 0.4-0.5 (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. To harvest, centrifuge cells in cold centrifuge bottles in a cold rotor at 4000 x $g_{max}$ for 15 minutes.

*4. Remove as much of the supernatant (medium) as possible. 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 400ml of ice-cold water taking care not to lyse them. Centrifuge as in step 3.

*6. Resuspend in 200ml of ice-cold water. Centrifuge as in step 3.

*7. Resuspend in 8 ml of ice-cold 10% glycerol. Centrifuge as in step 3.

* Keep the cells as close to 0 °C as possible (in an ice/water bath) throughout their preparation.

8. Resuspend to a **final** volume of 0.8ml in ice-cold 10% glycerol. The cell concentration should be about 1 - 3 x $10^{10}$ cells/ml.

9. This suspension is frozen in 40µl aliquots in liquid N2, and stored at -70 °C. The cells are good for at least 6 months under these conditions.

### B. Electro-transformation and Plating

1. Gently thaw the cells at room temperature and then immediately place them on ice. Remove the sterile cuvettes from their pouches and place them on ice. Place the white chamber slide on ice (Figure 7).

2. In a cold, 1.5 ml polypropylene 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[c]). Mix well and let sit on ice ~0.5 - 1 minute.

3. Set the E. coli Pulser apparatus to 2.50 kV when using the 0.2 cm cuvettes. Set it to 1.80 kV when using the 0.1 cm cuvettes. See Section 2 for operating instructions.

4. Transfer the mixture of cells and DNA to a cold electroporation cuvette, and shake the suspension to the bottom. Place the cuvette in a chilled safety chamber slide. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber (Figure 4).

5. Pulse once.

6. Remove the cuvette from the chamber and **immediately** add 1 ml of SOC[d] medium to the cuvette and quickly but gently resuspend the cells with a pasteur pipette. (This rapid addition of SOC after the pulse is very important in maximizing the recovery of transformants.)

7. Transfer the cell suspension to a 17 x 100 mm polypropylene tube and incubate at 37 °C for 1 hour. (Shaking the tubes at 225 rpm during this incubation may improve the recovery of transformants.)

8. 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).

9. Plate on selective medium.

a L-Broth: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

b 10% Glycerol: Prepare fresh weekly with sterilized water. Do not autoclave or filter-sterilize the glycerol solution.

c DNA containing too much salt will make the sample too conductive and cause arcing at high voltage. TE: 10 mM Tris-HCl pH 8.0, 1 mM EDTA.

d SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose.