Electroporation

For small volumes of electrocompetent cells:

1. Grow 2 ml overnight cultures in LB (*recA* mutants need to be used in early exponential phase but stationary phase cultures of most strains work fine.)
2. Centrifuge for 1 min in a microfuge at room temperature.
3. Resuspend the cell pellet in 2 ml cold sterile distilled water, vortex, recentrifuge.
4. Resuspend the cell pellet in 1 ml cold sterile distilled water, vortex, recentrifuge.
5. Resuspend the cell pellet in 100 μl cold sterile 10% glycerol, vortex, recentrifuge.
6. Resuspend the cell pellet in 40 μl cold sterile 10% glycerol.
7. Add 1-3 μl DNA and electroporate.

For large volumes and frozen electrocompetent cells:

1. Subculture 2 ml of a fresh overnight culture into 200 ml LB in a 500 ml flask. Grow on a 37°C shaker to midlog phase (about 60-100 Klett units).
2. Centrifuge the cells in a GSA rotor at 6000 rpm for 10 min at 4°C.
3. Resuspend the cells in 200 ml cold sterile water then recentrifuge the cells in a GSA rotor at 6000 rpm for 10 min at 4°C.
4. Resuspend the cells in 100 ml cold sterile water then recentrifuge the cells in a GSA rotor at 6000 rpm for 10 min at 4°C.
5. Resuspend the cells in 5 ml cold sterile 10% glycerol, transfer the cell suspension to a sterile SS34 tube and centrifuge the cells at 10,000 rpm for 5 min at 4°C.
6. Resuspend the cells in 0.75 ml cold sterile 10% glycerol. The final cell concentration should be about 10^{10}/ml. These cells can be used directly for electroporation or stored at -70°C indefinately before use.
7. To freeze place 50 μl aliquots of the cell suspension in small sterile microfuge tubes and freeze at -70°C. Before use remove the cells from the freezer, thaw briefly at room temperature, then place on ice before electroporation.

Electroporation (using a BioRad Gene Pulser):

1. If using cuvettes multiple times, check them carefully for cracks before use. Precool cuvettes in a refrigerator or on ice. Cool the cuvette chamber slide, cells, and DNA on ice.
2. Add 1-3 μl DNA to 40 μl electrocompetent cells. Mix gently with a pipetman.

3. For 0.2 mm cuvettes, set the Gene Pulser to 25 μF and 2.5 kV. (Use 1.5 kV for 0.1 mm cuvettes.) Set the Pulse controller to 200x.

4. Transfer the sample to a cuvette and tap gently to make sure the sample is evenly distributed between the sides of the cuvette. Insert the cuvette into the cold chamber slide and push the chamber slide into the chamber until the cuvette is seated between the contacts in the base of the chamber.

5. Pulse once by pressing the 2 red buttons at the same time until the Gene Pulser beeps. The time constant should be about 4.0 msec.

6. Add one ml plasmid broth (or LB) to the cuvette and transfer to a test tube. (This should be done relatively quickly after shocking the cells because cell viability will decrease over time.) Incubate for 30-60 min on a 37°C shaker to allow the cells to recover and for phenotypic expression.

7. To obtain single colonies from both high and low effeciency electroporations, use two selective plates per sample: spot 20 μl of the culture on the first plate, allow the spot to dry, then streak out for isolated colonies; centrifuge the rest of the culture for 1 min in a microfuge, resuspend in 0.1 ml medium, then spread the entire sample on a second plate.

8. To turn off the Gene Pulser, flip the on-off switch off, on, then off again to fully discharge the capacitors.

9. If reusing the cuvettes, wash them thoroughly immediately after use. Mark the side after each use, and discard the cuvettes after 5 uses.

To wash the cuvettes:

1. Rinse cuvettes after use with distilled water.

2. Fill cuvettes with 0.1 N NaOH. Leave for about 5 min.

3. Rinse well with distilled water.

4. Rinse once with 95% or 100% ethanol.

5. Fill cuvettes with ethanol. Cover with plastic cap. Invert the cuvette to wet the cap. Drain the ethanol. Allow cuvettes to air dry. After dry, cover with cap. Check cuvettes for cracks along the bottom. Discard any cuvettes with cracks.

Potential problems that can cause arcing:

1. Inadequate mixing of the cells and DNA or bubbles in the sample. Gently mix the cells and DNA before adding to the cuvette.

2. Cracks in the cuvettes. Carefully check any used cuvettes for cracks.

3. Wet cuvette chamber slide. Dry the chamber slide with a Kimwipe before use.

4. Failure of sample to contact both sides of the cuvette. Make sure to use an adequate volume of sample, tap the cuvette gently after adding the sample, and inspect before electroporation.

5. Excess salt in the DNA sample. Do not add electrocompetent cells directly to a tube used for ethanol precipitation of DNA. If the DNA sample has relatively high salt content (for example, ligation mixtures), drop dialize before electroporation (see Silhavy et al., 1984 or Maloy, 1990).

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