Bacterial Transformation by Electroporation

Preparation of electrocompetent cells

1. Inoculate 1 L of LB containing 1/2 the amount of NaCl as normal with 5 ml of an overnight culture. Shake at 37 °C until mid log OD(600) = 0.5. Incubate cells in ice water for 15 minutes.
2. Meanwhile, incubate 2 centrifuge bottles, 12 mL of 10% glycerol, 500 ml of sterile water, and a 50 ml conical tube on ice for at least 30 minutes.
3. Pour 500 ml of cells into each of the pre-chilled centrifuge bottles. Pellet cells at 6000xg for 15 minutes at 4 °C.
4. Pour off the supernatant and resuspend each pellet in 250 ml of ice-cold sterile water. Resuspend the cells while on ice by loosening the pellet by scraping with a sterile pipet, quickly vortexing for a few seconds and then shaking the bottles in a circular motion by hand until the cells are completely resuspended. Maintain cells on ice or ice water at all times.
5. Pellet cells at 6000xg for 15 minutes at 4 °C.
6. Pour off the water and resuspend each pellet in 2.5 ml of ice cold 10% glycerol. This can be accomplished by breaking the pellet with a cold pipet followed by tituration (sucking in and out of the pipet). Combine cells and transfer to an ice cold 50 ml conical tube.
7. Pellet cells at 600g for 15 minutes at 4 °C.
8. Carefully pour off the glycerol and resuspend the cells up to 2 ml using 10% glycerol.
9. Distribute cells in 80 µl aliquots into microfuge tubes and quick freeze in a -70 °C bath (liquid N2 is okay, too).

Transformation of electrocompetent cells

1. Chill cuvettes on ice for 5 minutes. Thaw cells on ice.
2. Add 5 pg - 5 µg plasmid DNA in 1 µl to the cells. Mix by tapping the tube or swirling the contents with a pipet tip.
3. Transfer the DNA and the cells into a cuvette. Keep on ice.
4. Set the electroporator to 2.5 kV, 25 µF, and 400 ohms.
5. Pulse, and record the actual voltage and time constant.
6. Immediately add 1 ml SOC medium (0.5% yeast extract, 2% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, and 20 mM glucose) and transfer to a sterile microfuge tube.
7. Incubate 30-60 minutes with moderate shaking at 37 °C.
8. Plate aliquots on LB plates containing the appropriate antibiotics