



شرکت سلول بافت زیست (سبز)

Transformation of *E. coli* by Electroporation

Preparation of Electrocompetent Cells

1. Add colony of desired bacterium to 5 ml LB; grow overnight.
2. Add 0.5 ml from overnight culture to 500 ml LB flask; grow until bacteria are in log phase ($OD_{600} = 0.4$).
3. Incubate on ice for 30 min. FROM HERE ON, ALWAYS KEEP CELLS COLD!!!
4. Centrifuge at 2600g for 15 min, 4 °C.
5. On ice, discard supernatant and resuspend cell pellets in 500ml cold distilled water.
6. Centrifuge as before; discard supernatant and resuspend in 250 ml; recentrifuge and discard supernatant.
7. On ice, suspend pelleted cells in 100 ml cold distilled water, Centrifuge as before; discard supernatant and resuspend in 4 ml of 10% glycerol; split into 100 μ L aliquots.
8. If you are not going to use them immediately, store at -80 °C.

Transformation of *E. coli* by Electroporation

Electroporation

1. Put the competent bacteria and electroporation cuvetts (2mm diameter between electrodes) on ice.
2. Add 100-1000 ng of plasmid to 100 μ l electrocompetent bacteria, mix and move to the cuvet (if the plasmid solution contains salt, it is better not to exceed 1-5 μ l) (for handling the bacteria, use blue sampler tips to minimize shear stress on the cells).
3. Dry the walls of the cuvet, put the cuvet in the electroporator and start it with
4. this setting: 2500V, 5 mseconds) (If you hear a cracking noise, your sample probably had too much salt and has exploded all over the inside of the chamber).
5. Add 1 ml of fresh LB or preferably 2xYT medium (without antibiotic) to the cuvet and move it to a new tube.
6. Incubate in 37 °C shaker incubator for 1 h and then move 100 μ l of it to an antibiotic containing plate. Centrifuge the rest of it, resuspend in 100 μ l medium and culture on another plate.