

Electroporation

Materials Needed

1. Electrocompetent cells
2. Electroporation cuvettes
3. Electroporator
4. Plasmid DNA
5. Ice + ice bucket
6. 1.5mL tubes
7. Plates + spreader
8. Pipets

Protocol

1. Place electroporation cuvettes on ice.
2. Dialyze your DNA sample(s) using a nitrocellulose filter and DI water.
 - a. Fill a Petri dish with DI water.
 - b. Place a single nitrocellulose filter paper on the surface of the water – shiny side up.
 - c. Spot 10uL DNA samples onto the surface of the paper.
 - i. Space out the samples a little bit because they will expand in volume as they dialyze.
 - ii. Dialyze your sample for 30 minutes.
 1. Important if the DNA has undergone any previous manipulations that have introduced salts (adding buffers for ligation, restriction enzyme digestions, etc.). Too much salt in the DNA will cause your sample to arc when electroporating. This will kill the cells and significantly reduce the efficiency of transformation. If your sample arcs, it may still be worthwhile to plate your cells. However, it is likely that you will get very few (if any) colonies. You should repeat this sample. Main factors that can cause arcing - too much salt in your DNA, water on the outside of the cuvette, oil on the outside of the cuvette from handling it too much without gloves, too much salt in the cells.
3. Thaw electrocompetent cells on ice (50uL per aliquot)
4. Turn on electroporator and set voltage (1250 V for 1 mm cuvettes.)
5. Add 2uL dialyzed DNA to 20uL thawed cells. Mix gently by flicking tube.
6. Add cell/DNA mixture to the electroporation cuvette.
7. Wipe off excess moisture from outside of cuvette using a kimwipe.
8. Place cuvette in electroporator. Close lid.
9. Shock cells.
10. Remove cuvette from the chamber and immediately add 250uL LB.
11. Transfer LB-cell mixture to a 50mL tube.
12. Incubate tube in 37°C shaker for 1 hour
13. Pre-warm plates
14. Plate 250uL transformation onto plate.
15. Incubate plate overnight at 37°C.

<http://mcb.berkeley.edu/labs/krantz/protocols/electroporation.pdf>

