

Electroporation Protocol #2

Adopted from Jeremy Minty's electroporation protocol

Goal: Transform *E. coli* DH5 α with your desired plasmid(s).

Materials: Each 2 mL O/N culture will give you ~ 200 μ L of cells, or 4 electroporations. Remember to count for controls, and making extra is a good idea! Each 2 mL O/N also translates to 100 mL of LB the next day. Suggested amount of materials to freeze per O/N (4 samples): 6 25 mL tips, 4 5 mL tips 160 mL dH₂O, a box of 200 tips, and 1.5 mL tubes (whole beaker). This should provide a few extras if you're conservative with materials.

Preparation of Electric Competent Cells

1. Inoculate 2 ml LB with *E. coli* DH5 α and let grow out overnight at 37C.
2. The next morning, turn on the cold room, place the vortexer, waste container, and the needed volume of sterile water inside. Also, put all needed pipettes, cuvettes, and eppendorf tubes in the -20C freezer (I put them in the 1224 freezer).
3. After 12-16 hours, inoculate 100 ml of LB with 2 mL O/N culture and cultivate until OD600 reaches 0.4 to 0.6 (early log phase). This should take ~3 hours.
4. Turn on the cold room in 1224 and make sure the centrifuge is ready to go, and move the vortexer and waste container into the cold room.
5. After 2-3 hours, get a bucket of ice from the ChE 360 lab, and check OD600; if culture is not sufficiently dense, return to incubator and check OD600 every $\frac{1}{2}$ hour until OD600 \geq 0.4.
6. Pipette the cultures into 50 mL centrifuge tubes (2x for each culture) and chill on ice 15-20 minutes. All steps after this point must be carried out and 4C and use chilled materials.
7. Harvesting cells: Centrifuge culture at 4200 rpm, 4C for 5 minutes. Decant supernatant.
8. Washing #1: Resuspend cell pellets by vortexing, and then add 50ml of cold sterile dH₂O, centrifuge at 3500 rpm, 4C for 15min, and pipette off supernatant with autopipette. Be sure to use chilled pipettes! After the first washing, the cell pellets will be much looser, so it is important to work carefully the autopipette and not suck up any of the pellet.
9. Washing #2: Resuspend cell pellets with 25ml of cold sterile dH₂O combine each 2 aliquots into one 50 mL centrifuge, centrifuge at 3500rpm for 15min, and

- pipette off supernatant. Caveats about loose cell pellets apply here as well. Be sure to use chilled pipettes!
10. Washing #3: Resuspend cell pellets with 2ml of cold sterile dH₂O, transfer to a 15 mL centrifuge tube, centrifuge it at 3500rpm for 15min, and pipette out the supernatant. Be sure to use chilled pipettes!
 11. Final Resuspension: Resuspend cell pellet in 200ul of cold sterile dH₂O (usually there is enough water left over after removing the supernatant). Keep cells on ice.
 12. Measure the density of cell suspension by making a 1:100 dilution of the suspension and measuring the OD600 of the dilution.
 - a. Put 10 µL of cells and 990 µL of dH₂O into a cuvette, and use 1 mL of dH₂O as a blank. The measured OD600 should be above 0.2.
 - b. If the density of the final cell suspension is less than 0.2 OD600 units ($\sim 2 \times 10^{10}$ cells/mL), transfer the cell suspension to an eppendorf tube and centrifuge at 5000 rpm, 4°C for 10 minutes. Pipette out supernatant, resuspend cell pellet in smaller amount of dH₂O to bring cell density to at least 0.2 OD600 units.
 - c. *Assuming that the starting culture was ~ 0.4 OD600 units (which corresponds to $\sim 4 \times 10^8$ cells/mL), the final concentration of the cell suspension should be $\sim 2 \times 10^{11}$ cells/mL. A 1:100 dilution would be $\sim 2 \times 10^9$ cells/mL, which should have an OD600 of ~ 2 . While $\sim 2 \times 10^{11}$ cells/mL is the ideal concentration for the electrocompetent cell suspension, more dilute cell suspensions (down to at least 2×10^{10} cells/mL) should work with electroporation, albeit at lower efficiency.*
 13. There should be enough for 3 different electroporations (extras will be available in case of errors/failure) + 1x control (using 50 µL volumes).

Electroporation

1. Get the electroporator from the Lin lab, and make sure no one is using it.
2. Transfer 50µl electrocompetent cells into a prechilled cuvette labeled “control-cells” on ice. Be sure to use chilled pipette tips and keep all materials on ice!
 - a. *These will be a negative control (no plasmid DNA added)*
3. Use a tissue paper to wipe off water around the cuvette labeled “control”, make sure the cell suspension goes down to the bottom of the cuvette (tap gently if needed), then place the cuvette into the sample chamber of the electroporator and apply the pulse. Monitor the actual voltage and the time constant. If arcing

- occurs, the cell suspension should be washed again to remove residual ions or possibly diluted (although this will reduce efficiency)
- a. *Time constant should be ~5 msec. Lower is not good, this means that the resistance of the sample is low. If the resistance is low enough, arcing will occur (audible “pop” or “bang”). Arcing will generally kill the cells in the cuvette, although occasionally electroporation will still be successful. Arcing may be highly mutagenic so arced samples should probably just be discarded.*
 4. Immediately add 1ml LB medium into the cuvette, pipette up and down 10 times, and transfer the mix (~1.05 mL total volume) into a 15ml Falcon tube labeled “control”.
 - a. LB should be room temp-37°C
 5. For each transformation, pipette 2 µL of the plasmid into a chilled eppendorf tube, then pipette in 50 µL of cell suspension. Pipette up and down several times to mix, then transfer mixture to appropriately labeled cuvettes. Use chilled materials! Also do a DNA-only control – pipette 50 µL dH₂O + 1 µL of plasmid into an eppendorf tube, mix, then pipette into a cuvette.
 6. Repeat steps 3-4 for other samples.
 7. Incubate all tubes for 1 hour, 37°C, with moderate shaking.
 8. Make two, 200 µL 1:10 serial dilutions of each culture.
 - a. Eg. Make a dilution with 180 µL dH₂O or LB, and after pipetting up and down several times, transfer 20 µL of that into another 180 µL of dH₂O or LB. Then you will have a 1:10 and a 1:100 dilution.
 - b. Transformation via electroporation can occur with very high efficiency. Plating out a dilution is a good practice, since if the efficiency is high enough undiluted plates might grow out as lawns rather than individual colonies
 9. Plate undiluted, 1:10, and 1:100 dilutions on appropriately labeled plates, with appropriate antibiotics. Control cells/DNA don't need to be diluted.
 10. Plate 100 µl of each culture on labeled plates, using sterile glass beads or a spreader.
 11. Incubate the plates overnight at 37°C, and place liquid recovery cultures in the 4°C in case your plates do not grow out or you need to make a greater dilution. Assuming everything goes smoothly, throw these cultures out once you see growth on the plates, don't let the fridge get cluttered.
 12. Check the plates the next day, preferably within 16 hours, and if there are cultures, move them to the 4 °C. If there are no cultures, leave them for a couple more days to see if anything grows out.