∅∅∅∅∅ Electroporation of Electrocompetent Cell Stock

This protocol will transform previously produced electro-competent cells via electroporation. Chilled pipette tips, centrifuge tubes, and ice will be required, but not for the entire protocol.

A total of five electroporations will be completed. Usually 1/4 the electroporation solution is used as a control to test for arcing in case the resistance of the culture is too low; the other 3 aliquots are used for transformation. A fifth electroporation will occur with the plasmid solution alone as a second control.

If a popping noise occurs when testing the control cells, the solution is arcing; electrical arcs will both kill your cells and destroy DNA/plasmids. While proper transformation may still be possible, proceed with caution if this does occur.

This protocol will take approximately 2 hours and consists of two main parts. The first component, consisting of *I,II,III,* is for transformation of the cells; a 1 hour incubation period is taken, then the second component *IV* and *V*, which plates the cells and test the efficiency of the electroporation, is done.

Solutions
Electrocompetent culture solution
Plasmid solution
LB solution (warmed to 37℃)
dH2O (distilled water)
Plates (with antibiotic)
Glass beads

Materials
Pipetman P5, tips (chilled to 4°C)
Pipetman P100, tips (chilled to 4°C)
Pipetman P200, tips
Pipetman P1000, tips
Electrocuvettes (chilled to 4°C)
1.5mL centrifuge tubes (chilled to 4°C)
15mL tube
Tissue paper
10mL beaker

You will also need access to:
ICE
Electroporator
Incubator with shaker

Procedure I

1. Fill a **10mL beaker** with room temperature water until level in the beaker is about **1cm**.

2. Place the **culture solution**'s centrifuge tube in the beaker. Wait until the liquid culture is fully thawed before proceeding. This will take about 10 minutes.

3. When thawed, remove the culture from the beaker and store in **ice.**

4. Gently open the culture solution's tube. Transfer **50µL** culture solution to a chilled, sterile **electrocuvette.** Tap or flick the electrocuvette, ensure all solution has collected in the bottom. Wipe

off the coelectroporatorndensation forming on the cuvette with a piece of **tissue paper**.

Condensation on the electrocuvette can do unfortunate things such as short-circuit the electroporator.

5. Slide the electrocuvette into the elecroporator and apply a pulse of electricity. The time constant should be around **5 milliseconds.** A lower time constant indicates low electrical resistance in the solution and can be accompanied by arcing.

6. Remove the electrocuvette from the electroporator and add **1mL LB** with a **P1000** pipette. Mix by repeatedly uptaking and expelling liquid from the pipette about 10 times.

7. Transfer the solution to a sterile **15mL tube** with a **P1000.** Label this tube "control" or something else that will remind you it is the control. The total volume in the tube will only be about 1.05 mL.

8. **Incubate** the tube at **37° C** for **1 hour** with gentle shaking.

The cell control is now complete. A second control should be completed on the plasmid solution to test its conductivity.

Procedure II

1. Add **50µL dH2O** to a **1.5mL centrifuge tube** with a **P100.**

2. Inject **1µL plasmid solution** into the bottom with a **P5.**

3. Mix by repeatedly uptaking and expelling liquid from the pipette several times.

4. Transfer the solution to an **electrocuvette** with a **P100.**

5. Insert into the **electroporator.** Pulse the sample, again checking to make sure the **time constant** is at or above **5 milliseconds**.

If no arcing occurred and the electroporator didn't explode, the following procedure can be used to finally transform the cells. Repeat this solution as many times as is necessary, or until you run out of electrocompetent cells.

It is important to add the LB quickly and return the cells to the incubator. Electroporation, while an efficient mode for transformation, also kills and heavily damages most of the cells in the culture. Giving the culture time and nutrients to repair and regrow after this method is important to prevent it from dieing off entirely.

Procedure III

1. Add **2µL plasmid solution** to **one** chilled **1.5 mL centrifuge tube** with a fresh, sterile, chilled **P5.**

2. Add **50µL electrocompetent culture** to the centrifuge tube with a fresh, chilled, sterile **P100**. Mix by uptaking and expelling the solution with your pipette, about 10 times.

3. Transfer the solution to a chilled **electrocuvette.** Wipe the condensation of the cuvette and place it in the electroporator.

4. Electroporate the solution for **5 seconds**.

5. **Quickly** remove the cuvette and add **1mL LB** with a **P1000** pipette. Mix by repeatedly uptaking and expelling liquid from the pipette about 10 times.

6. Remove the electrocuvette from the electroporator and add **1mL LB** with a **P1000** pipette. Mix by repeatedly uptaking and expelling liquid from the pipette about 10 times.

7. Transfer the solution to a sterile **15mL tube** with a **P1000.** Label the tube.

8. **Incubate** the tube at **37° C** for **1 hour** with gentle shaking.

Repeat this procedure with the remaining three cultures or until no electrocompetent cell solution is left in the tube. You will end up with 4 tubes of culture-three with your transformed plasmids, one with no plasmids as a control.

The next procedure will dilute the cells and plate them at various concentrations. The efficiency of electroporation is difficult to predict, and the culture could be within a wide range of transformed bacteria concentrations. Two dilutions will be produced: 1:10 and 1:00. Both will be plated.

This will be done 2-3 times, depending on the number of electroporations performed besides the control. Each separate culture will be diluted twice, for a total of three volumes~3 elecroporations will yield 9 total tubes and 9 plates. Controls will be plated once for each~1 plate for the cell solution control, 1 plate for the plasmid control.

Do not dilute cells from the control culture solution or control plasmid solution!

Procedure IV

1. Remove the tube of transformed culture from the incubator after 1 hour.

2. Add **180µL LB broth** to the tube with a sterile **P200.** The volume in the tube should be roughly 200µL.

3. Add **180µL LB broth** to a **second 15mL tube** with a sterile **P200.** You should now have two tubes: one containing culture and LB at 200µL, the other with plain LB at 180µL.

4. Transfer **20µL** of **culture** from the **first tube** to the **second tube.** The concentration of culture in the second tube will be 1:10 that of the first tube.

5. Add **180µL LB broth** to a **third 15mL tube** with a sterile **P200.** You should now have three tubes.

6. Transfer **20µL** of **culture** from the **second tube** to the **third tube.** The concentration of culture in the second tube will be 1:100 that of the first tube and 1:10 that of the second tube.

This final procedure will plate all solutions. 3 plates are needed per dilution. Plating is straitforward, but use glass beads to do the spreading.

Procedure V

1. Add **100µL from each culture** to a separate, labeled **agar plate** with a sterile **P100.** Drop along the edge with the pipette.

2. Add several **glass beads** to the transformed cultures (not control!). Roll around in the dish to spread out the solutions with a gentle swirling motion.

3. Remove beads carefully. **Incubate** at **37°C** for **14-16 hours**.

4. Check after 16 hours to see if colonies are present. If growth is visible, transfer to a **4°C environment**. If no colonies are visible, wait another **24** hours.

You can potentially wait longer than 24 hours if nothing is visible on the plate. If colonies are visible on the transformed plates but not the controls, the culture was transformed successfully and you deserve a pat on the back.