This protocol is adapted from Marcus Lehr's electroporation protocol which, in turn, was adapted from Jermy Minty's electroporation protocol
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∅∅∅∅∅ Production of electrocompetent cell stock

This protocol will generate a stock solution of bacteria suitable for transformation by electroporation-enough solution will be generated to electroporate 4 times. The protocol will take 2-3 days to complete, as cells must be grown overnight. E.Coli DH5α is recommended.

All Solutions
LB Broth stock
Plated E.Coli stock
dH2O stock
Liquid nitrogen

All Materials
25mL autopipette tips (chilled to 4℃)
5mL autopipette tips (chilled to 4℃)
15mL centrifuge tubes
50mL centrifuge tubes
125mL Erlenmeyer flask
Pipetman P10, tips (chilled to 4°C)
Pipetman P500, tips (chilled to 4°C)
Pipetman P1000, tips (chilled to 4°C)

You will also need access to an:
Incubator
Spectrophotometer
Centrifuge
Vortexer
ICE (preferably in a bucket or something)
Large freezer or other 4℃ environment (such as northern MI)
-80℃ freezer

Part I. The first component of this protocol must be completed in a **sterile** environment and will produce one 2mL culture.

Solutions
LB Broth stock
Plated E.Coli stock

Materials
15mL centrifuge tubes
Pipetman P10, tips

You will also need access to an:
Incubator

Procedure

1. Add **2mL LB broth** to the bottom of a **10mL centrifuge tube**.

2. With a **P10 pipette**, "poke" an isolated colony **E.Coli** plate. The bacterial will stick to the tip.

3. Place the **pipett**e into the **15mL tube** and into the **liquid LB**, *being careful not to touch the sides of the tube.* "Wash" the bacteria off the pipette tip by quickly drawing in and expelling fluid from the pipette. Wash about 5-10 times.

4. Place the **15mL tube** in the **incubator**. Leave the culture to grow at **37°C** for **12-16 hours**.

This "mini-culture" of bacteria will be used to jumpstart growth of the larger culture, from which we will make the stock.

Part II. The second component of this protocol must be **sterile** and must be completed **12-16 hours** after part I. One 125mL culture will be generated.

Solutions
LB Broth stock
2mL culture (made previously)

Materials (sterile)
25mL autopipette tips
5mL autopipette tips
50mL centrifuge tubes
125mL Erlenmeyer flask

You will also need access to an:
Incubator

Procedure

1. Remove the **2mL growth solution** from the incubator.

2. Using a **25mL autopipette**, add **100mL LB broth stock** to an empty, sterile **125mL Erlenmeyer flask.**

3. Using a **2mL autopipette**, empty the **2mL culture** into the **125mL Erlenmeyer flask**.

4. Incubate the **125mL Erlenmeyer flask** at **37° C** for **2-3 hours**.

During the growth time, the culture will grow and increase in density. Electrocompetent cell product occurs at an optimum cell density, which we will test for in **part III.**

Part III. This will wash the cultures and rid them of LB that may interfere with the freezing and electroporation process. The cells will be washed three times with distilled water (dH2O), then

This section is tedious. Please be patient with it.

Keep your tube racks in an ice bath to ensure chilling.

Solutions
dH2O (4°C, sterile)
100mL culture (made previously)

Materials (sterile)
25mL autopipette tips (chilled to 4℃)
50mL centrifuge tubes (chilled to 4°C)
Pipeteman P500 (tips chilled to 4°C)
15mL centrifuge tubes

You will also need access to a:
4℃ environment for general lab work
Centrifuge (at 4℃)
Ice bath
Vortexer

Procedure

1. Add **50mL** of the **100mL culture** to **one 50mL centrifuge tube** using a fresh, sterile, chilled **25mL autopipette.**

2. Add the **remaining amount** (about about 50mL) to a **second 50mL centrifuge tube** using a fresh, sterile, chilled **25mL autopipette.**

   You should now possess two 50mL centrifuge tubes, each one containing half the original solution.

3. Place the two tubes on an **ice bath.** Let them chill for **15-20 minutes.** Tubes should be stored in an ice bath when not in use.

4. **Centrifuge** the two tubes at **4200 rpm, 4° C** for **5 minutes.** A pellet of compacted bacteria should appear on the bottom. Decant the supernatant.

5. Add **50mL** chilled, sterile **dH2O** to each tube with a fresh, sterile, chilled **25mL autopipette**. **Vortex.** The pellet should be resuspended and the solution in each tube cloudy. *This will wash the bacteria once.*

6. **Centrifuge** the tube at **3500 rpm, 4° C** for **15 minutes**. A looser, more fragile pellet will form.

7. Pipette off the supernatant with a fresh, sterile, chilled **25mL autopipette** in each tube and discard**.** Be careful not to disturb or suck up pieces the fragile pellet.

8. Add **25mL** chilled, sterile **dH2O** to each tube with a fresh, sterile, chilled **25mL autopipette. Vortex** to resuspend. *This will was the bacteria a second time.*

9. Combine the solutions in both **50mL tubes** by carefully pouring the contents of one tube into the other tube. One tube should be empty; the other should be contain **50mL culture solution.**

10. **Centrifuge** the remaining tube at **3500 rpm, 4° C** for **15 minutes**.

11. Pipette off the supernatant with a fresh, sterile, chilled **25mL autopipette** in each tube and discard**.** Again, do not touch the pellet.

12. Add **2mL** chilled, sterile **dH2O** to each tube with a fresh, sterile, chilled **5mL autopipette. Vortex** to resuspend. *This will wash the bacteria a third (and final) time.*

13. Transfer the 2mL solution from the 50mL tube to a fresh, sterile, chilled **15mL centrifuge tube**.

14. **Centrifuge** the 15mL tube at **3500rpm, 4° C** for **15 minutes**. If you don't balance the centrifuge it will kill you.

15. Add **200µL** chilled, sterile **dH2O** to each tube with a **P200**, ensuring the tips are chilled**. Vortex** to resuspend. Store the tube in your ice bath.

The cells are now washed and in a state useful for electroporation. The next section will dilute them to the proper density.

Part IV. This protocol must be completed in a **semi-sterile, 4° C** environment. Density of the cultures will be tested using a spectrophotometer tuned to measure absorbance at the 600nm wavelength, or OD600; with this measurement, the optimum density for electroporation is **0.3±0.1.** If the OD600 is off, the cells must be centrifuged and resuspended with larger or smaller amounts of distilled water,depending on the concentration.

As the cultures may not have reached this optimum density, some trial and error may be necessary. *Again, please be patient with it.*

This protocol will produce one 1.5mL tube containing 100-200 microliters of solution.

Solutions
dH2O
200µL culture suspension (made previously)

Materials (sterile)
Pipeteman P20 (tips chilled to 4°C)
Pipeteman P500 (tips chilled to 4°C)
Pipeteman P1000 (tips chilled to 4°C)
1.5mL centrifuge tubes (chilled to 4°C)
Cuvettes

You will also need access to a:
Spectrophotometer
4℃ environment for general lab work
Centrifuge (at 4℃)
Ice bath
Vortexer

Procedure

1. Add **10µL culture suspension** solution to a **cuvette** with a fresh, chilled, sterile **Pipeteman P20 tip.**
2. Add **990µL dH2O** solution to a **cuvette** with a fresh, chilled, sterile **Pipeteman P1000 tip.**
3. Measure the OD600 of the cuvette with the **spectrophotometer.**
4. Transfer the remaining **~190µL culture** to a chilled, sterile **1.5mL centrifuge tube**.

⇒ If the OD600 is less than 0.2 units ($2x10^{10}$cells/mL), electroporation will be possible but inefficient. If you don't want that, complete the following:

Procedure

1. **Centrifuge** the **culture suspension** in its centrifuge tube at **5000rpm, 4°C** for **10 minutes**.
2. Pipette out the supernatant and discard using a fresh, chilled, sterile **Pipeteman P500 tip.**
3. Add **~150µL** chilled, sterile **dH2O**. **Vortex** to resuspend.
4. Add **10µL culture suspension** solution to a **cuvette** with a fresh, chilled, sterile **Pipeteman P20 tip.**
5. Measure the OD600 of the cuvette with the **spectrophotometer.** If it still reads less than 0.2 OD600 units, repeat the protocol with *increasingly smaller amounts of dH2O* in **step 4.**

OD600 measures concentration, so resuspending the same cell number in smaller amounts of dH2O will ultimately increase OD600 units.

Part V (optional). This will snap freeze the cell solution for long term storage.

You really shouldn't touch the tube once its been frozen in nitrogen.

Solutions
Culture suspension between 0.2 and 0.4 OD600 units (made previously)
Liquid N_2

Materials
10mL beaker
Tongs/thick gloves

You will also need access to a:
-80℃ freezer

Procedure

1. Fill flask with **liquid N_2**-not full, but to about the 40% of the height of a centrifuge tube.
2. Drop the **culture suspension** into the beaker until it freezes solid.
3. Store the tube of culture suspension in a **-80°C freezer.**

Note that once the solution is thawed, you must use all of it in an electroporation—refreezing and rethawing competent cells doesn't work very well.