This protocol is based off of a previous Team Michigan method as well as advice from the Jakob lab.
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∅∅∅∅∅ Competent Cell Production

Competent cells are prepared cell cultures capable of receiving plasmid DNA when transformed using a transformation protocol. These cells are generally grown to mid-log phase (OD600 of 0.6-0.8), cooled, washed with a salt solution, and frozen.

Please note that cells should not be warmed once they are cooled from a fresh culture, otherwise transformation efficiency drops severely.

This protocol will require the overnight growth of a culture, so plan ahead. Part I will produce a culture to be grown for 16 hours; part II will complete the preparation of the culture.

All Solutions
0.1M $CaCl_2$ (chilled to 4°C)
Growth media (with antibiotics)
A method of flash freezing (Liquid nitrogen, etc.)
Ethanol

All Materials
Plate containing isolated cultures
Pipetman P5, tips (chilled to 4°C)
25mL autopipette (chilled to 4°C)
5mL autopipette (chilled to 4°C)
125mL Erlenmeyer flask
25mL centrifuge tubes (chilled to 4°C)
1.5mL centrifuge tubes (chilled to 4°C)
15mL tubes
10mL beaker
Tissue wipes
ICE

You will also need access to a
Centrifuge at 4℃
Incubator at 37°C

Part I. This procedure will grow sufficient cultures for the preparation stage.
Note that none of the materials have to be be chilled.

All Solutions
Growth media (with antibiotics)
Ethanol

All Materials
Plate containing isolated cultures
Pipetman P5, tips
15mL tubes
.5mL centrifuge tubes
Tissue wipes

You will also need access to a
Incubator at 37°C

Procedure

1. Add **2mL growth media** to a **15mL tube** with a **P1000.** *This pipette does not need to be chilled.*

2. Sterilize the shaft of the **Pipeteman P5** with ethanol and tissues.

3. Using sterile technique, carefully "poke" a **colony** on the plate with the **P5.**

4. Being careful not to touch the sides of the tube, submerge the tip of the pipette in the LB of the 15mL tube. Wash the bacteria off the tip by uptaking and expelling the LB about 10 times.

5. **Incubate** the 15mL tube for **16 hours** at **37° C.**

Part II. The 16 hour growth will make a large volume of bacteria. The next two steps can be completed in the same day, but require an incubation time of 4 hours.

Procedure I

1. Add 100mL **growth media** to a **125mL Erlenmeyer flask** with a **25mL autopipette.**

2. Add the entire growth volume of the 15mLtube produced earlier to the Erlenmeyer flask full of growth media with a **P1000**or by **pouring.**

3. Incubate the growth media for **3.5 hours** at **37° C**.

⇒ After 3.5hours, check the OD600. It should reside between 0.6 and 0.8 for maximum cell quality.

⇒ Once the proper optical density has been reached, store the cultures on ICE. Do not let them heat beyond this temperature.

Procedure II

1. Transfer the ~100mL culture to **two 50mL centrifuge tubes** with a fresh, chilled, sterile **25mL autopipette.**

2. **Centrifuge** the 50mL tubes at **2500rpm** at **4°C** for **20 minutes.** Decant the supernatent.

3. Add **25mL 0.1M $CaCl_2$** with a fresh, chilled, sterile 25mL autopipette**.** Vortex to resuspend the pellets.

4. Let the two tubes stand on **ICE** for **30 minutes.**

5. **Centrifuge** the 50mL tubes at **2500rpm** at **4°C** for **20 minutes.** Decant the supernatent.

6. Add **2.5mL 0.1M $CaCl_2$** with a fresh, chilled, sterile **P1000.** Vortex to resuspend the pellets.

7. Transfer the cells in **200µL aliquots** into **1.5mL centrifuge tubes.** A total of ~12 tubes should be produced.

⇒ Snap freeze the tubes by placing them in a -80°C freezer.