This protocol adapted from protocols from Marcus Lehr and Marcus Ammerlaan.
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∅∅∅∅∅ Quick Transformation (QT) Competent Cell Production

Transformation can be achieved by a fast and simple 5 minute protocol involving simply mixing competent bacteria and plasmid solution together. This is far faster than electroporation, which requires many careful steps to properly dilute, clean, and freeze the bacteria. Electroporation transforms 10-100 times more efficient than QT, however, and therefor should be the primary method for transforming when a functional outcome is important; QT should be used when time is limited or an experiment is less critical.

As QT competent cells contain calcium ions, they are highly conductive and therefor not suitable for electroporation unless you intend to completely kill a bacterial culture in the most spectacular way possible.

0.1M solutions of $CaCl_2$ and $MgCl_2$ must be produced and refrigerated prior to completion of this protocol. Additionally, centrifuge rotors must be either refrigerated or chilled prior; an alternative solution is to use a cold room. It is imperative, however, that cultures are kept cold until transformation; any heating above 4°C will reduce competence by a significant amount.

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.

Solutions
0.1M $CaCl_2$ (chilled to 4°C)
0.1M $MgCl_2$ (chilled to 4°C)
Growth media such as LB
Source of cells (plate or cryostock)

Materials
5µL capable pipette, tips (chilled to 4°C)
25mL autopipette (chilled to 4°C)
5mL autopipette (chilled to 4°C)
125mL Erlenmeyer flasks
50mL centrifuge tubes (chilled to 4°C)
Paper towels/wipes

You will also need access to a
Centrifuge at 4°C (50mL tube capable)
Incubator at 37°C (with shaker)

External protocols
Innoculation from frozen stock
OD600 Optimization
Aliquoting for cryostorage

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

Procedure I

1. Add **5mL growth media** to a **50mL tube** with a **P1000.** *This pipette does not need to be chilled.*
2. Sterilize the shaft of the **5 µL pipette** with ethanol and tissues.
3. Using sterile technique, carefully “poke” a **colony** on the plate with the **5 µL pipette.**

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 50mL tube for **16 hours** at **37°C** with shaking**.**

⇒ Alternatively, if producing from a frozen stock, follow the Innoculation protocol instead to get a growth solution.

Procedure II

1. Add 100mL **growth media** to two sterile **125mL Erlenmeyer flasks** with a **25mL autopipette.** A total of 200mL LB should be used total. Cover with foil.

2. Incubate the sterile media overnight. The temperature should be identical to the inoculated media.

Part II. The 16 hour growth will make a large volume of bacteria. The next two steps can be completed in the same day.

Three procedures will be completed. *Procedure I* is very short and involves expanding the volume of solution. After 1 hour, *II* will be used to wash the cells and make the solution competent.

Procedure I

1. Add the contents of the **50mL tube** two both 125mL Erlenmeyer flasks. Split the 5mL in half, adding 2.5mL to each flask with a **1000 μL pipette.**

2. Incubate the growth media for **1 hours** at **37°C**.

⇒ After 1 hour, check the turbidity of the solution; it should be barely cloudy. If more precision is desired, optimize the optical density in accordance with the **OD600 optimization protocol** until the value is approximately 0.4.

Procedure II

1. Transfer the ~200mL culture to **four 50mL centrifuge tubes** with a fresh, chilled, sterile **25mL autopipette.**

2. **Centrifuge** the 50mL tubes at **5000rpm** at **4°C** for **5 minutes.** Decant the supernatent.

3. Place the tubes on a paper towel upside-down to ensure all media is drained. Wait ~**2 minutes.**

4. Add **30mL 0.1M $MgCl_2$** with a 25mL autopipette to one of the 50mL tubes**.** Vortex to resuspend the pellet.

5. Pour the suspension in the previous 50mL tube in another tube containing a pellet. Again, vortex to re-suspend. Repeat this process with each subsequent tube until all the pellets are consolidated into a single tube.

6. **Centrifuge** the 50mL tube at **5000rpm** at **4°C** for **5 minutes,** making sure to balance the centrifuge. Decant the supernatent.

7. Add **40mL 0.1M $CaCl_2$** to the 50mL tube with a 25mL autopipette.

8. Store the solution at **4°C.**

⇒ If desired, follow the *Aliquoting solutions for cryostorage* protocol.