This protocol was adapted from an online protocol by "MFT," as well as advice from Marc Ammerlaan and Michael Grey.
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∅∅∅∅∅ **Heat Shock Protocol**

Transformation of competent cells can be achieved using a fairly straightforward process. Heat shock (A) is a relatively quick method of transformation.

Procedure *I* will transform the bacteria; after a 20 minute recover stage, procedure *II* will grow them for 45min-2 hours, and procedure *III* will plate them.

⇒ Competent cells should be made in accordance with the **Competent Cell Production** protocol.

Compounds
Competent cell solution
Plasmid solution
Ethanol
SOC media

Materials
LB plates (labeled, with antibiotic)
1.5mL centrifuge tubes
5µL pipette, tips
200µL pipette, tips
1000µL pipette, tips
Spreader
ICE

You will also need access to an:
Water bath at 42°C
Thermomixer at 37°C
Centrifuge

External protocols:
Production of competent cells

Procedure I

1. Add **50ng plasmid solution** to a **1.5mL centrifuge tube** with a **5µL pipette.** Keep the tube on **ICE.**
   ⇒ *If you don't know, usually 1-2µL will do the trick.*

2. Add 5**0µL competent cell solution** to the tube with a fresh, chilled, sterile **1000µL pipette**. Mix the solutions by repeatedly uptaking and expelling it from your pipette about 2-3 times. Keep the comp cells on ICE.

3. Chill the centrifuge tubes for **20 minutes** on ICE.

4. Place the tubes in the **water bath** at **42°C** for **45 seconds.**

5. Immediately pipette **1000µL SOC media** into the tube. Place in a **thermomixer** and shake **45min-2h** at **550rpm**.

6. Place the tubes back on ice for **2 min.**

   ⇒ *This "phenotypic expression" stage will help the bacteria survive th antibiotic. If completing a 3A construction, incubate for 2 hours. Otherwise, 45 minutes is usually sufficient.*

Procedure II

1. Spin down the tubes at max speed for **30 seconds** and decant the supernatent. ~50µLshould remain in the tube.

2. Pipette the contents of the centrifuge tube onto the center of an **antibiotic LB plate** with a sterile **100µL pipette**.

3. Dip a **spreader** in **ethanol** and flame. Touch the spreader to fresh agar to coll, then spread the transformed colony solution evenly across the plate.

4. **Incubate** the plates for **16 hours** at **37°C** or until colonies are observed.

*If plating on ampicillin, this step is not necessary, but recommended. If construction is complex (IE: 3A or other multiple parts) this should be completed anyway.