Transformation Protocol

Before you begin:

-Turn on water bath and set to 42°C.
-Turn on incubator and shaker.
-Get ice.
-Take out the LB/agar plates with desired antibiotic so they can warm to room temperature.

1. Chill a 15ml polypropylene tube on ice.
2. Thaw DNA on ice. Meanwhile, get a vial of competent cells (we use JM109 cells) from -80° freezer. Be sure to keep on ice. The cells will thaw very quickly.
3. Transfer 1µl of DNA to the 15ml tube.
4. Add 50µl of competent cells to the 15ml tube.
5. Incubate on ice for 20 minutes.
6. Heat shock the cells by holding the tube in the water bath set to 42°C. Gently shake the tube back and forth. Do this for exactly 45 seconds.
7. Place tube back on ice for 2 minutes.
8. Add 950µl of SOC or LB medium (both are kept at 4°C; SOC medium is preferred).
9. Let the cells recover while shaking in the shaker at 37° for 1 hour. (This step can go longer than 1 hour if necessary).
10. Transfer the cells to a clean eppendorf tube and centrifuge at max speed for 1 minute at room temperature.
11. Remove 700-900µl of the supernatant with a pipette and resuspend the cells using a pipette in the remaining media (should be about 300µl).
12. Using the same pipette tip, plate the cells to an LB-agar + antibiotic plate.
13. Incubate the plate top side up for about 10 minutes in the incubator at 37°C.
14. Turn the plate over and incubate overnight (~16 hours).