Transformation and Plating Protocol (revised 6/8/2018)

The cells used in the following protocol are delicate and temperature sensitive.
Please keep your tubes on ice for all steps unless otherwise indicated.
Please pipette cell solutions slowly (2+ seconds up, 2+ seconds down)

Transforming Competent Cells

1. Add 3 µl of undiluted ligation product or 100 ng of plasmid to the very bottom of a labeled sterile 2 ml tube. Place tubes on ice in a square tube rack.

2. Before handling the competent *E. coli* cells, strategize how your group will handle them without bringing the tube above 0°C. Transformation efficiency drops dramatically every minute the cells are above 0°C.

3. Slowly add 20 µl of competent *E. coli* BL21-DE3 cells to each pre-cooled tube, visually verify that cells are placed at the bottom of the tube with the ligation or plasmid solution and mix gently by swirling the pipet tip. Place immediately back on ice in a white square floating tube rack.

4. Incubate the tubes on ice for 10 minutes.

5. Heat shock the cells by transferring the entire square rack with tubes to a 42°C water bath for 30 seconds.

6. Immediately transfer the rack and tubes back onto ice for 2 minutes.

7. Transfer tubes to a standard room temperature tube rack and add 300 µl of room temperature S.O.C. recovery medium to each tube.

8. Place the tubes into a rack in the shaking incubator at the back of 123 and shake (250 rpm) at 37°C for 60 minutes.

Plating Cells

9. Decide as a group how many of each type of plate will be needed (LB, LB+carb, or LB+carb+IPTG) and label each plate clearly on the bottom along the edge (lids can accidentally be swapped) with your group, section, plasmid, and media type.

10. Pipette 100 µl of transformed cells into the center of a plate and carefully add 4-5 sterile glass beads and replace the lid.

11. Spread the solution evenly across the surface of the plate by shaking it vigorously back and forth for at least 30 seconds while keeping it level with the counter top.

12. Pour the used glass beads into the Used Beads container. *Beads will be sterilized and reused.*

13. Obtain a metal petri dish holder label it with your section and group information. Stack plates upside down into the holder and incubate at room temperature until next week.