Bacterial Transformation (Short Protocol)

Materials

- 50mM $CaCl_2$
- Overnight MM294 E.coli plates
- LB Amplicillin plates
- LB Plates
- pGFP plasmid (0.008ug/ml)

Protocol

1. Take 2, 15mL snap-cap tubes and lable with your group number and mark one tube (+) and the other (-)
2. Add 250uL of $CaCl_2$ to teach tube. Keep these tubes on ice untill advised otherwise in the protocol.
3. Using a sterile loop, add a "clump" of bacteria to each tube, being sure to wash the bacteria off into the $CaCl_2$.
4. Using a blue pipette, thoroughly mix the bacteria into the $CaCl_2$ solution (pipette up and down slowly so that you do not contaiminate the pipette).
5. Add 10ul of the pGFP plasmid to **to the + tube only**; use your finger to tap and swirl the solution.
6. Let tubes rest on ice at least 5 minutes.
7. While tubes are on ice, label one LB AMP plate (+) and the other (-); label plates on the agar side (not the lid). Be sure to write on the circumfrence (edge) of the plates. Include your group number and date.
8. Heat shock both tubes by placing them directly from the ice into a 42C water bath for 90 seconds (use a timer). Then place tubes back on ice.
9. Add 250ul of LB to each tube. Be sure to change tips to avoid cross contaimination.
10. Add 100ul of cells from the + tube to the + plate and use glass beads to plate. Do the same for the - tube and the - plate.
11. Incubate plates at 37C overnight. Place them in the fidge the next morning.