Fluorescence Assay

The fluorescence assay will take one week to complete. First, we culture the bacteria containing our gene of interest in Luria-Bertani broth containing carbenicillin (LB+CARB), to allow the target bacteria to grow up to a large population size while preventing growth of off target bacteria. In the prep lab, this overnight culture will be transferred on the following day into a larger volume of fresh LB+CARB and allowed to grow for 2 hours up to roughly OD_{600} 0.8. At that point, IPTG will be added to a final concentration of 0.2 mM and shaken at room temperature for 16 hours. The IPTG induces expression of the fluorescence gene. Then, they will concentrate the bacteria into a smaller volume of LB+CARB and expose them to an excitation wavelength of light and record the fluorescent emission from each sample. We use a plate reader that can excite and detect emissions from samples in a 96-well plate (Bio-Tek Synergy plate reader, courtesy of Dr. Brian Hammer). The baseline against which these readings are detected is bacteria that contain the pET-15b plasmid without a fluorescent gene inserted ("empty plasmid").

Pick Colonies into Overnight Culture (Students in lab)

1. Review aseptic technique with your TA so that you can handle all of the sterile media without contaminating your cultures.
2. Use a piece of labeling tape to label one small culture tube of 2 ml LB-CARB (without IPTG) for each of your colonies and the ancestor to those colonies. The TA will label and inoculate a tube for the empty plasmid sample.
3. To "pick" the colony into the culture tube, carefully lift the plate from its lid, carefully tap the edge of the colony with the tip of a sterile toothpick to collect a few cells onto the toothpick, then drop the toothpick into the culture tube and put the lid on. HINT: Wipe your gloves down with ethanol before handling the toothpick, and only handle the end opposite where you'll tap the bacterial colony.
4. Grow culture tubes overnight at 37°C in the shaking incubator at 250 rpm.

Day 2 (Prep lab staff)

5. Transfer 300 µl of each overnight culture into 10 ml LB+CARB tubes.
6. Grow to an optical density of OD_{600} 0.8 at 37°C shaking, about 2 hours.
7. Add IPTG to a final concentration 0.2 mM, then culture, shaking at room temperature for 16 hours.

Day 3 (Prep lab staff) – Fluorescence Assay

8. Centrifuge 1 ml culture at 14,000 rpm for 5 minutes.
9. Carefully remove the supernatant with a sterile pipet without disturbing the cell pellet.
10. Resuspend the pellet in 200 µl of fresh LB+CARB broth.
11. Load samples into 96 well plates. Each group of an original RFP and all variants plus the empty plasmid pET-15b control is read as a unit so that the control can be used to standardize the readings for the other related samples.
12. Read the fluorescent emission spectrum for each sample, using the pET-15b as the blank, on a Bio-tek Synergy plate reader (equipment courtesy of Dr. Brian Hammer).