**Our Biofilm Assay Protocol**
Adapted from "Growing and analyzing static biofilms" (Merritt et al., 2005)

Materials Needed:
1 round-bottom 96-well plate
1 flat-bottom 96-well plate
LB Broth
M9 Minimal Media
Appropriate antibiotic stock
15 mL conical tubes or glass test tubes for growing liquid cultures
0.1% crystal violet
30% acetic acid
Paper towels
A large beaker
A tray or box that is slightly larger than a 96-well plate
A plate reader

1. Prepare 5mL LB liquid cultures (supplemented with appropriate antibiotics and inducers) from stock or a colony from the transformation plate of the desired strains. Allow the cultures to grow for 18-20 hours at 37 degrees Celsius in a shaking incubator.
3. Prepare 1:100 dilutions, with a total volume of 1 mL, with LB broth and M9 minimal media for each liquid culture.
4. Plate 100 µL of each dilution in sets of 4 wells in a round-bottom 96 well plate.
5. Cover the 96-well plate and incubate at 37°C for 48 hours.
6. Shake the plate out over a tray to remove all planktonic bacteria.
7. Rinse the 96-well plate in a large beaker of water and shake the water out over the tray.
8. Lay a paper towel out on the bench top. Hit the 96-well plate against the covered bench top until no liquid remains in the wells.
9. Stain all wells used in the assay with 125 µL of 0.1% crystal violet for 10 minutes.
10. Shake the 96-well plate over the tray again and rinse out the crystal violet in a large beaker of water.
11. Cover the bench top with more paper towels and hit the plate against the bench top until all wells are free of liquid crystal violet. Note: Make sure that the only crystal violet remaining is bound to a biofilm at the bottom of a well. Rings of crystal violet around a well are not indicative of biofilm formation and should be rinsed again, as excess stain will skew the results of the assay.
12. Leave the plate face up on the bench top overnight to dry.
13. Add 200 µL of 30% acetic acid to all wells that were stained to solubilize the crystal violet. Allow the acetic acid to sit for 10 minutes.
14. Pipette up and down the mix the acetic acid and crystal violet in the wells.
15. Transfer 125 µL of the acetic acid/crystal violet solution from each well into a well in a flat-bottom 96-well plate.
16. Read the $OD_{595nm}$ of each well in the flat-bottom plate with a plate reader.
17. Subtract the average of the blank wells from the OD of each well that contained a sample.
18. Calculate the average of the sets of wells containing the same sample.
19. Normalize the averages to the average of the control wells.