2019 Tacoma RAINmakers Part Characterization

Adapted from iGEM 2018 InterLab Study Protocol

Characterization of BBa_J23100, BBa_J23101, BBa_J23102, BBa_J23105, BBa_J23106, BBa_J23112

Instrument Information:	SpectraMax M2
	Molecular Devices
	Uses Top Optics Only
	Temperature Adjustable to RT

Cell growth, sampling, and assay

- ☐ Make a 1:10 dilution of each overnight culture in LB+Ampicillin (0.5mL of culture into 4.5mL of LB+Amp)
- ☐ Measure Abs_{660} of these 1:10 diluted cultures
- ☐ Record the data in your notebook
- ☐ Dilute the cultures further to a target Abs_{660} of 0.02 in a final volume of **12 ml** LB medium + Amp in 50 mL falcon tube (covered with foil to block light).
- ☐ Take 500 µL samples of the diluted cultures at 0 hours into 1.5 ml eppendorf tubes, prior to incubation. (At each time point 0 hours, 3 hours, and 5 hours take a sample from each sample). Place the samples on ice.
- ☐ Incubate the remainder of the cultures at 37°C and 220 rpm for 3 hours.
- ☐ Take 500 µL samples of the cultures at 3 hours of incubation into 1.5 ml eppendorf tubes. Place samples on ice.
- ☐ Incubate the remainder of the cultures at 37°C and 220 rpm for additional 2 hours.
- ☐ Take 500 µL samples of the cultures at 5 hours of incubation into 1.5 ml eppendorf tubes. Place samples on ice.
- ☐ At the end of sampling point you need to measure your samples (Abs_{660} and fluorescence measurement), see the below for details.
- ☐ Record data in your notebook
- ☐ Import data into Excel sheet provided (**fluorescence measurement tab**)

Measurement

Samples should be laid out according to the plate diagram below. Pipette 100 µl of each sample into each well. From 500 µl samples in a 1.5 ml eppendorf tube, 4 replicate samples should be pipetted into wells in rows A, B, C and D. Be sure to include 8 control wells containing 100uL each of only LB+ampicillin on each plate in column 9, as shown in the diagram below. Instrument temperature should be set to room temperature (approximately 20-25 C) if your instrument has variable temperature settings.

Layout for Abs_{660} and Fluorescence measurement

At the end of the experiment, you should have two plates to read. Each plate should be set up as shown below. On each plate you will read both fluorescence and absorbance.