Overview

We will be collecting supernatant from algae to test Lsr circuit activation, using E. coli supernatant for a positive control and LuxS- supernatant for a negative control. Assayed cells will be pelleted and re-suspended in equal volume of supernatant in order to preserve concentration of AI-2/mimic. Activity will be reported by GFP using a fluorescence spectrophotometer.

Needed Materials & Equipment

- Glucose
- Spectrophotometer

Collecting AI-2 from E. coli

- Inoculate 10 mL of LB in 50 mL tube and incubate overnight (12-16 hours) at 37 °C at 200 rpm.
- Dilute in 100 mL LB + 0.8% glucose (+ 100 µL of Amp and Kan when required) to an OD600 of 0.02 according to dilution protocol. (0.8g powdered glucose, or 2 mL of 40% glucose)
- Incubate cells at 37 °C, 225 rpm, in a 500 mL flask.
- Remove 25 mL aliquots with autopipette at 3, 4, and 5 hours (OD600 ~0.3-1.0) and put in 50 mL tubes.
- Immediately spin tubes down in centrifuge at 5,000 rpm for 10 minutes.
- Gently, without disturbing pellet, attach 50 mL filter tube to 50 mL tube with pellet and invert.
- Hook filter tube to 1224 sink hose and filter supernatant.
- Store filtered supernatant at -20 °C.
- Modified from Singapore 2008 iGEM team.

Collecting Supernatant from Algae

- Acquire about 40 mL of algae at maximum density/ready to harvest stage from Bobby Levine.
- Put algae in 50 mL tube and spin down in centrifuge at 10,000 rpm (?) for 10 minutes.
- Carefully, without disturbing pellet, attach 50 mL filter tube and invert.
- Hook filter tube to 1224 sink hose and filter supernatant.

- Store filtered supernatant at -20 °C.

Testing E. coli quorum sensing response to algae AI-2/mimic

- Inoculate 10 mL of LB + Ampicillin and Kanamycin in a 15 mL tube with MDAI-2 and incubate overnight (12-16 hours) at 30 °C, 200 rpm.
- Take OD reading to make sure the culture is between 0.5 and 1.0. If higher than 1.0, make a 1:2 dilution, allow to grow for 30-40 minutes and recheck OD.
- Aliquot 1 mL of culture into or 15 mL tubes.
- Spin tubes in centrifuge at 4,200 rpm for 10 minutes, then decant supernatant
- Label tubes appropriately and resuspend pellet with corresponding supernatant.
- Cultivate tubes for 1-2 hours.
- Pipette cultures into cuvettes and take OD and fluorescence readings on Lin Lab fluorescence spectrophotometer.