SAMPLE INSTRUCTOR PROTOCOLS FOR LAB PREPARATION AND SCALE UP

The following protocols were used to implement the described experiments in a junior-level Bioengineering undergraduate lab under the following conditions:

- Length of Lab: Six 3-hour sessions over 3 weeks (2 sessions/week)
- Students/section: Approximately 40 students (10 groups of 4)
- Number of Sections: 2 sections or 80 students total
- Sample preparation for all 80 students accomplished by a single staff member

Because the protocols were designed for sections that meet on alternate days, cell growth was determined to be complete after approximately 20 hours at room temperature in order to halve the amount of equipment by eliminating overlap between sections. Thus, these protocols are meant as guidelines, and the numbers and timing can be scaled to fit individual needs.

Experiment 1. Measuring Transfer Functions in Liquid Culture

Each group characterizes 3 "Receiver" strains in liquid culture over two lab sessions. In **Session 1**, groups induce GFP expression with 11 different AHL concentrations (Final [AHL]: 0 M (negative control) and every log order 10^{-13} - 10^{-4} M). In **Session 2**, groups measure steady-state GFP expression levels normalized by optical density.

Scale of Preparation:

- 2 mL * 2 duplicates * 11 concentrations * 10 groups = 440 mL of bacteria/strain total. Grow >500 mL of culture per strain for extra.
- Because each group is responsible for 66 inductions (3 strains x 11 concentrations x 2 replicates), we suggest using 48-well deep well plates rather than culture tubes to save on shaker space, as well as to facilitate sample tracking (see below for lab notebook guides).

Materials Required for Preparation:

- Sterile LB Media supplemented with 34 µg/mL chloramphenicol
- Sterile M9 Media supplemented with 34 µg/mL chloramphenicol
- One 2L baffled culture flask per strain
- One 37°C shaking incubator
- Sterile pipettes, pippetors, etc.
- Sterile 50 mL conical tubes

Instructor Protocol:

Session 1: Day Before Session

1. Start 5 mL cultures of in LB media of the strains that the students are going to test
2. Pre-aliquot 500 mL of M9 media into sterile, 2 L baffled culture flasks and leave at room temperature. This step can be done in the morning the next day if desired, but the media must be allowed to warm to room temperature before back-diluting.

Session 1: Day of Session, Approximately 5 hours Before Student Arrival

1. Dilute 5 mL of each strain into the 500 mL of M9 media that has been allowed to reach room temperature.
2. Place the 500 mL cultures into a shaking incubator (37°C, 250 rpm) for approximately 4 hours until OD600 ~ 0.3.

3. Before the training session, aliquot the strains into 50 mL aliquots for each group.
4. Set up the following materials for each lab group prior to lab:
    a. [50 mL] per strain of bacteria
    b. [300 µL] Stock of 10 mM N-(3-Oxohexanoyl)-L-homoserine lactone (AHL)
    c. [10 mL] Sterile-filtered H2O
    d. [2] 48-well deepwell plates
    e. [2] Breathe-easier sealing strips
    f. Pipettes and serological pipettors

Session 1: In Session

1. Students serially dilute the AHL and induce cells across the concentration range (0 M and 10^{-13} - 10^{-4} M, once per log order). By the end of the lab session, they hand in two 48-well plates to the lab staff.

Session 1: After Session

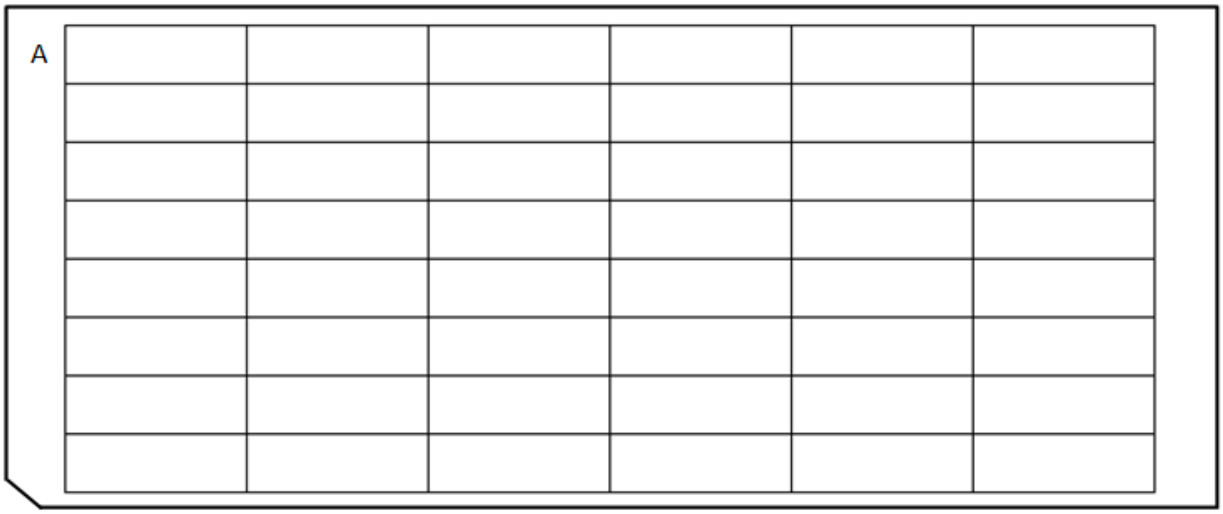
1. Incubate the 48-well plates at room temperature on a benchtop shaker for >20 hours.
2. >20 hours after the induction, move the induced plates to a refrigerator for storage until the next lab class in order to prevent overgrowth. The growth arrest has minimal impact on the response curves. If lab sessions are on consecutive days, skip this step and proceed to gathering transfer function data **(Session 2).**

Session 2: In Session

1. Students measure response curves or transfer functions, using OD-normalized GFP expression levels. Any multi-mode spectrophotometer is sufficient, including education-grade instruments (e.g. Vernier/Ocean Optics Red Tide). Set up will be specific to your model of spectrophotometer. Cells will need to be agitated prior to measurement due to sedimentation.

Accessory:

Cut-and-tape 48-well plate guides for notebooks to prevent sample mix-ups.



Experiment 2: Spatiotemporal Patterning of GFP Expression in Two Dimensions

Each group characterizes the 2D spatiotemporal patterning of GFP expression in one strain. In **Session 1,** groups induce the strain with a single bolus application of AHL on a filter disc centered on the plate. Four 60 mm plates can be simultaneously imaged on the transilluminator described in main text. Refer to Supporting Document 2 for automated data acquisition set up and protocols.

Scale of Preparation:

- 1 mL/plate * 4 plates * 10 groups = 40 mL of bacteria total
- Grow 250 mL of culture (suggested) for excess.

Materials Required for Preparation:

- Sterile LB Media supplemented with 34 µg/mL chloramphenicol
- Sterile M9 Media supplemented with 34 µg/mL chloramphenicol
- One 1 L baffled culture flask (per strain)
- One 37°C shaking incubator
- One 55°C water bath
- Sterile pipettes, pippetors, etc.
- Sterile 15 mL conical tubes and tube racks

Preparation Protocol:

Session 1: Day Before Session

1. Start 5 mL cultures of strains in LB media
2. Pre-aliquot 250 mL of M9 media into sterile, 1L baffled culture flasks and leave at room temperature. This step can be done in the morning the next day if desired, but the media must be allowed to warm to room temperature before back-diluting

Session 1: Day of Session, Approximately 5 hours before Student Arrival

1. Dilute 2.5 mL of each bacterial strain into the 250 mL of M9 media that has been allowed to reach room temperature
2. Place the 250 mL cultures into a shaking incubator (37°C, 250 rpm) for approximately 4 hours until the OD600 ~ 0.3.
3. Before class, aliquot the bacterial strains into 10 mL aliquots for each group.
4. Note: For best results, use strains with high levels of GFP expression (we suggest strain BC-A1-001, with 10 µL of 10 µM AHL). Conditions will need to be optimized on a strain-by-strain basis.
5. Set up the following materials for each lab group prior to lab:
    a. [5 mL] per strain of bacteria
    b. [4] 60 mm M9 Media Plates supplemented with Chloramphenicol
    c. [5 mL] of 0.7% Agarose solution (1 mL aliquots), located in 55ºC Hot Water Baths
    d. [4] Filter paper discs (5 mm diameter) in an empty 60 mm petri dish
    e. [100 µL] Stock of 10 mM N-(3-Oxohexanoyl)-L-homoserine lactone (AHL)
    f. [4] Sterile Forceps
    g. Kim Wipes, Sterile Pipettes, Tube Racks

Session 1: In Session

Students will induce plates and then place on custom transilluminator for data acquisition over one-to-several days. When students have completed the induction, follow the below steps:

1. Tape the outside of the plates with black masking tape (such as Thorlabs T743-1.0) in order to limit imaging artifacts at the plate edges.
2. If there is condensation on the inside lid of the plate, use a Kim Wipe to gently wipe it off, then place the plate face down on the transilluminator. This prevents future condensation, which can interfere with imaging.
3. Run the automated image acquisition script.
    a. Refer to Supporting Document 2 for automated data acquisition set up and protocols.
    a. Alternatively, any commercial blue-light transilluminator and hand-held camera are sufficient for end-point analysis. Cells can be stored in a 4°C refrigerator if needed, with limited change in the gene expression profile prior to imaging.

Session 2

1. After the completion of the experiment, plates can be stored in a refrigerator for students to physically examine.