2022 iGEM InterLab study

Experiment 3 – Challenge Cell measurement protocol

This year we plan to test protocols that will eventually be automated. For this reason, we will use 96-well plates instead of test tubes for culturing. Consequently, we want to evaluate how the performance of our plate culturing protocol compares to culturing in test tubes (e.g. 50mL falcon tube) on a global scale. This version of the interlab protocol involves 2 hr. time interval measurements and incubation inside a microplate reader/incubator.

At the end of the experiment, you will have two plates to be measured. You will measure both fluorescence and absorbance in each plate.

Before performing the cell measurements, you need to perform all the calibration measurements. Please do not proceed unless you have completed the calibration protocol. Completion of the calibrations will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions. For consistency and reproducibility, we are requiring all teams to use E. coli K-12 DH5-alpha. If you do not have access to this strain, you can request streaks of the transformed devices from another team near you. If you are absolutely unable to obtain the DH5-alpha strain, you may still participate in the InterLab study by contacting the Engineering Committee (interlab [at] igem [dot] org) to discuss your situation.

For all below indicated cell measurements, you must use the same type of plates and the same volumes that you used in your calibration protocol. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you used in your calibration measurements. If you do not use the same type of plates, volumes, and settings, the measurements will not be valid.

Protocol summary: You will transform the eight devices listed in Table 1 into E. coli K-12 DH5-alpha cells. The next day you will pick two colonies from each transformation (16 total) and use them to inoculate 12mL overnight cultures (this step is still in tubes). Each of these 16 overnight cultures will be used to inoculate four wells in a 96-well plate (200uLs each, 4 replicates) and three test tube (12mL). You will incubate this tubes for 2, 4 and 6 hrs, You will measure how fluorescence and optical density develops over 6 hours by taking measurements at time points 0 hour, 2 hour, 4 hour, and 6 hours. Follow the protocol below and the visual instructions in Figure 1 and Figure 2.

Protocol Outputs:

- baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint)
- 0 hr absorbance timepoint measurements of plate 1
- 0 hr fluorescence timepoint measurements of plate 1
- absorbance timepoint measurements of plate 1 at timepoints 2.0 hour, 4.0 hour, 6.0 hour
- fluorescence timepoint measurements of plate 1 at timepoints 2.0 hour, 4.0 hour, 6.0 hour
- absorbance timepoint measurements of Plates 2, 3, and 4
- fluorescence timepoint measurements of Plates 2, 3, and 4

Protocol Materials:

- *E. coli* DH5 alpha competent cells
- Negative control
- Positive control (I20270)
- Test Device 1 (J364000)
- Test Device 2 (J364001)
- Test Device 3 (J364002)
- Test Device 4 (J364007)
- Test Device 5 (J364008)
- Test Device 6 (J364009)
- LB Broth + Chloramphenicol (34 ug/mL)
- LB Agar + Chloramphenicol (34 ug/mL)
- Chloramphenicol stock solution (34 mg/mL)
- Ice
- Plate reader
- Shaking incubator
- Petri dish (x 8)
- culture tube (x 32)
- 1.5 mL microfuge tube (x 16)
- 50 ml conical tube (x 64)
- 96 well microplate black with flat bottom (x 4)
- microplate adhesive sealing film

Table 1: Part Locations in Distribution Kit

Device	Part Name	Coordinate
Negative Control	BBa_J428100	Kit Plate 1 Well 12M
Positive Control	BBa_I20270	Kit Plate 1 Well 1A
Test Device 1	BBa_J364000	Kit Plate 1 Well 1C
Test Device 2	BBa_J364001	Kit Plate 1 Well 1E
Test Device 3	BBa_J364002	Kit Plate 1 Well 1G
Test Device 4	BBa_J364007	Kit Plate 1 Well 1I
Test Device 5	BBa_J364008	Kit Plate 1 Well 1K
Test Device 6	BBa_J364009	Kit Plate 1 Well 1M

Protocol Steps:

Day 1

1. Obtain 8 x Petri dish containing LB Agar + Chloramphenicol (34 ug/mL) growth medium for culturing `transformant strains`
2. Transform `Negative control` DNA into *`E. coli`* `DH5 alpha competent cells`. Repeat for the remaining transformant DNA: `Positive control (I20270)`, `Test Device 1 (J364000)`, `Test Device 2 (J364001)`, `Test Device 3 (J364002)`, `Test Device 4 (J364007)`, `Test Device 5 (J364008)`, and `Test Device 6 (J364009)`. Plate transformants on LB Agar + Chloramphenicol (34 ug/mL) `transformant strains` plates. Incubate overnight (for 16 hour) at 37.0°C.

Day 2

3. Obtain 16 x culture tubes to contain `culture (day 1)`
4. Pick 2 colonies from each `transformant strains` plate.
5. Inoculate 2 colonies of each transformant strains, for a total of 16 cultures. Inoculate each into 5.0mL of LB Broth + Chloramphenicol (34 ug/mL) in culture (day 1) and grow overnight (for 16.0 hour) at 37.0°C and 220 rpm.

Day 3

6. Obtain 16 x culture tubes to contain `culture (day 2)`
7. Dilute each of 16 `culture (day 1)` samples with LB Broth + Chloramphenicol (34 ug/mL) into the culture tube at a 1:10 ratio and final volume of 5.0mL. Maintain at 4.0°C while performing dilutions. (This can be also performed on ice).
8. Obtain 16 x 1.5 mL microfuge tubes to contain `cultures (0 hr timepoint)`
9. Hold `cultures (0 hr timepoint)` on ice. This will prevent cell growth while transferring samples.

10. Transfer 1.0mL of each of 16 `culture (day 2)` samples to 1.5 mL microfuge tube containers to contain a total of 16 `cultures (0 hr timepoint)` samples. Maintain at 4.0°C during transfer. (This can be also performed on Ice).
11. Measure baseline absorbance of culture (day 2) of `cultures (0 hr timepoint)` at 600.0nm.
12. Obtain 16 x 50 ml conical tubes to contain `back-diluted culture` The conical tube should be opaque, amber-colored, or covered with foil.
13. Back-dilute each of 16 `culture (day 2)` samples to a target OD of 0.02 using LB Broth + Chloramphenicol (34 ug/mL) as diluent to a final volume of 40.0mL. Maintain at 4.0°C while performing dilutions.

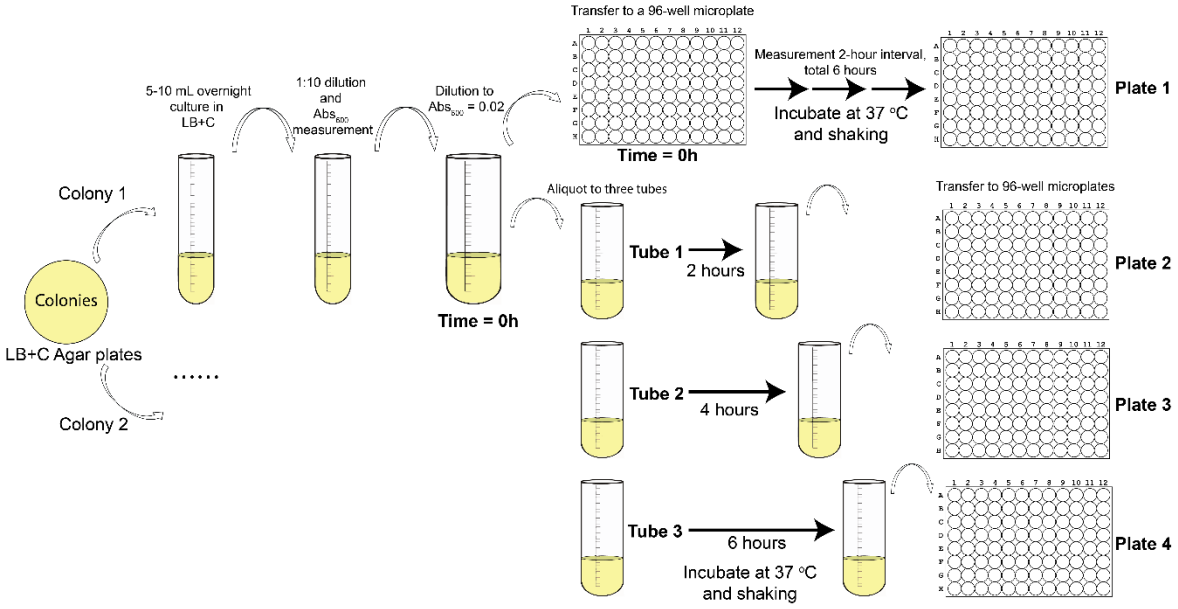

Fig 1: Visual representation of protocol

14. Obtain 16 x 3 x 50 ml conical tubes to contain `Tubes 1, 2 and 3.` The conical tubes should be opaque, amber-colored, or covered with foil. This make a total of 48 tubes.
15. Transfer 12.0mL of each of 16 `back-diluted culture` samples to 50 ml conical tube containers to contain a total of 16 `Tubes 1, 2 and 3` samples. Maintain at 4.0°C during transfer.
16. Obtain a 96 well microplate to contain `plate 1`
17. Hold `plate 1` on ice.
18. Transfer 200.0uL of each `back-diluted culture` sample to 96 well microplate `plate 1` in the wells indicated in the plate layout. Maintain at 4.0°C during transfer.
19. Transfer 200.0uL of `LB Broth + Chloramphenicol (34 ug/mL)` sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate `plate 1`. Maintain at 4.0°C during transfer. These samples are blanks.

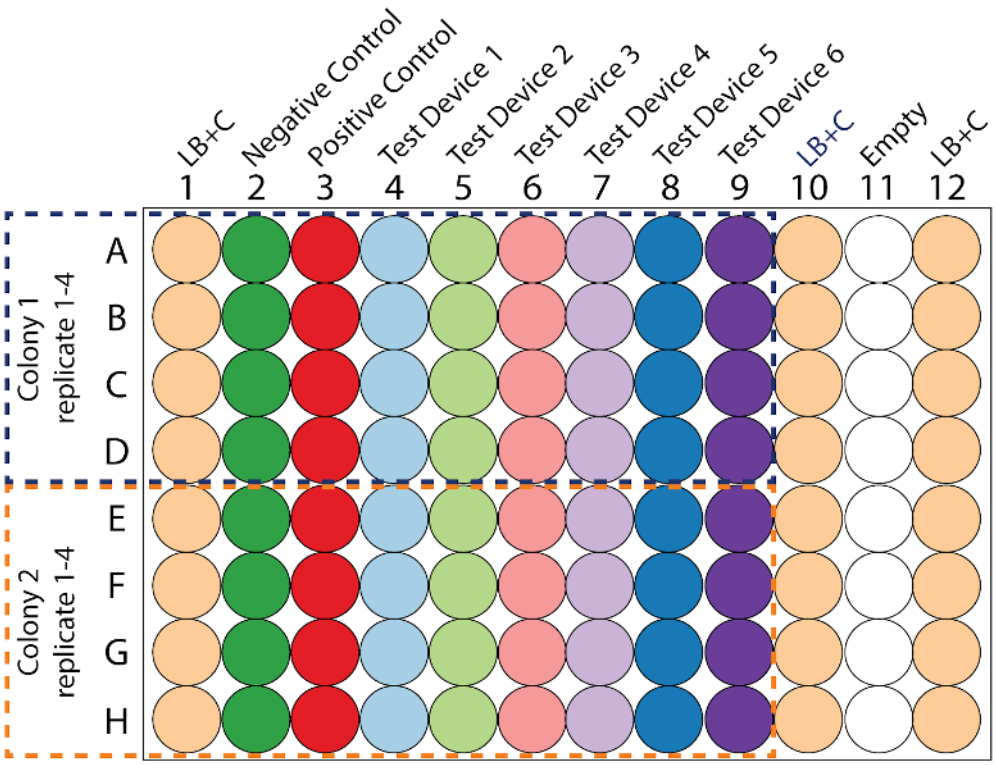

Fig 2: Plate layout

24. Measure 0 hr absorbance timepoint of `plate 1` at 600.0nm.
25. Measure 0 hr fluorescence timepoint of `plate 1` with excitation wavelength of 488.0nm and emission filter of 530.0nm and 30.0nm bandpass.
26. Cover `plate 1` samples in 96 well microplate with your choice of material to prevent evaporation.
27. Incubate all `plate 1` samples for 6.0 hour at 37.0°C at 220 rpm.
28. Measure absorbance timepoint of `plate 1` at 600.0nm at timepoints 2.0 hour, 4.0 hour, 6.0 hour.
29. Measure fluorescence timepoint of `plate 1` with excitation wavelength of 488.0nm and emission filter of 530.0nm and 30.0nm bandpass at timepoints 2.0 hour, 4.0 hour, 6.0 hour.
30. Incubate all `Tube 1` samples for 2.0 hour at 37.0°C at 220 rpm.
31. Hold all `Tube 1` samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
32. Incubate all `Tube 2` samples for 4.0 hour at 37.0°C at 220 rpm.
33. Hold all `Tube 2` samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
34. Incubate all `Tube 3` samples for 6.0 hour at 37.0°C at 220 rpm.
35. Hold all `Tube 3` samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
36. Obtain three (x3) 96 well microplates to contain `Plates 2, 3, and 4`

37. Transfer 200.0uL of each Tubes 1, 2 and 3 sample to 96 well microplate Plates 2, 3, and 4 in the wells indicated in the plate layout. Maintain at 4.0°C during transfer.
38. Transfer 200.0uL of LB Broth + Chloramphenicol (34 ug/mL) sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate Plates 2, 3, and 4. Maintain at 4.0°C during transfer. These samples are blanks.
39. Measure absorbance timepoint of Plates 2, 3, and 4 at 600.0nm.
40. Measure fluorescence timepoint of Plates 2, 3, and 4 with excitation wavelength of 488.0nm and emission filter of 530.0nm and 30.0nm bandpass.
41. Import data for baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint), 0 hr absorbance timepoint measurements of plate 1, 0 hr fluorescence timepoint measurements of plate 1, absorbance timepoint measurements of plate 1 at timepoints 2.0 hour, 4.0 hour, 6.0 hour, fluorescence timepoint measurements of plate 1 at timepoints 2.0 hour, 4.0 hour, 6.0 hour, absorbance timepoint measurements of Plates 2, 3, and 4, fluorescence timepoint measurements of Plates 2, 3, and 4 into provided Excel file.

Protocol version: 1.2b