

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](https://identifiers.org/taxonomy:668369)
* [Negative control](http://parts.igem.org/Part:BBa_J428100)
* [Positive control (I20270)](http://parts.igem.org/Part:BBa_I20270)
* [Test Device 1 (J364000)](http://parts.igem.org/Part:BBa_J364000)
* [Test Device 2 (J364001)](http://parts.igem.org/Part:BBa_J364001)
* [Test Device 3 (J364002)](http://parts.igem.org/Part:BBa_J364002)
* [Test Device 4 (J364007)](http://parts.igem.org/Part:BBa_J364007)
* [Test Device 5 (J364008)](http://parts.igem.org/Part:BBa_J364008)
* [Test Device 6 (J364009)](http://parts.igem.org/Part:BBa_J364009)
* LB Broth + Chloramphenicol (34 ug/mL)
* LB Agar + Chloramphenicol (34 ug/mL)
* [Chloramphenicol stock solution (34 mg/mL)](https://pubchem.ncbi.nlm.nih.gov/compound/5959)
* 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

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| |  |  |  | | --- | --- | --- | | 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**

1. Obtain 16 x culture tubes to contain culture (day 1)
2. Pick 2 colonies from each transformant strains plate.
3. 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**

1. Obtain 16 x culture tubes to contain culture (day 2)
2. 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).
3. Obtain 16 x 1.5 mL microfuge tubes to contain cultures (0 hr timepoint)
4. Hold cultures (0 hr timepoint) on ice. This will prevent cell growth while transferring samples.
5. 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).
6. Measure baseline absorbance of culture (day 2) of cultures (0 hr timepoint) at 600.0nm.
7. Obtain 16 x 50 ml conical tubes to contain back-diluted culture The conical tube should be opaque, amber-colored, or covered with foil.
8. 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.

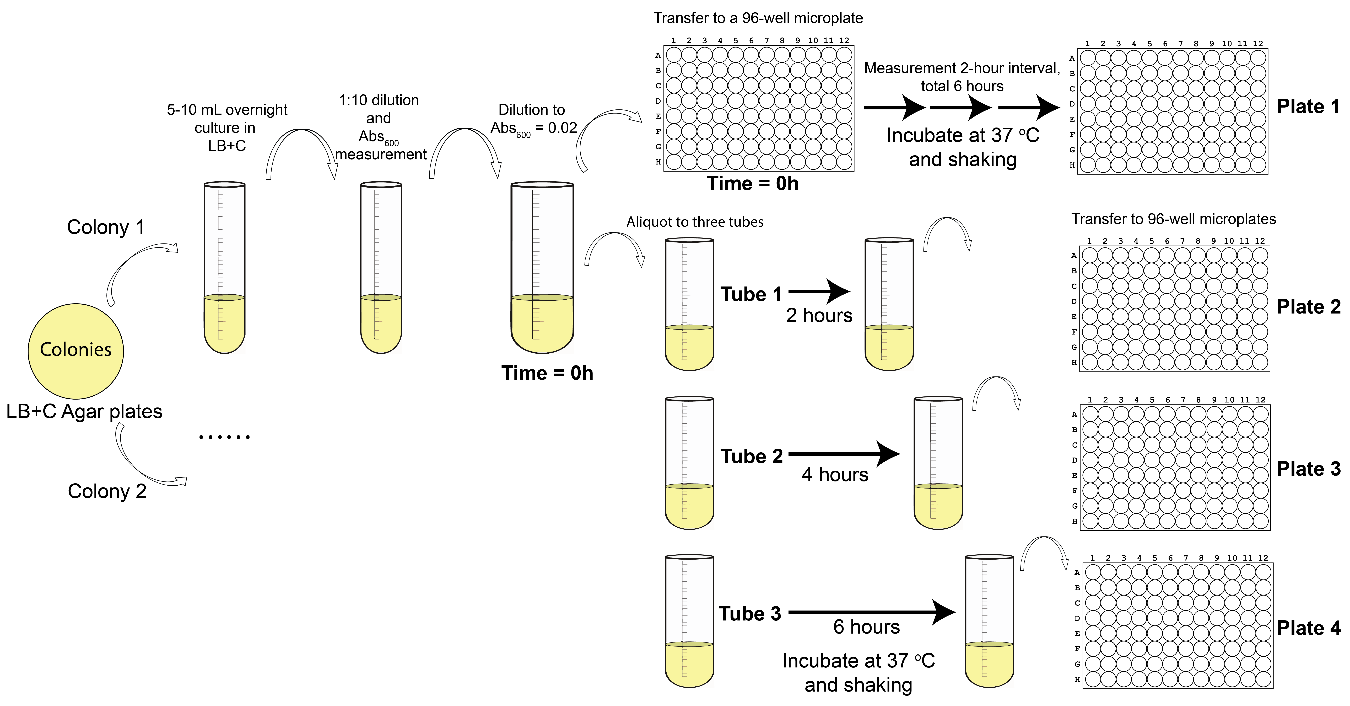


Fig 1: Visual representation of protocol

1. 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.
2. 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.
3. Obtain a 96 well microplate to contain plate 1
4. Hold plate 1 on ice.
5. 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.
6. 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.

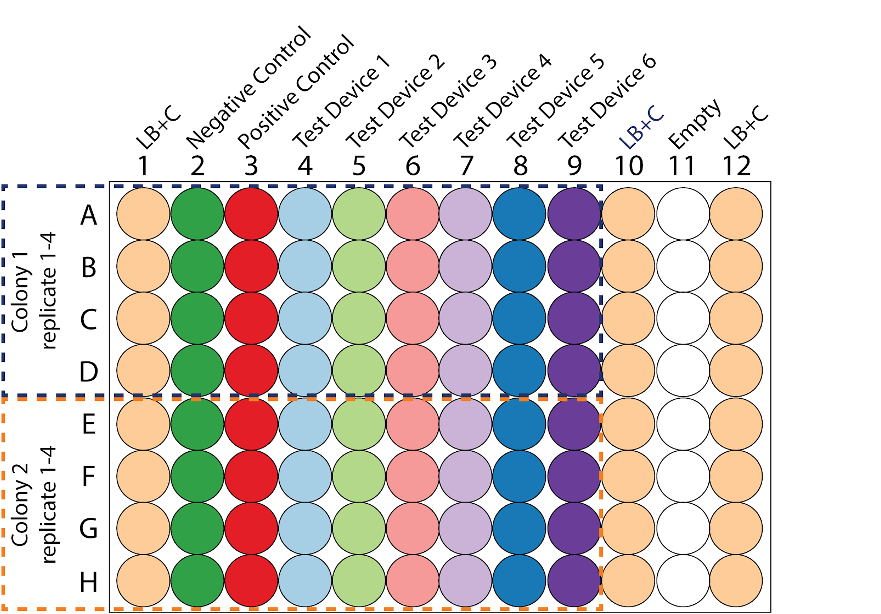


Fig 2: Plate layout

1. Measure 0 hr absorbance timepoint of plate 1 at 600.0nm.
2. Measure 0 hr fluorescence timepoint of plate 1 with excitation wavelength of 488.0nm and emission filter of 530.0nm and 30.0nm bandpass.
3. Cover plate 1 samples in 96 well microplate with your choice of material to prevent evaporation.
4. Incubate all plate 1 samples for 6.0 hour at 37.0°C at 220 rpm.
5. Measure absorbance timepoint of plate 1 at 600.0nm at timepoints 2.0 hour, 4.0 hour, 6.0 hour.
6. 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.
7. Incubate all Tube 1 samples for 2.0 hour at 37.0°C at 220 rpm.
8. Hold all Tube 1 samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
9. Incubate all Tube 2 samples for 4.0 hour at 37.0°C at 220 rpm.
10. Hold all Tube 2 samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
11. Incubate all Tube 3 samples for 6.0 hour at 37.0°C at 220 rpm.
12. Hold all Tube 3 samples on ice. Reserve until the end of the experiment for absorbance and fluorescence measurements.
13. Obtain three (x3) 96 well microplates to contain Plates 2, 3, and 4
14. 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.
15. 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.
16. Measure absorbance timepoint of Plates 2, 3, and 4 at 600.0nm.
17. 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.
18. 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