Experiment 1 - Testing Efficacy of pfur# promoter constructs

Created by Heaven, Nathan, Alex, and Wangui

Protocol examples:

- **http://2013.igem.org/Team:Evry/Sensor**
- http://2018.igem.org/Team:ECUST/Fur_Inverter
- *iGEM plate reader calibration protocol for red fluorescent protein:*

Experiment 1 Outline:

- Grow E.coli in growth media containing multiple iron concentrations
- Observe to see which construct yields the lowest fluorescent output
- IF SUCCESSFUL:
    - Repete with Cyanobacteria instead of E. coli

Experiment 1 Steps:

(Adapting from *iGEM's protocol for testing fluorescence of transformed bacteria*, page 9-12) - This experiment was part of an InterLab study to compare fluorescence measurements of several constructs (all expressing GFP) across lab environments. They do this study taking two measurements (one at 0 hrs, another at 6 hrs). We'll copy that, but instead focus on taking at least 2 measurements (one with iron, and one without iron).

Disclaimer: Prior to performing the cell measurements you should perform all three of the calibration measurements (link). Please do not proceed unless you have completed the three calibration protocols. 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 all of these cell measurements, you must use the same plates and 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 plates, volumes, and settings, the measurements will not be valid.

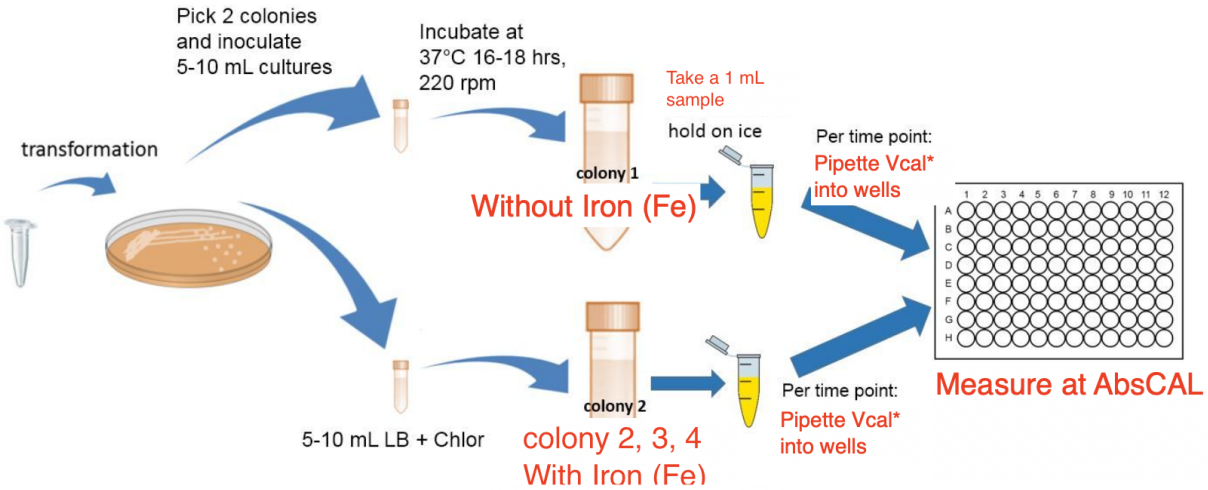
Record Calibration Settings:
Volume in Well (Vcal): _100 uL_ _ (this calibration uses 200 uL)
Wavelength used in calibration (AbsCAL): _(We're still in process of calibrating)_
Temperature: 23.9C
Fluorescence settings: _ Excitation: 550 nm, Emission: 595 nm

Materials:

- LB (Luria Bertani) media
- Antibiotic (To match transformations, stock concentration 25 mg/mL dissolved in EtOH)
- 50 ml Falcon tube (or equivalent, preferably amber or covered in foil to block light)
- Incubator at 37°C
- 1.5 ml Eppendorf tubes for sample storage
- Ice bucket with ice
- Micropipettes and tips
- 96 well plate, black with clear flat bottom preferred (provided by team)
- Transformed bacteria (Synthesized by our team):

Device Function	BBa_#	Short description
Negative Control (Shouldn't be any fluorescence here)	BBa_R0040 (or any part from kit without a fluorescent protein)	TetR repressible promoter
Positive Control	BBa_K3651036	J23100-mcherry
Test Construct 1	BBa_K3651026	pfur1-mCherry
Test Construct 2	BBa_K3651028	pfur2-mCherry
Test Construct 3	BBa_K3651037	pfur13-mCherry
Test Construct 4	BBa_K3651023	pfur23-mCherry
Test Construct 5	Bba_K3651029	pfur12-mCherry
Test Construct 6	Bba_K3651031	pfur123-mCherry
Test Construct 7	BBa_K3651038	pfur3-mCherry

Workflow



Method

Day 1 & 2: Transform and Pick colonies

Should have bacteria transformed with our constructs that have been sequence verified. We need to pick colonies, do this the night before, so we can start with liquid cultures for the next day.

Pick 4 colonies from each of the transformation plates and inoculate in 5-10 mL LB medium + antibiotic. For 3 cultures of each transformation add iron to the following final concentrations:

- low iron (0.1 µM)
- medium iron (1 µM)
- high iron (10 µM) (similar to ECUST 2018)

Grow the cells overnight (16-18 hours) at 37°C and 220 rpm.

Day 3: Cell growth, sampling, and assay

- [ ] Take 1 mL samples of the cultures Place the samples on ice.

- [ ] Measure your samples (Abs_{CAL} and fluorescence measurement), see the below for details.

- [ ] Record data in your notebook

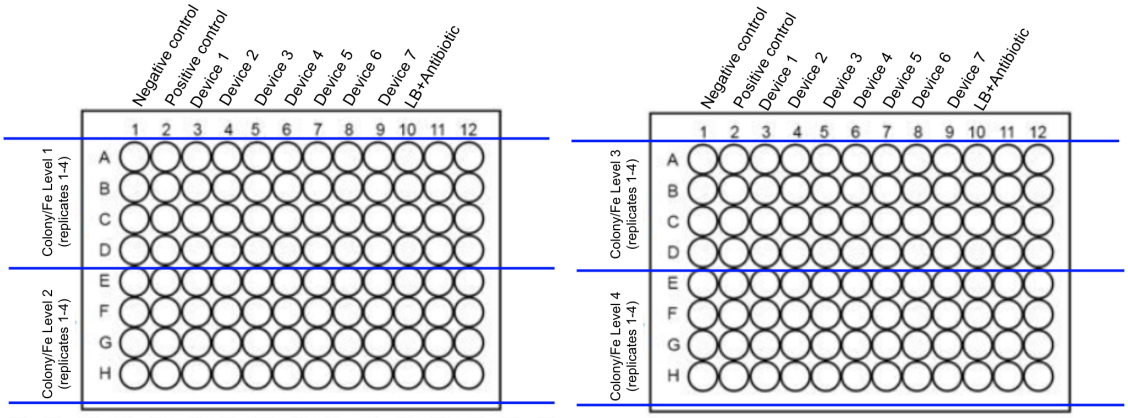
Import data into Excel sheet provided (**fluorescence measurement tab**)

Measurement

Samples should be laid out according to the plate diagram below. Pipette Vcal µl of each sample into each well. From 1 mL samples in a 1.5 ml Eppendorf tube, pipette 4 replicates for each of the 4 cultures into the wells, as illustrated below. Set the instrument settings as those that gave the best results in your calibration curves (no measurements off scale). If necessary, you can test more than one of the previously calibrated settings to get the best data (no measurements off scale). Instrument temperature should be set to room temperature (approximately 20-25 C) if your instrument has variable temperature settings.

Layout for AbsCAL and Fluorescence Measurement - From original protocol.

At the end of the experiment, you should have two plates to read. Each plate should be set up as shown below.



Help Debugging: - From original protocol (no changes made)

- If you have measurements that are off scale ("OVERFLOW"), that data will not be usable. You need to adjust your settings so that the data will be in range and re-run your calibration.
- If your Abs600 measurements for your cell colonies are very close to that of your LB+antibiotic, then your cells have probably not been transformed correctly or grown correctly.
- If your negative and positive control values are very close to each other, that probably means something has gone wrong in your protocol or measurement.