## MATERIALS AND METHODS

## PLATE READER SETUP

Plate Reader: Perkin Elmer- Multimode platereader Enspire

##### **Abs OD600**

* Wavelengths: 600nm
* Read Speed: Normal

**Fluorescence**

* Excitation: 485nm
* Emission: 530nm
* Optics: Top
* Temperature :29.8 degree Celsius
* No. of flashes per well: 10
* Orbital averaging: 7.5

**OD600 REFERENCE POINT**

##### **Materials**

* 1 mL LUDOX
* dH2O
* 96 well plate

##### **Methods**

1. 100 uL of LUDOX-S40 from the InterLab Measurement Kit added into wells A1, B1, C1, and D1
2. 100 uL of dH2O added to wells A2, B2, C2, D2
3. Absorbance at 600 nm taken for all samples in setup described above
4. Data used to obtain correction factor

## PROTOCOL FOR FLUORESCEIN FLUORESCENCE STANDARD CURVE

##### **Materials**

* Fluorescein
* 10 mL 1xPBS (phosphate buffered saline)
* 96 well plate

##### **Methods**

1. Fluorescein stock tube from InterLab Measurement Kit spun down to make sure pellet is at bottom of tube
2. 2x fluorescein stock solution (100 uM) prepared by resuspending fluorescein in 1 mL of 1xPBS
3. 500 uL of 2x fluorescein stock solution diluted with 500 uL of 1xPBS to make 1mL of 50 uM (1x) fluorescein solution
4. Serial dilutions of fluorescein:

	1. 100 uL of PBS added into wells A2, B2, C2, D2... A12, B12
	2. 200 uL of fluorescein 1x stock solution added to A1, B1, C1, D1
	3. 100 uL fluorescein stock solution transferred from A1 into A2
	4. A2 mixed by pipetting up and down, then 100 uL transferred to A3
	5. A3 mixed by pipetting up and down, then 100 uL transferred to A4
	6. A4 mixed by pipetting up and down, then 100 uL transferred to A5
	7. A5 mixed by pipetting up and down, then 100 uL transferred to A6
	8. A6 mixed by pipetting up and down, then 100 uL transferred to A7
	9. A8 mixed by pipetting up and down, then 100 uL transferred to A9
	10. A9 mixed by pipetting up and down, then 100 uL transferred to A10
	11. A10 mixed by pipetting up and down, then 100 uL transferred to A11
	12. A11 mixed by pipetting up and down, then 100 uL transferred to liquid waste
5. Step 4 repeated for rows B-D
6. Fluorescence for all samples measured using setup described above

**CELL MEASUREMENT PROTOCOL**

##### **Materials**

* Competent Cells (*Escherichia coli* strain DH5 alpha)
* LB media
* Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH, working stock 25 ug/mL)
* 50 mL Falcon tubes covered in foil
* Incubator at 37ºC
* Devices from InterLab Measurement Kit
	+ Positive Control
	+ Negative Control
	+ Test Device 1: J23101 + I13504
	+ Test Device 2: J23106 + I13504
	+ Test Device 3: J23117 + I13504
	+ Test Device 4: J23101.BCD2.E0040.B0015
	+ Test Device 5: J23106.BCD2.E0040.B0015
	+ Test Device 6: J23117.BCD2.E0040.B0015

##### **Methods**

1. Each of the plasmids listed above were located on Plate 7 of the distribution kit and resuspended in 10 uL of dH2O
2. Each device was transformed into 10 uL of competent cells (see here for the full transformation protocol)
3. Transformations were spot plated onto an agar plate with chloramphenicol and grown overnight(see here for the full spot plating protocol)
4. On Day 2, 2 colonies were picked from each plate and inoculated in 10 mL of LB medium + Chloramphenicol in 50 mL Falcon tubes covered with foil. These cultures were grown overnight in an incubator at 37ºC and 220 rpm.
5. On Day 3, 100 uL of each culture was pipetted into a 96 well plate for an OD600 reading, which was then used for the Dilution Calculations in the Excel sheet provided by iGEM
6. Each culture was diluted to a target OD of 0.02 following the preloading culture and media volumes calculated by the Dilution excel sheet (see OD Readings and Dilutions Calculations in the Results section below) in 12 mL of LB media + chloramphenicol in 50 mL falcon tubes wrapped in foil
7. Cultures were placed in incubator at 37ºC and 220 rpm
8. 4 replicates of 100 uL samples were taken from each culture at 0, 2, 4, and 6 hours of incubation and placed in a 96 well plate for OD and fluorescence measurements using the setup described above