Interlab calibration protocols

Project: Interlab Study iGEM 2017
Authors: Allison Harpel
Dates: 2017-06-29 to 2017-06-30

THURSDAY, 6/29/17

We will be doing the calibration protocols for inter-lab right now. We are in the M215 lab where the plate readers are kept. The first portion of the calibration protocols was the calibration of the absorption plate reader with LUDOX and H_2O.
Absorption plate reader: BioTek model EL808, SN 214093
Fluorescence plate reader: Spetramax model Gemini X5 software by Molecular devices
To pipette the materials to the plate, sterile tips were retrieved from the Moench lab. 100µl of the LUDOX solution provided in the 2017 inter-lab kit was pipetted into cells A1, B1, C1, and D1. 100µl of sterile ultrapure H_2O was pipetted into cells A2, B2, C2, and D2.

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	LUDOX	H2O										
B	LUDOX	H2O										
C	LUDOX	H2O										
D	LUDOX	H2O										
E												
F												
G												
H												

Figure 1: Layout of well plate with LUDOX and H_2O

Measurements for absorbance were taken on the absorbance plate reader at 630nm - the closest filter we had to 600nm. Data was collected using GEN5 software. The data was exported to an Excel sheet named "igem 2017 interlab part 1"
Our data was as follows:

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.037	0.031										
B	0.038	0.031										
C	0.039	0.030										
D	0.039	0.030										
E												
F												
G												
H												

Figure 2: Results from measuring OD at 630nm of H_2O as compared to LUDOX at 630 nm. H_2O seems to have a lower absorbance. Results in au (absorbance units)

The data was then placed in the Excel sheet provided by iGEM HQ.

Optical Density (OD600) Standard Measurement

Introduction

You will use LUDOX-S40 as a single point reference to obtain a ratiometric conversion factor to transform your absorbance data into a standard OD600 measurement. YOU MUST THEREFORE TURN OFF PATHLENGTH CORRECTION. To measure your standard LUDOX Abs600 you must use the same plates and volumes (suggestion: use 100 µL for plate reader measurement) that you will use in your cell based assays.

Prepare a column of 4 wells with 100 µL 100% LUDOX and 4 wells containing 100 µL dH2O, as outlined in the protocol document. Repeat the measurement in all relevant modes used in your experiments (e.g. settings for orbital averaging).

PDF protocol: http://2017.igem.org/wiki/images/8/85/InterLab_2017_Plate_Reader_Protocol.pdf

Materials

- 1ml LUDOX
- H_2O
- 96 well plate, black with a flat bottom preferred.

Procedure

1. Add 100µl LUDOX into wells A1, B1, C1, D1 (or 1 mL LUDOX into cuvette).
2. Add 100µl of H_2O into wells A2, B2, C2, D2 (or 1 mL H_2O into cuvette).
3. Measure absorbance 600nm of all samples in all standard measurement modes in instrument.
4. Record the data in your notebook.
5. Import data into Excel (OD600 reference point tab) Sheet_1 provided.

Interlab calibration protocols

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FRIDAY, 6/30/17

To start this portion we made PBS (Phosphate Buffered Saline). To do this a Sigma PBS packet (Lot 118K8208) was added to 1L of water. The beaker was then repeatedly inverted to mix the two. (This was all done in the Moench lab).
To create the fluorescein stock solution we then spun down the fluorescein that came in the inter-lab kit. (This was done by placing it in the mini centrifuge for 30s). Then 1ml of PBS (1x) was pipetted into the fluorescein tube to rehydrate it. During the initial attempt to do this, the fluorescein was spilled and as a result, a new container had to be spun down and the process restarted. The 1ml of PBS was pipetted successfully into the fluorescein tube the second time, and then the mix was pipetted up and down several times until the mix was clear green.
2ml of 1x solution was made by pipetting 1ml of PBS into a falcon tube along with 1ml of flourescein. After combination this was gently mixed by swirling.
We then moved to the Meyer 215ab.
The materials were transported to the M215 lab to for fluorescence measurements on the plate reader. The 96 well plate was dried from the previous day. 100µl of PBS was added to cells A2 through D12 as indicated in Figure 3 (Well 3). 200µl of fluorescein 1x was added to wells A1, B1, C1, and D1, as is also indicated in Figure 3.

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Fluor	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
B	Fluor	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
C	Fluor	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
D	Fluor	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
E												
F												
G												
H												

Figure 3: Layout of 96-well plate with fluorescein and PBS. The places where 100µl of PBS were placed in a well are marked with "PBS". Whereas 200µl of fluorescein was added is indicated by "Fluor".

Well D6 may have slighly less PBS due to a pipetting error. Cells C1 and C2 were redone during dilutions due to an error in pipetting.
100µl of fluorescein was transfered from A1 to A2 and mixed by pipetting up and down 3 times. This process was repeated on cells A2 and A3, A3 and A4, A4 and A5, A5 and A6, A6 and A7, A7 and A8, A8 and A9, A9 and A10, A10 and A11, and then 100µl of PBS and fluorescein was taken out of A11 and discareded. This serial dilution process was repeated for rows B, C, and D.
The samples in the 96 well plate were placed in the fluorescence plate reader. The excitation wavelength was set to 501nm in Softmax Pro 4.6. The emission wavelength was set to 511 nm.
The tests were started with the software set to low sensitivity and the results are shown in Figure 4.

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	14489…	97374…	60982…	34723…	24337…	13462…	3439.…	7423.…	12917…	7599.…	4370.…	3053.…
B	11827…	93067…	58801…	32420…	19356…	12677…	6622.…	11964…	5179.…	3499.…	9349.…	3181.…
C	12781…	97919…	61426…	30546…	18127…	10923…	6465.…	48055…	6672.…	5123.…	5678.…	4107.…
D	74523…	41091…	55547…	35993…	18758…	22269…	11446…	31880…	6201.…	5358.…	2842.…	4577.…
E												
F												
G												
H												

Figure 4: Results from plate reader for fluorescence with low sensitivity. Shows a marked decrease in the fluorescence as the samples become more dilute.

Well5

	1	2	3	4	5	6	7	8	9	10	11	12
A	SAT	SAT	SAT	SAT	SAT	11751…	4409.…	8073.…	7814.…	7945.…	5077.…	3706.…
B	SAT	SAT	SAT	SAT	SAT	12069…	7361.…	9834.…	4997.…	4288.…	9903.…	3733.…
C	SAT	SAT	SAT	SAT	15794…	11503…	7131.…	SAT	5595.…	5752.…	6555.…	4620.…
D	SAT	SAT	SAT	SAT	SAT	SAT	11391…	719.6…	5656.…	6193.…	3399.…	4283.…
E												
F												
G												
H												

Figure 5: Results from plate reader testing fluorescence with medium sensitivity. SAT means that the plate was at saturation at that point.

Well6

	1	2	3	4	5	6	7	8	9	10	11	12
A	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT
B	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT
C	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT
D	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT	SAT
E												
F												
G												
H												

Figure 6: Results from plate reader testing fluorescence with high sensitivity. All wells were seen as fully saturated by the plate reader.

From these results we were able to determine that all future tests should be run at Low sensitivity to avoid our samples passing the saturation threshold.

Making -80 cultures for Transformations (6/30)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-06-30 to 2017-07-01

FRIDAY, 6/30/17

This was done to preserve the transformed cells made during **Transformation (Backbone, Inter-lab, and Reizman)**. This was done using *Cultures containing Glycerol* from Molecular Cloning, A Laboratory Manual, 3rd Edition by Sambrook and Russell.
We will be storing: BBa_I20270 from the 6/29 liquid culture, BBa_R0040 from 6/29 liquid culture, BBa_J364000 from 6/29 liquid culture, BBa_J364001 from the 6/29 liquid culture, BBa_J364002 from the 6/29 liquid culture, BBa_J364003 from the 6/29 liquid culture, and BBa_J364005 from 6/29 culture. We did not use our BBa_J23111 liquid culture because the culture did not appear to have any bacteria growing in it.
To start 7 cryovials had 250µl of glycerol was placed in them. We then added 750µl of the appropriate cells to each vial. These vials were then mixed and flash frozen using liquid nitrogen. They were then placed in the -80°C freezer.

Storage of Bacterial Cultures in Liquid media

Introduction

Originally from Molecular Cloning: A Laboratory Manual third edition by Sambrook and Russell.

Materials

›

Procedure

1. To 1.5 ml of bacterial culture, add .5ml of steril 60% glycerol (sterilized by autoclaving for 20 minutes at 15psi [1.05kg/cm^2] on liquid cycle).
2. Vortex the culture to ensure that the glycerol is evenly dispersed.
3. Transfer the culture to a labeled storage tube equipped with a screw cap and an air-tight gasket.
4. Freeze the culture in ethanol-dry ice or in liquid nitrogen, and then transfer the tube to -70°C for long-term storage.
5. To recover the bacteria, scrape the frozen surface of the culture with a sterile inoculating loop, and then immediately streak the bacteria that adhere to the needle onto the surface of an LB agar plate containing the appropriate antibiotic. Return culture to storage at -70°C. Incubate the plate overnight at 37°C.

Making -80 cultures for Transformations (6/30)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-06-30 to 2017-07-01

SATURDAY, 7/1/17

The cultures were checked for growth. The BBa_J364004 was shown to have growth, but the BBa_J23111 (in BBa_J61002) did not. Faulty AMP selection plates were suspected so more, new AMP plates were made for a retransformation on the next Wednesday.
The cultures had about 27 hours to incubate. The 250µl of glycerol (60%, sterile) was placed in the cryovial with the 750µl of the J364004 culture. This was flash frozen in liquid nitrogen and then it was placed in the -80°C freezer.

Inter-lab part 3 (1st try)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-07-05 to 2017-07-06

WEDNESDAY, 7/5/17

A toothpick being a colony was used to transfer this colony to a prefaced 5 µl LB chloramphenicol Falcon tubes.
This process was repeated for 2 colonies on each of the following Plates:
Plate #: Date:
BBa_J364002 6-28-17
BBa_J364003 6-28-17
BBa_I20270 6-28-17
BBa_J364005 6-28-17
BBa_J364001 6-28-17
BBa_J364004 6-28-17
BBa_J364000 6-28-17
BBa_R0040 6-28-17
The cultures were placed in the shaker at around 4 pm (4:25) at 220 rpm and 37 °C.
They were set to incubate for 16-18 hours.

Inter-lab part 3 (1st try)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-07-05 to 2017-07-06

THURSDAY, 7/6/17

Tubes were in the incubators for 17-18 hours.
Initially, 100 µl of each sample was pipetted into the 96-well plate as an initial OD630 reading was to be taken. OD630 was used because there was no access to a 600 nm filter.
Here is what the different parts and controls part numbers are:
Part#: Part:
BBa_I20270 + control
BBa_R0040 - control
BBa_J364000 Part 1
BBa_J364001 Part 2
BBa_J364002 Part 3
BBa_J364003 Part 4
BBa_J364004 Part 5
BBa_J364005 Part 6
LB LB and Chloramphenicol

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	+ control	- control	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6	LB			
B	+ control	- control	Part 1	Part 2	Part 3	Part 4	Part 5	Part 6	LB			
C												
D												
E												
F												
G												
H												

Well Plate 1: Layout of the well plate.

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.287	0.321	0.283	0.395	0.42	0.295	0.403	0.351	0.041			
B	0.424	0.363	0.448	0.375	0.58	0.338	0.26	0.267	0.044			
C												
D												
E												
F												
G												
H												

Well Plate 2: Absorbance Levels of the overnight samples. Samples were measured at OD630. All numbers are in Au (absorbance units).

All absorbance measurements were taken at 630 nm. The data was inserted into the excel document provided, named “2017_Interlab_Dilution_Calculation_Sheet”
To ensure that the OD630 did not change too much, the samples were put on ice. To prepare the big cultures to incubate, 9M of LB+Chloramphenicol were pipetted into each of 19 foil-wrapped tubes with a serological pipette. This was done in a laminar flow hood. Then the remainder from the following table-based off of the excel document named above was added.
This was done using the Excel sheet 2017_Interlab_Dilution_Calculation_Sheet, which was supplied by Inter-lab HQ
Our target absorbance at Abs630 is .02 and the target volume was 10ml.

Table1

	Sample	Abs630 Reading	Preloading Culture (mL)	Volume Preloading Media (mL)
1	positive control	0.287	0.81300813	9.18699187
2	negative control	0.321	0.714285714	9.285714286
3	device 1	0.283	0.826446281	9.173553719
4	device 2	0.395	0.564971751	9.435028249
5	device 3	0.42	0.527704485	9.472295515
6	device 4	0.295	0.787401575	9.212598425
7	device 5	0.403	0.552486188	9.447513812
8	device 6	0.351	0.64516129	9.35483871
9	media+chl	0.041		

Table 1: How much culture, of Sample 1, to place the appropriate amount of media based on our Absorbance measurements from immediately after our cultures were allowed to incubate overnight (16-18 hours).

Table2

	Sample	Abs630 Reading	Preloading Culture	Volume Preloading Media
1	positive control	0.424	0.526315789	9.473684211
2	negative control	0.363	0.626959248	9.373040752
3	device 1	0.448	0.495049505	9.504950495
4	device 2	0.375	0.604229607	9.395770393
5	device 3	0.58	0.373134328	9.626865672
6	device 4	0.338	0.680272109	9.319727891
7	device 5	0.26	0.925925926	9.074074074
8	device 6	0.267	0.896860987	9.103139013
9	media+chl	0.044		

Table 2: How much culture, of Sample 2, to place in the appropriate amount of media based on our Absorbance measurements from immediately after our cultures were allowed to incubate overnight (16-18 hours).

Then, the appropriate amount of culture was added to each tube via micropipette.
500ul were removed from each sample and placed in a 1.5 ml Eppendorf microcentrifuge tube. These tubes were placed on ice to be transported to the M215 lab for analysis at 12:00 on the dot.
The falcon tubes (foil-wrapped) were then placed into the shaker at 220 rpm and at 37.4 °C.
In M215 t=0 culture analysis began.
First 100 µl of culture were placed into the wells of a 96-well plate as shown below in well 3, (1) means the sample came from tube 1 of that part, and (2) means the sample came from tube 2 of that part.

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	- control (1)	+ control (1)	Part 1 (1)	Part 2 (1)	Part 3 (1)	Part 4 (1)	Part 5 (1)	Part 6 (1)	LB			
B	- control (1)	+ control (1)	Part 1 (1)	Part 2 (1)	Part 3 (1)	Part 4 (1)	Part 5 (1)	Part 6 (1)	LB			
C	- control (1)	+ control (1)	Part 1 (1)	Part 2 (1)	Part 3 (1)	Part 4 (1)	Part 5 (1)	Part 6 (1)	LB			
D	- control (1)	+ control (1)	Part 1 (1)	Part 2 (1)	Part 3 (1)	Part 4 (1)	Part 5 (1)	Part 6 (1)	LB			
E	- control (2)	+ control (2)	Part 1 (2)	Part 2 (2)	Part 3 (2)	Part 4 (2)	Part 5 (2)	Part 6 (2)	LB			
F	- control (2)	+ control (2)	Part 1 (2)	Part 2 (2)	Part 3 (2)	Part 4 (2)	Part 5 (2)	Part 6 (2)	LB			
G	- control (2)	+ control (2)	Part 1 (2)	Part 2 (2)	Part 3 (2)	Part 4 (2)	Part 5 (2)	Part 6 (2)	LB			
H	- control (2)	+ control (2)	Part 1 (2)	Part 2 (2)	Part 3 (2)	Part 4 (2)	Part 5 (2)	Part 6 (2)	LB			

Well Plate 3: Layout of samples in the well plate that was used for tests run on two-hour increments. (1) means the sample comes from tube 1 and (2) means the sample comes from tube 2. LB has wells with LB and Chloramphenicol.

After transfer to the 96-well plate, the OD639 measurements were taken. The measurement was taken at 630nm. The temperature in the absorbance reader was 29°C.

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.074	0.099	0.07	0.057	0.057	0.07	0.062	0.074	0.042			
B	0.079	0.083	0.066	0.058	0.059	0.067	0.071	0.076	0.044			
C	0.08	0.076	0.077	0.058	0.055	0.07	0.071	0.074	0.04			
D	0.077	0.101	0.069	0.056	0.055	0.073	0.068	0.076	0.041			
E	0.062	0.063	0.06	0.065	0.061	0.047	0.061	0.072	0.04			
F	0.062	0.062	0.053	0.087	0.062	0.674	0.062	0.073	0.041			
G	0.059	0.063	0.058	0.09	0.062	0.048	0.06	0.072	0.04			
H	0.055	0.065	0.058	0.086	0.06	0.056	0.055	0.072	0.039			

Well Plate 4: Absorbance measurements were taken at 0h. Based on the layout shown in well plate 3. All measurements are in Au.

This data was exported to an excel filed named “Interlab day 3 on samples OD630”
All fluorescence readings were exported to Excel sheets on fluorescence measurements (low sensitivity). Excitation was set to 501nm and emission was set at 511nm. The temperature in the fluorescence plate reader was 24.9°C. We ran this plate at medium and high sensitivity as well to see how the test looked at those levels.

Well5

	1	2	3	4	5	6	7	8	9	10	11	12
A	16539…	57049…	14524…	32820…	33727…	28776…	16197…	235098	23314…			
B	17953…	700435	19002…	20526…	17484…	76334…	55154…	24551…	40625…			
C	15178…	28140…	39281…	86947…	12077…	33254…	21829…	24228…	56090…			
D	10001…	211908	25598…	47048…	13492…	87128…	44744…	29518…	56459…			
E	14154…	36599…	25766…	33580…	91305…	59129…	31500…	67661…	34568…			
F	21186…	37890…	39887…	45094…	65029…	2816.…	37721…	32732…	29202…			
G	12753…	59497…	45263…	52422…	59832…	35515…	53803…	35362…	42769…			
H	39717…	56226…	49445…	43234…	57192…	572165	30069…	18545…	64215…			

Well Plate 5: Fluorescence measurement at low sensitivity of 0h sample.

Well6

	1	2	3	4	5	6	7	8	9	10	11	12
A	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
B	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
C	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
D	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
E	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
F	#Sat	#Sat	#Sat	#Sat	#Sat	3459.…	#Sat	#Sat	#Sat			
G	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
H	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			

Well Plate 6: Fluorescence measurement at medium sensitivity of 0h sample. This test was not used in the final write up but was done to see how things looked at this sensitivity.

Well7

	1	2	3	4	5	6	7	8	9	10	11	12
A	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
B	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
C	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
D	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
E	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
F	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
G	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			
H	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat	#Sat			

Well Plate 7: Fluorescence measurement at high sensitivity 0h sample. This test was not used in the final write up but was done to see how things looked at this sensitivity.

The 96-well plate was then removed and cleaned for later use.
At 2:00 pm we came back to the synthetic Bio lab and took 500 µl from each of our Falcon tubes to run the next round of tests. They had incubated for 2 hours since we last took samples. The samples were then put on ice and taken to M215.
In M215, the samples were pipetted (100 µl) into their respective wells in the plate as instructed by the provided plate chart (a recreation of this is Well Plate 3). The absorbance reader temperature read at 28.5 °C and the fluorescence reader was at 25.1 °C.

Well8

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.197	0.195	0.144	0.114	0.138	0.11	0.152	0.18	0.044			
B	0.203	0.218	0.149	0.109	0.134	0.083	0.145	0.19	0.044			
C	0.196	0.192	0.157	0.1	0.137	0.069	0.145	0.12	0.04			
D	0.194	0.18	0.154	0.108	0.139	0.099	0.125	0.154	0.042			
E	0.155	0.125	0.075	0.18	0.14	0.082	0.136	0.18	0.042			
F	0.153	0.138	0.071	0.178	0.094	0.082	0.147	0.172	0.042			
G	0.151	0.138	0.065	0.179	0.126	0.083	0.142	0.15	0.04			
H	0.149	0.102	0.066	0.16	0.132	0.081	0.131	0.177	0.042			

Well Plate 8: Absorbance measured at 630nm of samples. Samples were placed like well plate 3. Samples were taken at 2h. All units are Au

Well9

	1	2	3	4	5	6	7	8	9	10	11	12
A	19647…	30281…	45230…	20548…	36577…	304243	28144…	18652…	52443…			
B	209897	60348…	34622…	29163…	22611…	79650…	40444…	29163…	49890…			
C	17049…	31312…	295253	16802…	23214…	61153…	38038…	51956…	27982…			
D	14569…	36354…	23927…	37315…	23978…	42308…	62004…	47250…	54169…			
E	20486…	47152…	45910…	39208…	24780…	64060…	28318…	58198…	62459…			
F	32120…	42156…	61214…	45484…	52870…	46117…	46362…	65822…	45876…			
G	23025…	66131…	65704…	46148…	53080…	37705…	46524…	61454…	57557…			
H	328992	51474…	37359…	63889…	57705…	51916…	63741…	66839…	60985…			

Well Plate 9: Fluorescence measured with Excitation was set to 501nm and emission was set at 511nm. Samples were the 2h samples in the placement shown in well plate 3.

At 4:00 pm, in the synthetic biology lab, we pipetted 500 µl of culture into 1.5 ml Eppendorf micro centrifuge tubes. These samples were placed on ice to inoculate the cultures in the falcon tubes were again set to incubate in the shaker at 37.4 °C and 220 rpm.
Please note that at this point it was noticed that some of the foil wrapping were looking a tad rough: showing some small rips.
The micro centrifuge tubes were carried on ice to the M215 lab. Once the 96-well plate was thoroughly dried, 100 µl of each sample was placed into the wells in the same exact manner and pattern as the previous two trials.
When pipetting cultures, the culture destined from well D8 was pipetted into D9. The fluid was transferred to the proper well and D9 was attempted to be cleaned.
The OD630 measurements were taken at 630 nm. Using GEN 5 software, the spectrophotometer was the same used in all other trials.
The spectrophotometer was running at 27.6 °C.

Well10

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.204	0.154	0.168	0.164	0.189	0.148	0.201	0.214	0.041			
B	0.218	0.147	0.164	0.121	0.197	0.142	0.195	0.238	0.042			
C	0.198	0.153	0.173	0.159	0.196	0.142	0.196	0.226	0.039			
D	0.191	0.153	0.162	0.132	0.188	0.145	0.19	0.238	0.04			
E	0.197	0.19	0.078	0.271	0.204	0.108	0.199	0.259	0.04			
F	0.202	0.19	0.082	0.246	0.2	0.103	0.193	0.259	0.04			
G	0.198	0.19	0.074	0.273	0.194	0.109	0.189	0.251	0.04			
H	0.199	0.186	0.068	0.264	0.188	0.101	0.19	0.249	0.04			

Well Plate 10: Absorbance measured at 630nm of samples. Samples were placed like well plate 3. Samples were taken at 4h. All units are Au.

The file was exported to an excel sheet named “Interlab Day 3 4 hour samples OD630”.
After which, the fluorescence was measured in the plate reader. The plate reader was run at 25 °C.
Excitation λ: 501 nm
Emission λ: 511 nm
At low sensitivity

Well11

	1	2	3	4	5	6	7	8	9	10	11	12
A	20296…	36376…	34441…	19820…	21706…	267611	23708…	30816…	65934…			
B	26688…	53390…	45611…	46463…	25290…	49082…	27934…	27544…	419197			
C	18166…	325984	28209…	15824…	23065…	50627…	40629…	39778…	41077…			
D	15817…	23101…	29855…	63819…	45602…	331242	53299…	75036…	30240…			
E	21910…	41746…	40608…	455399	23762…	69549…	34430…	352090	43172…			
F	20183…	35679…	35662…	55554…	56206…	40513…	36301…	24189…	34048…			
G	25482…	553783	55081…	45087…	62056…	44491…	429897	62937…	47207…			
H	36260…	29291…	38921…	61905…	70795…	65762…	47383…	64280…	34602…			

Well Plate 11: Fluorescence measured with Excitation was set to 501nm and emission was set at 511nm. Samples were the 4h samples in the placement shown in well plate 3.

The file was exported to an excel sheet called “Interlab Day 3 4h samples fluorescence”.
Once it was verified that the data were transferred to the Excel sheets, the 96-well plate was cleaned and the aliquots disposed of. At 6:00 pm, the tubes were taken out of the shaker and 500 µl of culture was pipetted into the respective micro centrifuge tubes. The microcentrifuge tube was immediately put on ice and taken to the M215 lab. There, 100 µl of each culture was pipetted into plate wells as indicated by Well Plate 3. However, well H4 appears to have less than 100ul in it. The plate was placed in the plate reader where fluorescence and OD 630 uncertainty was measured.

Well12

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.677	0.272	0.254	0.214	0.232	0.192	0.31	0.273	0.046			
B	0.679	0.289	0.26	0.215	0.27	0.187	0.286	0.258	0.042			
C	0.618	0.288	0.248	0.218	0.248	0.19	0.317	0.285	0.04			
D	0.519	0.282	0.266	0.213	0.245	0.193	0.31	0.162	0.039			
E	0.239	0.231	0.098	0.272	0.294	0.156	0.254	0.349	0.04			
F	0.223	0.231	0.099	0.277	0.271	0.162	0.26	0.353	0.042			
G	0.248	0.229	0.101	0.278	0.287	0.161	0.264	0.358	0.038			
H	0.256	0.262	0.096	0.116	0.248	0.147	0.262	0.355	0.039			

Well Plate 12: Absorbance measured at 630nm of samples. Samples were placed like well plate 3. Samples were taken at 6h. All units are Au.

The OD630 measurement was 630 nm and the temperature was 26 °C since the GEN 5 software and the same plate reader. The file was exported to an Excel sheet and saved to a flash drive under the name “Interlab day 3 6h samples OD 630”.

Well13

	1	2	3	4	5	6	7	8	9	10	11	12
A	188730	33736…	40258…	24477…	39674…	37116…	28119…	30996…	60236…			
B	18111…	64446…	44092…	29056…	20411…	30960…	37442…	51010…	46196…			
C	33929…	34603…	29190…	31828…	29063…	45285…	24509…	58912…	49742…			
D	48152…	26967…	39059…	44277…	33910…	45365…	60287…	84528…	45232…			
E	28538…	49775…	32189…	39432…	29116…	73425…	35073…	50331…	64160…			
F	34490…	48932…	425884	31840…	46203…	33750…	20997…	42982…	29684…			
G	32409…	54582…	46419…	55036…	64210…	50809…	34876…	62622…	62307…			
H	46865…	19848…	51269…	22253…	726158	63150…	46746…	65789…	61263…			

Well Plate 13: Fluorescence measured with Excitation was set to 501nm and emission was set at 511nm. Samples were the 6h samples in the placement shown in well plate 3.

The fluorescence reader software is called SoftMax Pro. The temperature was 24.9 °C. Excitation wavelength was 501 nm. The emission wavelength was 511 nm. The plate was read at low sensitivity. File was exported and named “Interlab day 3 6 hr sample fluorescence”

Streaking new Colonies for Inter-lab

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-07-10 to 2017-07-12

MONDAY, 7/10/17

Today we streaked new plates from old plates of BBa_J364004 and BBa_J364005. We did this because we did not have a sufficient number of colonies of these for the inter-lab redo. We got plates from the 4°C refrigerator. We would then select part of the colony we had. In the case of BBa_J364005, we only had part of a colony remaining. We then made a streak plate using this colony. The loop was flamed in between making each plate to ensure no cross contamination. BBa_J364004 consisted of 3 small colonies growing together so we did our best to only pull from one of those.
At approximately 1:30 pm, colonies were taken from the plates started on 6/28/2017 for BBa_I20270 (+ control) and BBa_R0040 (- control). Three cultures of each were started, two containing LB+chloramphenicol and one with just LB. The colonies were transferred with toothpicks to Falcon tubes loaded with 3 ml of their respective media. These cultures were placed in the shaker for overnight at 37.4 °C and 220 rpm.

Interlab Part 2 redo

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Date: 2017-07-11

TUESDAY, 7/11/17

Preparing fluorescein stock solution
A new tube of fluorescein sodium salt was taken from the tube and centrifuged for 30 seconds to ensure that the pellet was at the bottom. 1000µl of PBS was added to the tube and pipetted up and down until thoroughly dissolved. 500µl of this 2x stock solution was transferred to a micro centrifuge tube to make a 1x stock solution. The micro centrifuge tube was then wrapped in foil.
Cell wash and other preparations
To determine what may have caused our results last time, we are running some additional samples: BBa_R0040 in PBS, LB, and LB with chloramphenicol, as well as BBa_I20270 in PBS, LB, and LB with chloramphenicol. LB, LB with chloramphenicol and chloramphenicol were also run. We did this to help determine why our initial tests LB had such a high fluorescence reading.
Initially, the cells for our PBS with cells samples were stored in LB and chloramphenicol so we had to wash them. To do this, we placed 1 ml of cells in a centrifuge at 10xg for 2 min. The media was then poured out and what wouldn't pour was removed by pipette. These pellets were then re-suspended in 1 ml PBS.
We then moved to the M215 Lab
We put the serial dilutions inter-lab called for into the well plate. 100µl of PBS was added to cells A2 through D12 as indicated in. 200µl of fluorescein 1x was added to wells A1, B1, C1, and D1. Then a serial dilution was performed so that the fluorescence curve could be made.
100µl BBa_R0040 (in PBS washed) was placed into E1. BBa_I20270 (100µl in PBS wash) was loaded into F1. LB and chloramphenicol BBa_R0040 in E2 (100µl). 100µl of LB and chloramphenicol BBa_I20270 was placed in F2. 100µl of BBa_R0040 in LB was laced in E3. 100µl BBa_I20270 in LB was placed in F3. 100µl LB was placed into G1. 100µl of LB and chloramphenicol (100µg/ml) was placed into G2. Then 100µl of 50µg/ml chloramphenicol stock solution was placed into G3. The uptake was placed in the plate reader. The optical density was read at OP630. The machine was at 26.3 °C. The fluorescence was measured.

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	100% Fluor	50% Fluor	25% Fluor	12.5% Fluor	6.25% Fluor	3.125% Fluor	1.562% Fluor	.781% Fluor	.391% Fluor	.195% Fluor	.098% Fluor	PBS
B	100% Fluor	50% Fluor	25% Fluor	12.5% Fluor	6.25% Fluor	3.125% Fluor	1.562% Fluor	.781% Fluor	.391% Fluor	.195% Fluor	.098% Fluor	PBS
C	100% Fluor	50% Fluor	25% Fluor	12.5% Fluor	6.25% Fluor	3.125% Fluor	1.562% Fluor	.781% Fluor	.391% Fluor	.195% Fluor	.098% Fluor	PBS
D	100% Fluor	50% Fluor	25% Fluor	12.5% Fluor	6.25% Fluor	3.125% Fluor	1.562% Fluor	.781% Fluor	.391% Fluor	.195% Fluor	.098% Fluor	PBS
E	R0040 (PBS)	R0040 (LB + Chlor)	R0040 (LB)									
F	I20270 (PBS)	I20270 (LB + Chlor)	I20270 (LB)									
G	LB	LB + Chlor	Chlor									
H												

Well Plate 1: The layout of well plate used for this round of tests. Shows dilutions at each step as well as the extra samples included to help us determine why our LB was so fluorescent in the last round of tests.

Excitation: 488nm

Emission: 530nm, 525nm.
Temperature in machines: 24.0 °C to 24.2 °C
Each of Emission was run 3x to compare.

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.032	0.035	0.033	0.036	0.033	0.033	0.033	0.033	0.033	0.033	0.034	0.031
B	0.037	0.033	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031
C	0.031	0.031	0.031	0.031	0.031	0.031	0.032	0.031	0.031	0.031	0.031	0.031
D	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031
E	0.668	0.434	0.806									
F	0.655	0.704	0.704									
G	0.037	0.037	0.035									
H												

Well Plate 2: Optical Density of samples as shown in Well Plate 1. Samples are in Au.

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	16764…	96224…	61514…	34935…	18750…	9921.…	4757.…	2568.…	1200.…	632.2…	297.0…	-28.551
B	14720…	10722…	54243…	35363…	18772…	9549.…	4748.…	2469.…	1195.…	628.2…	333.6…	49.912
C	14105…	10370…	64727…	32508…	18605…	11085…	4362.…	2083.…	1066.2	519.3…	251.71	-76.571
D	14039…	99004…	62168…	34836…	18490…	9330.…	4728.…	2484.…	1233.…	653.07	321.2…	-18.332
E	32.056	62.381	110.6…	-37.022	-12.865	10.957	-17.435	13.381	-39.04	-31.858	37.868	-55.603
F	1849.…	1801.…	2335.…	-17.981	-5.277	16.061	5.224	1.569	-19.942	32.113	-19.985	-4.466
G	40.834	95.389	-43.118	-1.972	-29.965	25.376	-32.744	-27.497	-6.207	-4.357	-15.482	-54.393
H												

Well Plate 3: First test for fluorescence of Well Plate 1 with the Emission set at 525nm. Units are in Fu.

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	16804…	96383…	61901…	34937…	18736…	9893.…	4732.…	2566.…	1215.…	670.6…	315.3…	-18.428
B	14750…	10765…	54368…	35473…	18807…	9569.…	4830.…	2522.…	1220.…	666.85	330.2…	3.144
C	14195…	10416…	64839…	32699…	18679…	11123…	4401.…	2178.…	1013.…	472.4…	279.9…	37.473
D	14104…	99203…	62473…	34958…	18614…	9430.…	4814.…	2481.…	1313.…	690.3…	349.56	32.01
E	3.139	107.7…	94.335	-1.921	48.167	15.402	40.271	10.385	15.54	-11.576	4.467	-27.937
F	1907.…	1852.…	2437.…	-10.575	-29.443	50.428	20.562	-13.715	16.929	65.386	-14.874	31.704
G	69.361	103.2…	0.354	1.843	36.31	-8.984	34.042	5.569	36.522	48.831	33.729	-73.488
H												

Well Plate 4: Second test for fluorescence of Well Plate 1 with the Emission set at 525nm. Units are in Fu.

Well5

	1	2	3	4	5	6	7	8	9	10	11	12
A	167645	95966…	61705…	34788…	18659…	9865.…	4724.…	2521.…	1190.…	605.3…	290.2…	-0.695
B	14731…	10751…	54305…	35448…	18820…	9617.…	4841.…	2515.…	1208.…	648.7…	330.8…	3.107
C	14160…	10390…	64779…	32603…	18637…	11068…	4369.…	2149.…	1051.…	461.6…	266.3…	-13.918
D	14082…	99045…	62412…	34883…	18483…	9410.…	4761.…	2501.…	1307.…	629.68	352.2…	33.338
E	-6.18	86.006	109.6…	-74.162	7.985	-48.001	-43.645	-27.208	40.071	-38.878	7.114	-82.439
F	1828.…	1862.…	2447.…	-41.608	-2.829	8.977	-16.502	42.149	-6.101	54.962	-23.467	-3.104
G	54.082	65.458	-5.554	-62.427	-58.352	-46.173	-43.801	13.236	-28.087	-0.785	1.757	-51.314
H												

Well Plate 5: Third test for fluorescence of Well Plate 1 with the Emission set at 525nm. Units are in Fu.

Well6

	1	2	3	4	5	6	7	8	9	10	11	12
A	10690…	59112…	38091…	21146…	11365…	6062.…	2841.…	1488.…	759.78	379.4…	199.6…	-22.311
B	93506…	67212…	33641…	22008…	11655…	5934.…	3028.…	1565.…	706.2…	379.04	195.9…	30.573
C	89859…	65018…	40430…	20172…	11489…	6819.…	2660.…	1295.…	665.7…	335.7…	153.0…	-26.561
D	89445…	61950…	38981…	21494…	11430…	5793.…	2966.…	1532.…	777.5…	374.3…	220.5…	-11.815
E	-7.491	50.34	97.919	42.418	50.401	23.759	-4.961	-44.613	28.244	-24.013	57.475	-10.102
F	1099.…	1080.…	1361.…	-13.63	-5.601	2.742	-17.257	-4.324	-9.289	-1.001	-40.409	19.74
G	46.628	68.558	-18.836	12.736	-26.684	-62.313	-0.908	42.244	-2.568	1.847	46.541	23.167
H												

Well Plate 6: First test for fluorescence of Well Plate 1 with the Emission set at 530nm. Units are in Fu.

Well7

	1	2	3	4	5	6	7	8	9	10	11	12
A	10686…	59481…	38236…	21368	11393…	6061.…	2893.…	1530.…	734.4…	374.2…	231.2…	-14.02
B	93593…	67285…	33698…	21944…	11519…	5953.…	2971.…	1582.…	751.9…	390.28	188.3…	-3.758
C	89876…	65028…	40306…	20160…	11486…	6835.…	2620.…	1340.…	667.4…	303.7…	155.5…	-27.922
D	89396…	61951…	38964…	21488…	11462…	5825.…	2961.…	1568.…	808.2…	394.8…	230.1…	-25.259
E	37.522	132.1…	124.1…	-2.605	8.637	-41.552	27.287	-5.643	44.705	-16.774	41.791	-26.205
F	1018.…	1044.…	1387.…	13.54	-2.86	-16.449	0.843	69.461	17.081	40.83	-17.328	-8.795
G	70.743	69.277	-4.16	18.122	-8.125	10.514	14.626	-14.943	33.105	8.147	-25.931	41.139
H												

Well Plate 7: Second test for fluorescence of Well Plate 1 with the Emission set at 530nm. Units are Fu.

Well8

	1	2	3	4	5	6	7	8	9	10	11	12
A	167645	95966…	61705…	34788…	18659…	9865.…	4724.…	2521.…	1190.…	605.3…	290.2…	-0.695
B	14731…	10751…	54305…	35448…	18820…	9617.…	4841.…	2515.…	1208.…	648.7…	330.8…	3.107
C	14160…	10390…	64779…	32603…	18637…	11068…	4369.…	2149.…	1051.…	461.6…	266.3…	-13.918
D	14082…	99045…	62412…	34883…	18483…	9410.…	4761.…	2501.…	1307.…	629.68	352.2…	33.338
E	-6.18	86.006	109.6…	-74.162	7.985	-48.001	-43.645	-27.208	40.071	-38.878	7.114	-82.439
F	1828.…	1862.…	2447.…	-41.608	-2.829	8.977	-16.502	42.149	-6.101	54.962	-23.467	-3.104
G	54.082	65.458	-5.554	-62.427	-58.352	-46.173	-43.801	13.236	-28.087	-0.785	1.757	-51.314
H												

Well Plate 8: Third test for fluorescence of Well Plate 1 with the Emission set at 530nm. Units are Fu.

Interlab Part 3 (2nd try)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-07-12 to 2017-07-13

WEDNESDAY, 7/12/17

The first step of redoing Part 3 of inter-lab was making cultures to incubate overnight (16-18hours).
5 ml of LB and Chloramphenicol (33 µg/ml) were placed in 14ml SD Falcon tubes. The broth was inoculated with colonies from the below plates (with each culture from the same plate colony from a different colony).
BBa_I20270 come from the 6/28/2017 plate
BBa_R0040 from the 6/12/2017 plate
BBa_J364000 from the 6/12/2017 plate
BBa_J364001 from the 6/12/2017 plate
BBa_J364002 from the 6/12/2017 plate
BBa_J364003 from the 6/12/2017 plate
BBa_J364004 from the 7/10/2017 plate
BBa_J364005 from the 7/10/2017 plate
The culture was inoculated using an autoclave sterilized toothpick and an alcohol rinsed gloved hands. The colony was scraped up with the toothpick and deposited into the culture tube. At approximate 4:35 pm the Falcon tubes were put into the shaker at 220 rpm and 37.4 °C.

Streaking new Colonies for Inter-lab

Project: Interlab Study iGEM 2017

Authors: Julia Walsh

Dates: 2017-07-10 to 2017-07-12

WEDNESDAY, 7/12/17

Results:

The plates were checked at 3:00pm on 7/12/17. The parts BBa_J364004 and BBa_J364005 both showed excellent growth.

Interlab Part 3 (2nd try)

Project: Interlab Study iGEM 2017
Authors: Julia Walsh
Dates: 2017-07-12 to 2017-07-13

THURSDAY, 7/13/17

At 9:00 am the cultures were removed from the incubator and placed on ice to be transported to the M215 lab. The cultures were well-mixed (not vortexed, merely inverted). Then, 100 µl of each were placed in the 96-well plate as shown below.

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	I20270 (1)	R0040 (1)	J364000 (1)	J364001 (1)	J364002 (1)	J364003 (1)	J364004 (1)	J364005 (1)	LB			
B	I2020 (2)	R0040 (2)	J364000 (2)	J364001 (2)	J364002 (2)	J364003 (2)	J364004 (2)	J364005 (2)	LB			
C												
D												
E												
F												
G												
H												

Well Plate 1: The layout used for running the samples of the overnight cultures.

Measurements were delayed slightly after the plate was made, as LB and Chloramphenicol had to be retrieved from the Synthetic Biology Laboratory (B206).
The OD measurements were taken at 630nm wavelength using GEN 5 software, the Bio-Tek EL 805 plate reader (SN# 214093). All bubbles were popped before the measurements were taken, at 24.7 °C. Results are shown in Well Plate 2.

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.352	0.445	0.46	0.555	0.431	0.417	0.568	1.509	0.039			
B	0.377	0.434	0.411	0.339	0.422	0.436	0.44	0.429	0.038			
C												
D												
E												
F												
G												
H												

Well Plate 2: Results OD test of overnight samples. Results in Au. Measured at OD630.

The Data was saved as an Excel document titled “Interlab 7/13/17 OD630 Dilution Measurement”

The OD 630 measurements were applied to the 7/13/2017 inter-lab Dilution Calculation Sheet in Excel. These numbers were used to make the cultures to be incubated at t=0. (The exact values can be viewed in Table 1 and 2 below.)
The appropriate quantity of media was pipetted into each foil-wrapped 14 ml Falcon tube. Each tube was inoculated with the appropriate amount of culture then placed on ice. Then 500 µl of each culture was placed in the appropriate micro centrifuge tube to be measured. Both the foil tubes and the micro centrifuge tubes were placed on ice after. The Falcon tubes were placed in the incubator at 220 rpm and 37 °C.

Table1

	Sample	Abs630 Reading	Volume of Preloading Culture	Volume of Preloading Media
1	positive control	0.352	0.638977636	9.361022364
2	negative control	0.445	0.492610837	9.507389163
3	device 1	0.46	0.475059382	9.524940618
4	device 2	0.555	0.387596899	9.612403101
5	device 3	0.431	0.510204082	9.489795918
6	device 4	0.417	0.529100529	9.470899471
7	device 5	0.568	0.378071834	9.621928166
8	device 6	1.509	0.136054422	9.863945578
9	media+chl	0.039		

Table 1: Set 1 of quantities used for making cultures to incubate. All volumes in mL.

Table2

	Sample	Abs630 Reading	Volume of Preloading Culture	Volume of Preloading Media
1	positive control	0.377	0.591715976	9.408284024
2	negative control	0.434	0.506329114	9.493670886
3	device 1	0.411	0.537634409	9.462365591
4	device 2	0.339	0.666666667	9.333333333
5	device 3	0.422	0.522193211	9.477806789
6	device 4	0.436	0.503778338	9.496221662
7	device 5	0.44	0.498753117	9.501246883
8	device 6	0.429	0.512820513	9.487179487
9	media+chl	0.038		

Table 2: Set 2 of quantities used for making cultures to incubate. All volumes in mL.

The microcentrifuge tubes were transported to the M215 lab. (The cooler with the ice was dropped at one point, but all samples were perfectly recovered.)

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	R0040 (1)	I20270(1)	J36400 0 (1)	J36400 1 (1)	J36400 2 (1)	J36400 3 (1)	J36400 4 (1)	J36400 5 (1)	LB			
B	R0040 (1)	I20270 (1)	J36400 0 (1)	J36400 1 (1)	J36400 2 (1)	J36400 3 (1)	J36400 4 (1)	J36400 5 (1)	LB			
C	R0040 (1)	I20270 (1)	J36400 0 (1)	J36400 1 (1)	J36400 2 (1)	J36400 3 (1)	J36400 4 (1)	J36400 5 (1)	LB			
D	R0040 (1)	I20270 (1)	J36400 0 (1)	J36400 1 (1)	J36400 2 (1)	J36400 3 (1)	J36400 4 (1)	J36400 5 (1)	LB			
E	R0040 (2)	I20270 (2)	J36400 0 (2)	J36400 1 (2)	J36400 2 (2)	J36400 3 (2)	J36400 4 (2)	J36400 5 (2)	LB			
F	R0040 (2)	I20270 (2)	J36400 0 (2)	J36400 1 (2)	J36400 2 (2)	J36400 3 (2)	J36400 4 (2)	J36400 5 (2)	LB			
G	R0040 (2)	I20270 (2)	J36400 0 (2)	J36400 1 (2)	J36400 2 (2)	J36400 3 (2)	J36400 4 (2)	J36400 5 (2)	LB			
H	R0040 (2)	I20270 (2)	J36400 0 (2)	J36400 1 (2)	J36400 2 (2)	J36400 3 (2)	J36400 4 (2)	J36400 5 (2)	LB			

Well Plate 3: Layout of well plate used for all samples taken on time intervals t=0 and t=6. LB means the well has LB with chloramphenicol in it.

OD630 measurements were taken on the same machine at 30.2 °C and 630 nm. All measurements are in AU.

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.063	0.064	0.058	0.058	0.061	0.06	0.056	0.046	0.043			
B	0.057	0.065	0.057	0.06	0.065	0.045	0.057	0.047	0.04			
C	0.054	0.064	0.059	0.066	0.058	0.069	0.041	0.042	0.037			
D	0.057	0.059	0.057	0.057	0.057	0.05	0.059	0.06	0.038			
E	0.056	0.057	0.061	0.052	0.057	0.057	0.058	0.135	0.037			
F	0.055	0.059	0.064	0.06	0.06	0.062	0.057	0.05	0.038			
G	0.056	0.06	0.063	0.061	0.063	0.046	0.061	0.052	0.038			
H	0.054	0.06	0.065	0.055	0.06	0.053	0.057	0.047	0.037			

Well Plate 4: OD630 measurements for samples taken when time is zero. All units are AU.

The fluorescence measurements were then taken at low sensitivity, Excitation λ = 488, and Emission λ = 525, 530 each. They were read at 24.3 °C on the molecular devices Spectramax Gemini XS (SN# XS03150).

Well5

	1	2	3	4	5	6	7	8	9	10	11	12
A	76.744	214.1…	319.6…	237.3…	118.8…	36.603	69.897	79.261	101.2…			
B	89.309	220.8	468.6…	217.0…	87.054	26.237	82.484	71.402	126.2…			
C	113.0…	211.1…	423.7…	260.8…	101.3…	161.9…	38.821	66.319	90.991			
D	115.9…	200.8…	419.6…	213.2…	88.946	68.728	100.7…	28.168	142.8…			
E	113.2…	185.4…	455.0…	136.5…	68.025	99.31	100.7…	36.458	74.114			
F	70.905	137.0…	506.0…	199.3…	99.994	151.0…	32.193	40.739	107.9			
G	46.576	151.8…	400.5…	115.2…	103.26	60.992	88.192	87.09	91.441			
H	90.723	150.7…	385.6…	147.7…	87.266	132.1…	63.829	68.06	97.684			

Well Plate 5: Fluorescence measurements of the samples taken when the time was zero. Emission was set at 525nm. All units are FU.

Well6

	1	2	3	4	5	6	7	8	9	10	11	12
A	34.757	107.8…	181.4…	144.2…	53.697	95.229	87.286	93.909	44.409			
B	5.798	63.621	240.3…	82.58	91.007	60.364	71.651	86.595	107.6…			
C	62.4	106.0…	222.7…	163.04	67.708	170.51	-18.875	42.077	107.9…			
D	72.069	130.2…	210.5…	132.2…	41.459	43.607	114.9…	-1.969	45.515			
E	78.767	78.461	363.8…	117.9…	44.681	114.8…	108.2…	20.737	93.454			
F	77.019	101.4…	302.58	104.9…	81.714	196.7…	54.28	24.776	93.216			
G	38.34	161.5…	240.0…	97.838	47.93	83.706	43.106	141.6…	127.3…			
H	49.86	98.829	215.8…	119.5…	60.342	111.2…	57.189	63.964	34.43			

Well Plate 6: Fluorescence measurements of the samples taken when the time was zero. Emission was set at 530nm. All units are FU.

Both files were exported as text files and saved as “Interlab 7-13-17 FL 525nm t0” and “Interlab 7-13-17 Fl 530nm t0”.
Two hours later, 500 µl of each culture were placed in micro centrifuge tubes and transferred to the M215 lab. 100 µl of sample was pipetted into the appropriate wells.

Well7

	1	2	3	4	5	6	7	8	9	10	11	12
A				LB	J36400 5 (1)	J36400 4 (1)	J36400 3 (1)	J36400 2 (1)	J36400 1 (1)	J36400 0 (1)	I20270(1)	R0040 (1)
B				LB	J36400 5 (1)	J36400 4 (1)	J36400 3 (1)	J36400 2 (1)	J36400 1 (1)	J36400 0 (1)	I20270 (1)	R0040 (1)
C				LB	J36400 5 (1)	J36400 4 (1)	J36400 3 (1)	J36400 2 (1)	J36400 1 (1)	J36400 0 (1)	I20270 (1)	R0040 (1)
D				LB	J36400 5 (1)	J36400 4 (1)	J36400 3 (1)	J36400 2 (1)	J36400 1 (1)	J36400 0 (1)	I20270 (1)	R0040 (1)
E				LB	J36400 5 (2)	J36400 4 (2)	J36400 3 (2)	J36400 2 (2)	J36400 1 (2)	J36400 0 (2)	I20270 (2)	R0040 (2)
F				LB	J36400 5 (2)	J36400 4 (2)	J36400 3 (2)	J36400 2 (2)	J36400 1 (2)	J36400 0 (2)	I20270 (2)	R0040 (2)
G				LB	J36400 5 (2)	J36400 4 (2)	J36400 3 (2)	J36400 2 (2)	J36400 1 (2)	J36400 0 (2)	I20270 (2)	R0040 (2)
H				LB	J36400 5 (2)	J36400 4 (2)	J36400 3 (2)	J36400 2 (2)	J36400 1 (2)	J36400 0 (2)	I20270 (2)	R0040 (2)

Well Plate 7: Layout of well plate used for all samples taken on time intervals t=2 and t=4. LB means the well has LB with chloramphenicol in it.

The location of samples in the well plate was flipped on both ends from the desired orientation. Thus the orientation shown in well plate 7 was used for the samples taken at 2 and 4 hours.
The OD630 measurements were taken at 630nm and 31.3°C. A hair was found near row G or F and removed.

Well8

	1	2	3	4	5	6	7	8	9	10	11	12
A				0.038	0.119	0.124	0.077	0.139	0.125	0.067	0.116	0.135
B				0.039	0.12	0.127	0.071	0.137	0.126	0.066	0.122	0.138
C				0.151	0.118	0.126	0.077	0.131	0.12	0.065	0.116	0.136
D				0.13	0.117	0.122	0.08	0.131	0.141	0.065	0.119	0.123
E				0.11	0.069	0.13	0.075	0.121	0.107	0.058	0.109	0.13
F				0.204	0.076	0.134	0.077	0.124	0.112	0.059	0.113	0.132
G				0.249	0.069	0.133	0.074	0.121	0.113	0.06	0.112	0.133
H				0.18	0.067	0.126	0.072	0.12	0.11	0.059	0.125	0.127

Well Plate 8: OD630 measurements for samples taken when time is two hours. All units are AU.

Fluorescence Measurements were taken at 25 °C and low sensitivity. Excitation was $\lambda = 488$ and emission was $\lambda = 525$ or 530.

Well9

	1	2	3	4	5	6	7	8	9	10	11	12
A				86.21	77.294	306.5…	412.52	109.57	668.7…	755.6…	780.0…	138.7…
B				45.991	124.5…	312.8…	360.6…	102.8…	578.1…	723.2…	758.4…	122.1…
C				13.227	113.1…	320.16	348.3…	108.6…	585.9…	627.1…	731.5…	74.206
D				58.418	96.675	228.9…	432.6…	101.1…	675.6…	636.3…	627.7…	2.041
E				36.766	57.96	287.2	61.233	164.3…	419.9…	517.2…	484.1…	92.25
F				114.97	54.115	284.8…	47.499	114.0…	496.3…	429.8…	541.03	85.485
G				46.539	61.66	263.74	45.886	66.926	533.2…	440.9	512.0…	71.604
H				126.5…	101.2…	170.4…	84.549	106.4…	494.7…	427.3…	503.6…	62.967

Well Plate 9: Fluorescence measurements of the samples taken when the time was two hours. Emission was set at 525nm. All units are FU.

Well10

	1	2	3	4	5	6	7	8	9	10	11	12
A				42.294	46.811	178.1…	284.1…	95.616	318.2…	469.1…	417.8…	43.215
B				58.064	27.006	246.8…	225.7…	42.009	363.6…	400.4…	397.1…	20.959
C				77.231	45.535	164.3…	244.9…	11.003	312.8…	346.3…	367.2…	53.874
D				-17.545	103.6…	177.0…	228.3…	20.929	384.1…	318.07	396.5…	83.36
E				101.0…	68.374	140.6…	89.499	69.607	277.8…	242.1…	238.8…	33.194
F				147.7…	16.781	158.8…	76.953	122.4…	265.1…	312.9…	304.2…	64.959
G				80.807	19.082	181.1…	27.265	22.955	326.3…	275.11	309.43	123.2…
H				97.024	23.109	148.8…	47.207	80.265	300.2…	246.3…	280.32	88.748

Well Plate 10: Fluorescence measurements of the samples taken when the time was two hours. Emission was set at 530nm. All units are FU.

Inter-lab t-4 Measurements time = 3:00 pm
When we went back to take measurements we noticed the shaker wasn't on. After inverting each of the Falcon tubes 5x each, 500 µl of culture was transferred to t=3 micro centrifuge tubes. The micro centrifuge tubes were then put on ice and the Falcon tubes were recovered in full and placed back into the shaker for another 2 hours. Cultures were put into the wells of the plate flipped from what inter-lab protocol ended. OD630 was measured at 30.5 °C, fluorescence was then measured. *flipped Orientation same as indicated in t=2. (Well Plate 7)
Excitation was set at λ =488nm and Emission was set so λ= 525 or 530.Temperature was 24.5 °C and the fluorescence plate reader set to low sensitivity

Well11

	1	2	3	4	5	6	7	8	9	10	11	12
A				0.037	0.145	0.136	0.109	0.16	0.156	0.084	0.145	0.174
B				0.037	0.153	0.135	0.107	0.188	0.143	0.079	0.153	0.176
C				0.039	0.151	0.101	0.109	0.173	0.156	0.084	0.152	0.17
D				0.037	0.145	0.154	0.107	0.142	0.153	0.083	0.15	0.169
E				0.037	0.118	0.149	0.097	0.153	0.15	0.075	0.148	0.164
F				0.037	0.12	0.153	0.108	0.152	0.126	0.078	0.141	0.162
G				0.037	0.122	0.169	0.111	0.148	0.157	0.077	0.151	0.176
H				0.037	0.111	0.165	0.103	0.158	0.144	0.071	0.151	0.16

Well Plate 11: OD630 measurements for samples taken when time is four hours. All units are AU.

Well12

	1	2	3	4	5	6	7	8	9	10	11	12
A				10.441	109.2…	229.7…	523.3…	133.8…	511.4…	1043.…	630.0…	68.57
B				39.756	46.135	138.4…	439.6…	86.202	503.6…	834.2…	595.6…	71.96
C				28.98	9.265	89.852	539.6…	41.719	551.7…	895.2…	610.3…	119.8…
D				44.637	124.1…	210.31	486.4…	34.086	483.67	823.0…	521.44	54.315
E				59.891	46.234	157.3…	-16.356	73.948	536.3…	704.64	483.1…	87.209
F				92.928	75.826	146.8…	35.038	71.22	386.7…	645.7…	386.7…	28.069
G				21.992	73.613	219.9	86.469	32.378	512.8…	632.5…	434.7…	43.785
H				23.456	41.476	150.9…	23.664	29.33	405.7…	539.6…	392.8…	97.752

Well Plate 12: Fluorescence measurements of the samples taken when the time was four hours. Emission was set at 525nm. All units are FU.

Well13

	1	2	3	4	5	6	7	8	9	10	11	12
A				14.387	28.95	108.8…	301.0…	42.698	358.5…	601.2…	337.1…	156.3…
B				138.4…	26.969	56.934	305.1…	36.52	332.33	427.0…	390.7…	51.688
C				70.474	66.69	59.323	287.9…	55.211	322.34	496.4…	349.1	117.4…
D				112.2…	88.51	138.6…	290.3…	50.769	328.3…	506.82	396.9…	125.0…
E				146.8…	51.555	137.4…	72.534	78.641	355.0…	447.4…	270.47	92.983
F				27.81	85.912	142.7…	21.326	83.059	254.5…	385.4…	263.7…	123.0…
G				28.358	86.34	158.6…	83.8	65.918	344.12	360.7…	284.1…	49.099
H				115.1…	59.908	147.3…	80.144	76.077	309.7…	361.7…	285.0…	99.999

Well Plate 13: Fluorescence measurements of the samples taken when the time was four hours. Emission was set at 530nm. All units are FU.

Inter-lab t=6 hours Measurements

After an additional 2 hours, 500 µl of each culture was placed into respective micro centrifuge tubes which were immediately put on ice. Falcon tube culture was taken to the 4 °C and the microcentrifuge tubes were taken to the M215 lab. 100 µl of each culture was placed into the wells indicated by the inter-lab protocol. This time they were NOT FLIPPED. The plate was measured by OD630 and fluorescence plate readers.

Excitation was set so that λ= 488nm and emission was set so λ= 525nm or 530nm. The temperature in the fluorescence plate reader was. 24.8 °C and it was set to low sensitivity. The optical density plate reader's internal temperature was 27.0 °C. These plates had their samples oriented like the samples in Well Plate 3.

Well14

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.267	0.215	0.142	0.231	0.245	0.151	0.24	0.181	0.04			
B	0.263	0.211	0.142	0.226	0.209	0.157	0.233	0.193	0.04			
C	0.254	0.213	0.133	0.234	0.21	0.163	0.232	0.197	0.039			
D	0.252	0.212	0.144	0.213	0.208	0.161	0.227	0.22	0.043			
E	0.247	0.228	0.153	0.213	0.25	0.17	0.221	0.245	0.038			
F	0.242	0.222	0.148	0.225	0.249	0.185	0.216	0.232	0.042			
G	0.245	0.229	0.147	0.223	0.236	0.175	0.222	0.228	0.039			
H	0.238	0.228	0.135	0.222	0.256	0.176	0.215	0.223	0.039			

Well Plate 14: OD630 measurements for samples taken when time is six hours. All units are AU.

Well15

	1	2	3	4	5	6	7	8	9	10	11	12
A	139.7…	1456.…	2186.…	1766.…	163.7…	135.3…	408.7…	107.7…	119.9…			
B	140.2…	1448.…	2015.…	1727.…	154.1…	130.7…	399.5…	116.8…	108.4…			
C	137.3…	1411.…	1990.…	1772.…	155.8…	129.5…	400.1…	116.9…	114.2…			
D	128.0…	1384.…	2007.…	1450.…	152.0…	125.8…	389.7…	131.5…	119.1…			
E	123.43	1513.…	2150.…	1126.…	141.0…	1016.…	325.91	122.8…	100.8…			
F	119.5…	1343.…	1861.…	1194.…	126.4…	1129.…	310.21	116.1…	91.239			
G	113.1…	1348.…	1860.…	1089.…	117.2…	1006.…	301.4…	108.5…	92.039			
H	102.47	1367.…	1648.…	1102.…	126.95	984.6…	280.2…	101.4…	95.877			

Well Plate 15: Fluorescence measurements of the samples taken when the time was six hours. Emission was set at 525nm. All units are FU.

Well16

	1	2	3	4	5	6	7	8	9	10	11	12
A	104.5…	843.9…	1257.…	1010.…	117.13	100.0…	259.9	78.984	88.91			
B	104.4…	839.0…	1152.…	993.7…	112.4…	97.909	249.3…	87.632	80.751			
C	102.2…	820.52	1130.…	1016.…	110.8…	94.647	254.8…	85.075	86.305			
D	95.79	800.2…	1138.…	833.2…	110.0…	95.086	241.8…	98.491	88.751			
E	91.692	872.8…	1225.…	659.2…	101.0…	590.5…	210.2…	89.678	72.731			
F	88.841	767.0…	1064.…	692.1…	90.661	645.3…	199.8…	84.974	65.21			
G	83.867	774.9…	1056.…	629.4…	82.277	576.6…	194.4…	76.894	67.944			
H	75.666	782.9…	919.1…	631.9…	93.1	563.51	177.5…	73.586	68.775			

Well Plate 16: Fluorescence measurements of the samples taken when the time was six hours. Emission was set at 530nm. All units are FU.