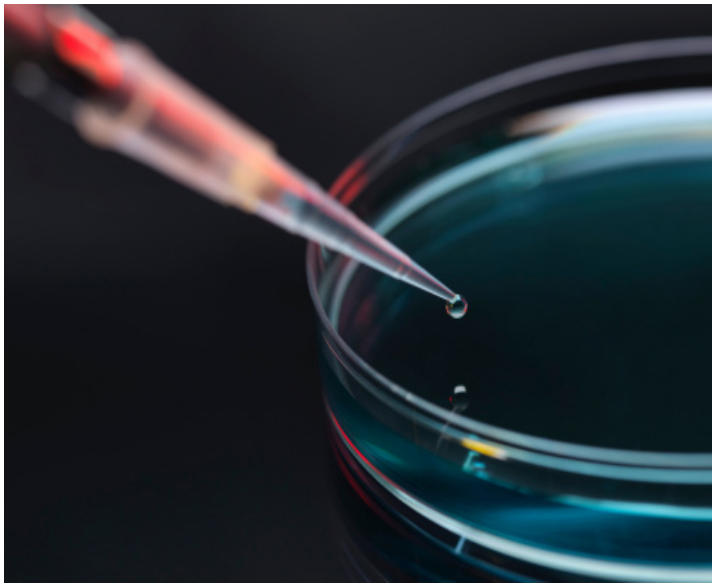

CCA_San_Diego Interlab

Getty Images

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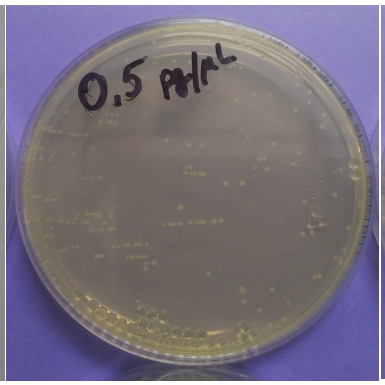
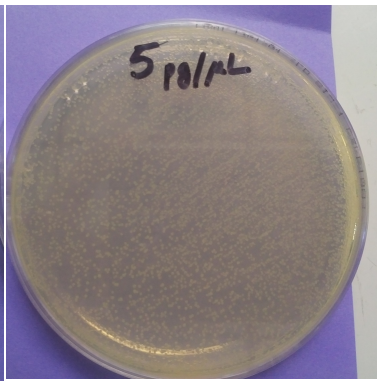
PURPOSE

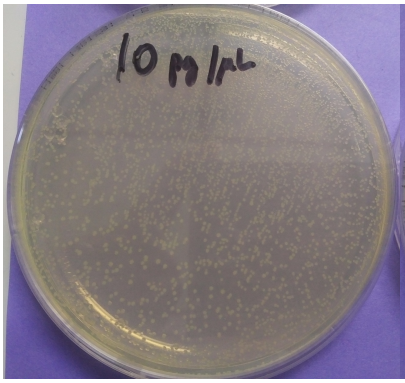
Establish a GFP measurement protocol based on engineering principles that anyone with a plate reader can use in their lab. This means that through e the same exact protocol around the world everyone can produce common, comparable units for measuring GFP with different plate readers.

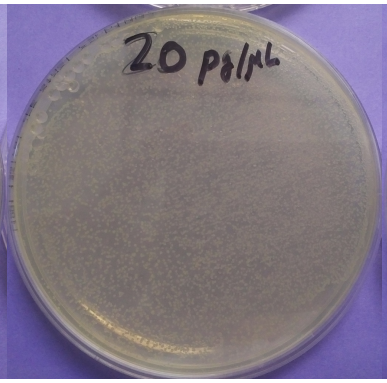
CELL COMPETENCY TEST

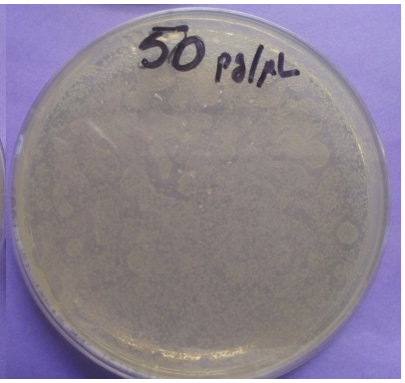
Procedure adhered to indicated under iGEM Interlab Protocols Page: Cell Comp. Test











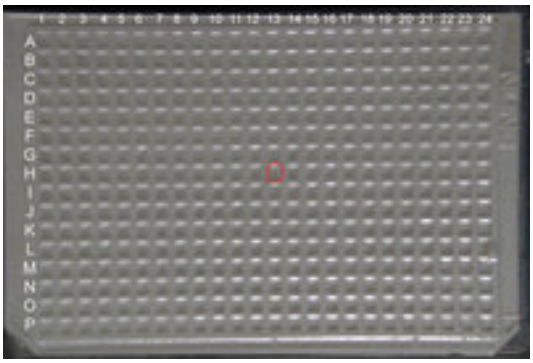
RESULTS

$V_{DNA\ transformation}$ [µl]	1					
V_{total} [µl]	251					
V_{plated} [µl]	20					
DNA concentration [pg/µl]	**0.5**	**5**	**10**	**20**	**50**	
# of colonies	13	125	289	477	517	
# of colonies	17	178	371	510	532	*<< sample data,*
# of colonies	11	138	321	588	572	
average # of colonies	13.7	147.0	327.0	525.0	540.3	
efficiency	#######	3.69E+08	4.10E+08	3.29E+08	1.36E+08	
Results	**efficiency**					
average	3.2E+08		*average of the efficiencies calculated for each DNA concentrati*			
weighted	2.3E+08		*gives more importance to higher concentrations*			

DNA PREPERATION

The DNA of the 8 plasmids listed below was resuspended in 10 µL of distilled water (diH_2O). The liquid turned red to indicating a successful resuspension of the plasmid (dried down the plasmid DNA with cresol red dye by IGEM).

The 8 plasmids are transformed (Positive Control, Negative Control, Test Device 1, Test Device 2, Test Device 3, Test Device 4, Test Device 5, and Test Device 6 - locations listed below) from Kit Plate 7 into *E. coli* DH5-alpha cells. Parts are taken from kit plate 7.



Kit Plate 7 InterLab Part Locations

- Positive Control (BBa_I20270): well 21B
- Negative Control (BBa_R0040): well 21D
- Test Device 1 (BBa_J364000): well 21F
- Test Device 2 (BBa_J364001): well 21H
- Test Device 3 (**BBa_J364002**): well 21J
- Test Device 4 (**BBa_J364003**): well 21L
- Test Device 5 (**BBa_J364004**): well 21N

TRANSFORMATION

We employed a similar transformation protocol, and have included the methodology below

MATERIALS

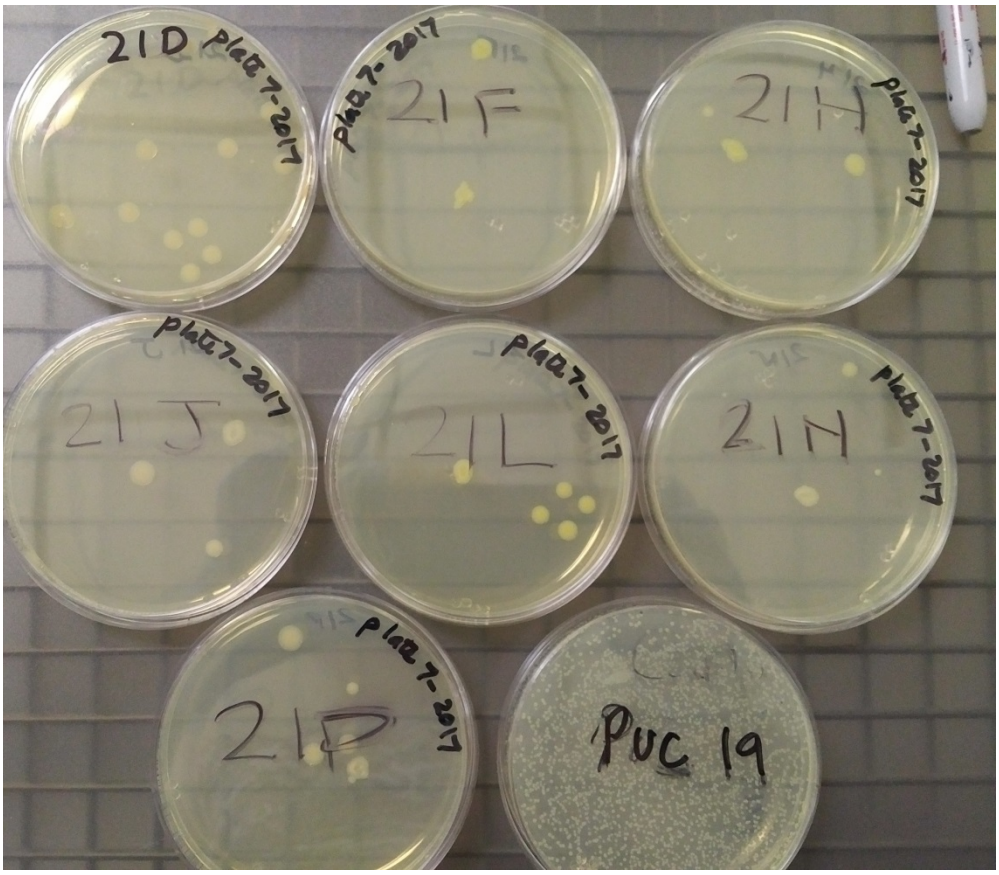
a. DNA samples
b. Transformation efficiency DNA, pUC 19, 10 pg/ µL, Lucigen, Cat No. F92078-1
c. LB Chloramphenicol agar plates, Cat No. Teknova, L1017
d. LB Carbenicillin 50 agar plates, Cat No. Teknova, L1801 for pUC19 transformation
e. DH5a competent cells, Invitrogen, Cat 18265-017
f. SOC (Recovery Medium), Lucigen, Cat No. F98226
g. 15 mL culture tube
h. Vortex
i. Tooth pick
j. Incubator shaker
k. 42°C Water bath
l. Ice and ice bucket
m. Pipet

PROCEDURE

a. Transform 2 µL of 8 different DNA mini-preparation samples aliquoted from 2017 IGEM distribution plate 7 into E coli DH5a chemically competent cells
b. Transform 1 µL of DNA of pUC19 as transformation efficiency control into E coli DH5a chemically competent cells
c. Turn on incubator-shaker at 37°C.
d. Turn on incubator for plates at 37°C.
e. Set up water bath 42°C.
f. Bring to room temperature S.O.C medium.
g. Bring LB plates supplemented with appropriate antibiotic (chloramphenicol for IGEM clones and Carbanicillin for pUC19) at room temperature and label them.
h. Thaw competent cells on ice.
i. Aliquots competent cells in as many tubes as needed.
j. Add 2.0 µl DNA preparation to 50 µl competent cells to DNA and swirl gently to mix
k. Incubate on ice for 20 minutes
l. Heat shock at 42°C in heat block for 30 seconds. Quickly return to ice and let it sit for 2 min.
m. Add 200 µl of 18-25°C SOC medium and transfer mixture to 15mL Falcon tube
n. Incubate in shaker at 37°C, 225 rpm for 60 min
o. Plate 150µL of the mixture on LB agar plates supplemented with Chloramphenicol 34 µg/mL
p. Plate 150µL of the mixture with pUC19 on LB agar plates supplemented with Carbenicillin 100 µg/mL
q. Incubate plates at 37°C overnight

RESULTS

The transformation of plasmid DNA into E.coli DH5a were plated on LB agar plates supplemented with Chloramphenicol (34 µg/mL). pUC19 transformed into E.coli DH5a was plated on LB agar plates supplemented with Carbenicillin (100 µg/mL). The plates are shown below.



200 uL transformation volume, plated 50 uL, 1 uL DNA for pUC = 10 pg
For the IGEM plasmids: 200 uL transformation volume, plated 50 uL, 2 uL DNA for pUC (estimated 20 pg)

CALIBRATION

Procedure provided by iGEM Interlab: Plate Reading Protocol

Cultures were started in 4 mL medium overnight.

Plate Layout of Overnight Cultures:

Colony 1		Colony 2			
Replicate 1	Replicate 2	Replicate 1	Replicate 2		
positive control	positive control	positive control	positive control	Media	Media
negative control	negative control	negative control	negative control	Media	Media
device 1	device 1	device 1	device 1		
device 2	device 2	device 2	device 2		
device 3	device 3	device 3	device 3		
device 4	device 4	device 4	device 4		
device 5	device 5	device 5	device 5		
device 6	device 6	device 6	device 6		

The OD readout of the overnight cultures was determined using a spectrophotometer. The values and the plate layout are shown below. As directed by IGEM, we use LUDOX-S40 as a single point reference to obtain a radiometric conversion factor to transform the absorbance data into a standard OD 600 measurement. The path length correction was turned off. To measure the standard LUDOX Abs 600 we have used the same plate and volumes used in the cell based assays, so 100 uL.
The table below shows the data for OD 600 measured by a spectrophotometer and a plate reader for the wells containing water or LUDOX. The corrected Abs 600 was calculated by subtracting the water readout values. The reference OD 600 was defined as that measured by the reference spectrophotometer. The correction factor to convert measured Abs 600 to OD 600 is thus the Reference OD 600 divided by Abs 600.

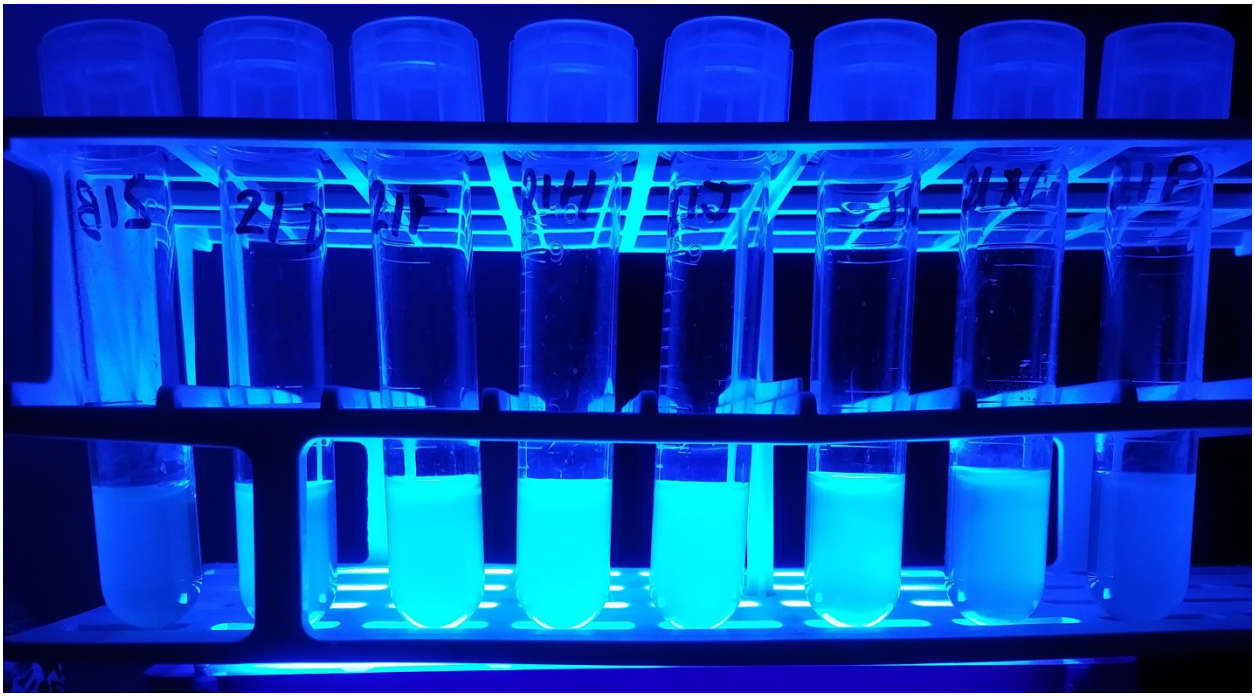
All cell density readings using this instrument with the same settings and volume can be converted to OD 600 by multiplying by (in this instance) 0.685.

0.3464	0.3569	0.3666	0.3752	0.0431	0.0433
0.2981	0.2913	0.2959	0.2994	0.0413	0.0416
0.4116	0.412	0.4426	0.4012		
0.3943	0.4242	0.4233	0.2905		
0.4258	0.4187	0.4315	0.4347		
0.3749	0.3653	0.3400	0.3532		
0.5449	0.3865	0.3196	0.3076		
0.3085	0.2973	0.2932	0.2883		

OD600 Reference Point

	LUDOX-HS4(	H2O
Replicate 1	0.048	0.041
Replicate 2	0.0483	0.0393
Replicate 3	0.0481	0.0392
Replicate 4	0.0484	0.0397

Data: (Excel will be linked to wiki page so data can be edited with)



Overnight cultures of 8 different Interlab plasmids placed on a blue light table. We can observe a difference in fluorescence amongst the cultures.

STANDARD CURVE

A serial dilution of fluorescein in 4 replicates was prepared in a 96 well plate and the fluorescence was
read in a plate reader. A standard curve was prepared from these values allowing to convert the concentration of GFP in the unknown samples.

MATERIALS

A. Fluorescein (provided in kit)
B. 10ml 1xPBS (phosphate buffered saline)
C. 96 well plate, black with flat, transparent/clear bottom preferred (provided by team)

METHODS

Prepare the fluorescein stock solution:

√ Spin down fluorescein stock tube to make sure pellet is at the bottom of tube.

√ Prepare 2x fluorescein stock solution (100 µM) by resuspending fluorescein in 1 mL of 1xPBS.

√ Dilute the 2x fluorescein stock solution with 1xPBS to make a 1x fluorescein solution and resulting concentration of fluorescein stock solution 50 µM (500µL of 2x fluorescein in 500 µL 1x PBS will make 1 mL of 50 µM (1x) fluorescein solution.)

Prepare the serial dilutions of fluorescein:

√ Serial dilutions is performed across columns 1-11. COLUMN 12 MUST CONTAIN PBS BUFFER ONLY. Initially you will setup the plate with the fluorescein stock in column 1 and an equal volume of 1xPBS in columns 2 to 12. You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.

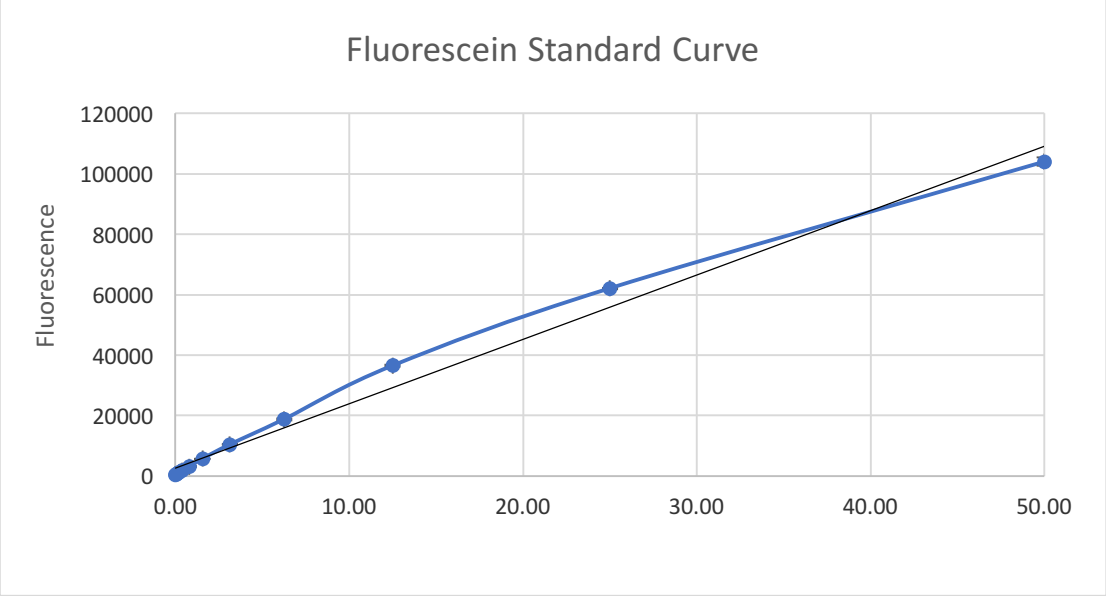
√ You must now measure the plate in your plate reader. TURN OFF path length correction if available.

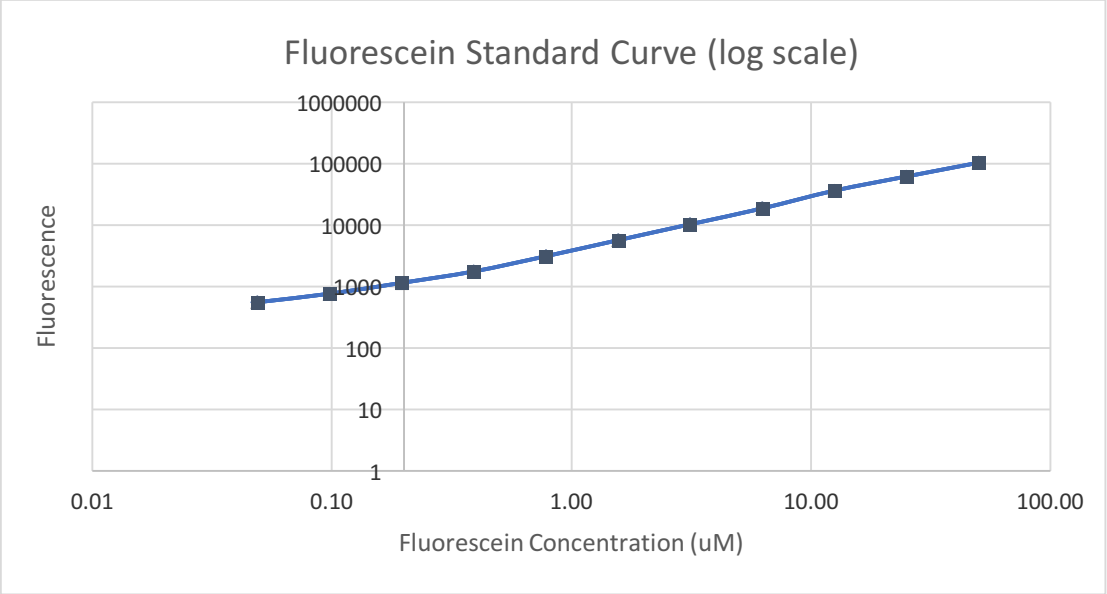
Excitation 485nm

Emission 538nm (closest to 530nm)

RESULTS

uM Fluorescein	50.00	25	12.5	6.25	3.125	1.5625	0.78125	0.390625	0.195313	0.097656	0.048828	0
Replicate 1	102378.4	62146.65	36862.65	19102.65	10254.02	5729.659	3149.86	1777.35	1163.581	780.153	584.045	404.551
Replicate 2	105307.7	62364.06	36378.38	18837.12	10543.58	5633.964	3113.961	1751.873	1145.869	738.402	577.443	430.199
Replicate 3	104839.5	61806.4	36602.77	18779.53	10394.65	5770.622	3147.845	1803.165	1157.979	775.331	519.503	419.139
Replicate 4	103415.3	61990.36	36171.02	18312.95	10302.82	5714.439	3130.597	1763.338	1162.116	788.131	561.975	417.007
Arith. Mean	103985.2	62076.87	36503.7	18758.06	10373.77	5712.171	3135.566	1773.932	1157.386	770.5043	560.7415	417.724
Arith. Std.Dev.	1339.836	236.6348	297.2314	328.4186	127.3417	57.28242	16.79841	22.09889	8.036248	22.04278	29.00649	10.51459
%CV	0.012885	0.003812	0.008142	0.017508	0.012275	0.010028	0.005357	0.012458	0.006943	0.028608	0.051729	0.025171





CELL MEASUREMENTS

MATERIALS

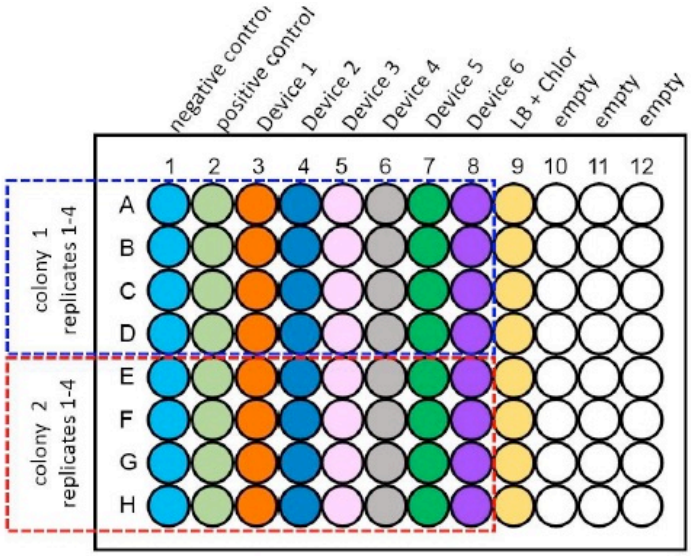
a. Cultures of clones in transformed in Escherichia coli strain DH5α
b. LB (Luria Bertani) media
c. Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH - working stock 25 ug/mL)
d. 50 ml Falcon tube with vented cap
e. Incubator at 37°C
f. 1.5 ml Eppendorf tubes for sample storage
g. Ice bucket with ice
h. Pipettes
i. 96 well plate with flat transparent bottom Devices

PROCEDURE

√ Set your instrument to read OD600 (as OD calibration setting)
√ Measure OD600 of the overnight cultures
√ Record data in your notebook
√ Import data into Excel (Dilution Calculation) Sheet_1 provided
√ Dilute the cultures to a target OD 600 of 0.02 (see the volume of preloading culture and media in Excel (Dilution Calculation) Sheet_1) in 12 mL LB medium + Chloramphenicol in 50 mL falcon
tube (amber, or covered with foil to block light).
√ Incubate the cultures at 37°C and 220 rpm.
√ Take 500 µL samples of the cultures at 0, 2, 4, and 6 hours of incubation. (At each time point, you will take a sample from each of the 8 devices, two colonies per device, for a total of 16 samples per time point)

√ Place samples on ice.
√ At the end of sampling point you need to measure your samples (OD and FI measurement)
√ Use the same instrument settings that you used when measuring the fluorescein standard curve. This
includes using the sample volume (100 ul) you used for the fluorescein measurement.
√ Samples are laid out according to Fig below.

√ Pipette 100 µl of each sample into each well. Replicate samples of colony #1 are pipetted into wells in
rows A, B, C and D. Replicate of colony #2 are pipetted into wells in rows E,F,G, and H.



RESULTS

Dilution Calculation to OD=0.02 to initiate the 4 replicates of 2 cultures (colony 1 and colony 2):

Colony 1

target Abs600 0.02
target volume (mL) 12

sample	Abs600 Reading	Volume of Preloading Culture	Volume of Preloading Media
positive control	0.371	0.730	11.270
negative control	0.298	0.940	11.060
device 1	0.422	0.632	11.368
device 2	0.357	0.763	11.237
device 3	0.433	0.614	11.386
device 4	0.347	0.789	11.211
device 5	0.314	0.885	11.115
device 6	0.291	0.966	11.034
Media+Chl	0.042		

Colony 2

target Abs600 0.02

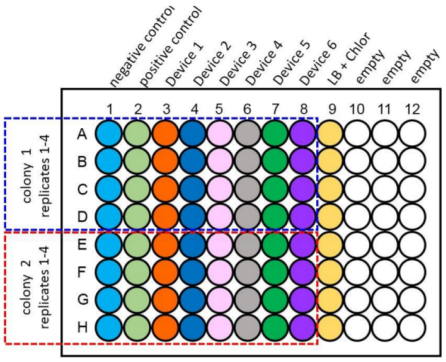
target volume (mL) 12

sample	Abs600 Reading	Volume of Preloading Culture	Volume of Preloading Media
positive control	0.352	0.776	11.224
negative control	0.295	0.951	11.049
device 1	0.412	0.650	11.350
device 2	0.409	0.654	11.346
device 3	0.422	0.632	11.368
device 4	0.370	0.732	11.268
device 5	0.466	0.567	11.433
device 6	0.303	0.921	11.079
Media+Chl	0.042		

Abs600 Raw Readings:									
Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.0628	0.0626	0.063	0.0618	0.0624	0.0587	0.0575	0.0618	0.0428
Colony 1, Replicate 2	0.0609	0.0599	0.0599	0.0598	0.0607	0.0575	0.0559	0.0591	0.0415
Colony 1, Replicate 3	0.0615	0.0615	0.0624	0.0603	0.0603	0.0568	0.0576	0.0583	0.0414
Colony 1, Replicate 4	0.0624	0.0643	0.0619	0.06	0.0668	0.0582	0.0556	0.0601	0.0428
Colony 2, Replicate 1	0.0626	0.064	0.0655	0.062	0.0616	0.0592	0.0549	0.0596	0.0426
Colony 2, Replicate 2	0.0623	0.0627	0.0611	0.0638	0.0648	0.0597	0.0555	0.06	0.0421
Colony 2, Replicate 3	0.0606	0.0623	0.0628	0.0594	0.0616	0.0591	0.0546	0.0602	0.0408
Colony 2, Replicate 4	0.0636	0.0652	0.0634	0.0669	0.0649	0.0605	0.0572	0.0622	0.0419
Hour 2:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.1564	0.089	0.0918	0.0996	0.1505	0.0696	0.1232	0.1615	0.0429
Colony 1, Replicate 2	0.1544	0.0843	0.0871	0.0965	0.152	0.0652	0.1218	0.1658	0.0416
Colony 1, Replicate 3	0.1573	0.0887	0.0911	0.0968	0.149	0.0644	0.1202	0.1592	0.0414
Colony 1, Replicate 4	0.1567	0.0865	0.0935	0.0994	0.1503	0.0678	0.118	0.1584	0.0426
Colony 2, Replicate 1	0.1625	0.0884	0.0946	0.1012	0.1603	0.0671	0.1179	0.1596	0.0423
Colony 2, Replicate 2	0.1587	0.087	0.0935	0.1029	0.1536	0.0688	0.1172	0.1613	0.0421
Colony 2, Replicate 3	0.1589	0.0877	0.0946	0.0985	0.1519	0.0663	0.1225	0.1552	0.0413
Colony 2, Replicate 4	0.1576	0.0888	0.0928	0.1001	0.1565	0.0689	0.1248	0.1644	0.0421
Hour 4:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.3548	0.1619	0.2178	0.2549	0.3859	0.0804	0.3383	0.3796	0.0429
Colony 1, Replicate 2	0.355	0.1599	0.2062	0.2571	0.3776	0.0775	0.3286	0.3769	0.0419
Colony 1, Replicate 3	0.3557	0.1604	0.2153	0.2565	0.3757	0.0766	0.3283	0.3503	0.0415
Colony 1, Replicate 4	0.3603	0.168	0.214	0.2586	0.377	0.0783	0.331	0.3944	0.0427
Colony 2, Replicate 1	0.3608	0.1645	0.2214	0.254	0.382	0.0796	0.3343	0.3951	0.0425
Colony 2, Replicate 2	0.3643	0.1641	0.2178	0.2533	0.3832	0.081	0.3514	0.3903	0.0423
Colony 2, Replicate 3	0.3563	0.1627	0.2134	0.2525	0.3786	0.0788	0.3401	0.3744	0.0412
Colony 2, Replicate 4	0.3703	0.1577	0.216	0.2664	0.4182	0.0807	0.3494	0.3867	0.0424
Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.4933	0.3107	0.4209	0.4434	0.5488	0.1162	0.5295	0.5395	0.0431
Colony 1, Replicate 2	0.5021	0.3132	0.4057	0.4513	0.5537	0.1168	0.5291	0.5349	0.0422
Colony 1, Replicate 3	0.515	0.3144	0.4101	0.456	0.5566	0.1157	0.5324	0.54	0.0417
Colony 1, Replicate 4	0.5095	0.3217	0.4203	0.4554	0.5562	0.1188	0.5273	0.5313	0.043
Colony 2, Replicate 1	0.5083	0.3363	0.4102	0.4648	0.5594	0.1197	0.5217	0.5319	0.0426
Colony 2, Replicate 2	0.5053	0.3226	0.4382	0.4595	0.5223	0.1221	0.5291	0.5348	0.0419
Colony 2, Replicate 3	0.5058	0.3158	0.4245	0.4491	0.5478	0.1173	0.5221	0.5212	0.0407
Colony 2, Replicate 4	0.5035	0.3265	0.4344	0.4607	0.5592	0.1246	0.5486	0.5494	0.0422

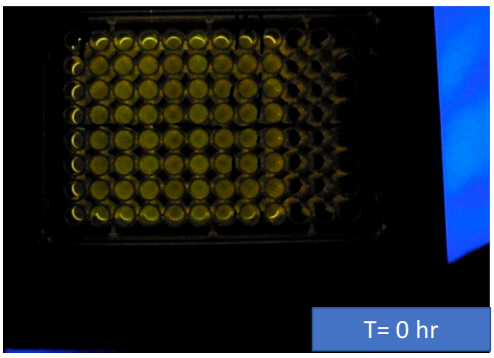
FLOURESCENCE READOUT

Plates the same as for OD600 Readouts



RESULTS

Fluorescence Raw Readings:									
Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	639.039	876.819	1002.001	984.099	627.191	844.673	641.301	575.006	556.9
Colony 1, Replicate 2	658.122	876.075	996.348	989.839	643.452	841.438	653.845	596.907	554.709
Colony 1, Replicate 3	652.247	871.77	1001.334	971.5	632.049	827.25	644.022	587.588	555.819
Colony 1, Replicate 4	685.599	896.498	1007.292	974.149	665.718	869.759	654.278	594.145	573.041
Colony 2, Replicate 1	659.15	904.583	1104.876	976.576	677.544	860.363	614.853	607.006	554.534
Colony 2, Replicate 2	666.345	883.248	985.797	974.838	680.843	882.338	671.78	625.105	563.569
Colony 2, Replicate 3	645.236	878.384	1015.622	969.491	661.721	873.782	643.47	617.404	578.173
Colony 2, Replicate 4	684.367	917.016	1012.982	1006.289	668.384	875.069	683.671	606.954	579.899
Hour 2:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	675.535	1178.837	1380.768	1537.652	665.724	1047.891	837.617	648.867	593.584
Colony 1, Replicate 2	709.119	1185.903	1369.002	1522.732	718.449	1026.331	848.512	677.144	591.557
Colony 1, Replicate 3	701.375	1184.292	1381.985	1503.531	696.34	1016.46	834.599	662.224	599.415
Colony 1, Replicate 4	726.215	1179.853	1386.768	1564.586	711.063	1066.379	843.069	660.222	611.27
Colony 2, Replicate 1	712.457	1187.455	1381.75	1526.597	735.436	1065.101	814.686	685.928	573.096
Colony 2, Replicate 2	715.224	1162.665	1384.474	1539.715	722.297	1060.352	789.485	686.216	615.464
Colony 2, Replicate 3	706.206	1151.63	1377.79	1488.296	711.987	1026.531	848.301	671.165	605.868
Colony 2, Replicate 4	709.31	1225.13	1413.006	1584.61	718.885	1070.845	867.182	664.987	626.014
Hour 4:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	679.393	1801.302	2318.029	2484.398	707.603	1236.102	1072.394	642.131	603.668
Colony 1, Replicate 2	700.75	1794.859	2304.969	2547.489	731.377	1176.681	1064.576	662.42	616.874
Colony 1, Replicate 3	704.179	1775.401	2288.218	2521.389	730.359	1216.018	1066.725	615.883	593.906
Colony 1, Replicate 4	705.545	1768.847	2277.982	2520.054	732.107	1187.94	1076.648	694.978	622.504
Colony 2, Replicate 1	712.948	1766.234	2245.244	2487.1	743.408	1178.376	1082.689	693.25	589.861
Colony 2, Replicate 2	715.679	1744.9	2212.406	2459.711	744.278	1202.483	1091.909	702.495	625.857
Colony 2, Replicate 3	695.219	1758.699	2302.713	2475.792	730.761	1180.393	1069.855	664.074	623.747
Colony 2, Replicate 4	705.462	1888.984	2435.06	2691.537	797.165	1231.467	1150.67	665.576	596.606
Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	688.042	2958.299	4086.77	4480.092	751.651	1438.117	1266.879	667.285	597.99
Colony 1, Replicate 2	721.775	2983.996	4151.869	4540.519	772.489	1491.011	1272.811	698.842	598.49
Colony 1, Replicate 3	737.362	2986.367	4122.943	4539.867	774.329	1472.8	1260.473	685.352	590.31
Colony 1, Replicate 4	717.205	2990.239	4231.925	4671.553	793.163	1497.549	1289.59	682.078	616.338
Colony 2, Replicate 1	715.585	3056.579	4085.275	4590.163	796.494	1483.725	1269.903	695.458	567.451
Colony 2, Replicate 2	727.04	2935.253	4163.875	4560.014	757.4	1525.377	1291.595	706.649	609.566
Colony 2, Replicate 3	728.696	2944.103	4157.588	4648.288	787.928	1501.519	1292.701	682.097	618.432
Colony 2, Replicate 4	733.543	3244.165	4538.759	4963.57	817.42	1570.379	1373.909	710.827	613.334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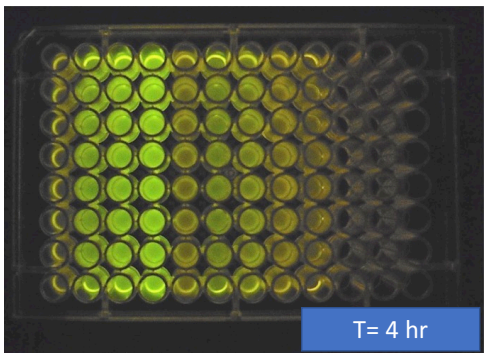
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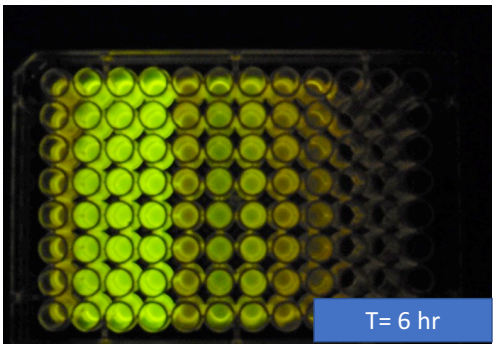
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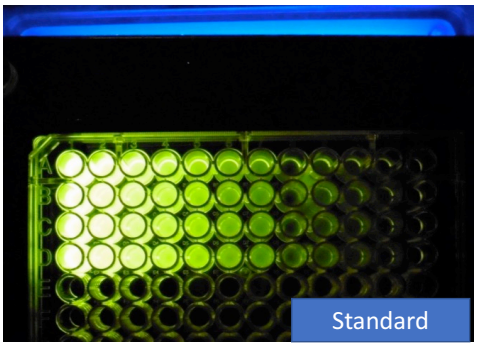
Standard