

COLOR CODING KEY

Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culturing/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Week 15

September 13, 2018

Biobrick Group (Natalie/Manvi/Priya)

- Miniprep of cpc560 for biobrick
- RE digest with EcoRV and EcoRI

Cyanobacteria Transformation Group (Natalie/Priya)

- Inoculated two PsbA2 colonies for cyanobacteria

Experimental Verification (Priya/Elon/Karthik)

- First standard luciferase expression experiment (nighttime standard) at 8:30 PM
- Standard luciferase expression experiment protocol:
 - Pipette 95 uL of cells into well plate.
 - Add 5 uL of decanal.
 - Parafilm plate along the edges.
 - Measure relative luminometer units
- Wells A1-A6: WT, B1-D6: idiA (B - col #1, C - col#2, D - col#3, E1-F6&A7-B12: cpc-560 (E - col2#1, F - col2#3, A - col3#1, B - col3#2), C7-F12: cpc (C - col2#1, D - col2#3, E - col3#1, F - col3#2)

30 minutes after induction

4	0	0	2	0	8	289	303	333	318	178	366
4	2	2	2	0	2	460	324	343	318	379	299
0	4	2	2	0	8	182	186	211	140	333	77
2	4	4	0	2	4	29	31	33	48	15	36
506	680	709	475	735	517	61	94	27	82	63	48
783	527	540	469	881	1415	100	228	155	100	96	155

1 hour after induction

2	0	0	4	0	2	270	306	352	255	236	278
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4	2	2	0	2	2	329	337	389	320	331	257
6	10	4	10	8	6	130	163	144	130	324	71
4	6	4	8	8	8	38	42	33	46	29	23
525	458	605	521	751	682	65	80	52	65	71	50
684	504	594	586	758	1969	130	222	172	134	117	96

1.5 hours

0	0	0	4	2	2	82	134	107	100	130	100
4	6	2	0	4	15	109	186	188	96	90	82
2	36	13	6	6	0	27	126	132	61	379	21
6	4	6	4	10	2	27	25	15	15	15	15
230	270	314	486	619	396	31	31	21	23	40	29
329	149	243	295	435	1948	96	71	92	67	103	52

Abs595 (will only give a relative idea of cell density)

0.4487	0.5908	0.4684	0.4644	0.5958	0.8685	0.8377	0.7298	0.9313	0.913	0.8787	0.8531
0.6481	0.6702	0.677	0.759	0.557	0.5383	0.77	0.9074	0.7278	0.8222	0.7207	0.7272
0.6489	0.6879	0.6688	0.562	0.6451	0.5601	1.1069	1.1534	1.261	1.1132	1.5161	1.2184
0.6316	0.6563	0.6444	0.6192	0.5291	0.657	0.4424	0.4947	0.4538	0.4737	0.4506	0.4624
1.0016	1.0144	1.1073	0.8059	1.0853	1.0799	0.9828	0.9956	1.0759	1.0485	1.2601	0.9422
1.0225	0.9735	1.0236	0.9898	1.1014	1.1367	0.8213	1.0281	1.0405	1.0516	0.8638	0.981

September 14, 2018

Biobrick Group (Matt/Natalie/Lin)

- RE digest from yesterday(cpc 560) run on gel
- Made 25x LB and CAM plates
- Transformed cpc

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Experimental Verification (Priya/Sara/Elon)

- Standard luciferase expression experiment (daytime standard) at 9:30 AM
- Standard luciferase expression experiment protocol:
 - Pipette 95 uL of cells into well plate.
 - Add 5 uL of decanal.
 - Parafilm plate along the edges.
 - Measure relative luminometer units
 - Wells A1-A6: WT, B1-D6: idiA (B - col #1, C - col#2, D - col#3, E1-F6&A7-B12: cpc-560 (E - col2#1, F - col2#3, A - col3#1, B - col3#2), C7-F12: cpc (C - col2#1, D - col2#3, E - col3#1, F - col3#2)

10 minutes after induction

4	2	8	4	2	6	167	339	140	236	159	155
2	2	0	0	2	4	182	153	186	174	205	205
2	2	2	4	2	6	190	207	144	132	84	136
0	2	10	2	0	2	23	25	174	15	19	15
534	963	582	532	289	197	48	27	46	63	42	48
458	400	293	272	404	303	92	180	167	157	149	100

Abs595 (will not inform us of actual cell count / OD but can give a bit of an estimate)

0.4932	0.5652	0.8112	0.6363	0.5168	0.6495	0.8345	1.4746	0.7347	1.3839	0.8585	0.8434
0.4738	0.6985	0.5989	0.5541	0.519	0.4828	0.4226	0.5706	0.5407	0.4975	0.7972	0.616
0.4119	0.6786	0.519	0.4855	0.5085	0.5353	1.4569	1.3805	0.8886	1.5179	0.9557	1.068
0.9121	0.4668	0.5235	0.4869	0.4828	0.5032	0.2641	0.2638	1.9919	0.3216	0.2936	0.2754
1.0907	1.7788	1.113	1.0463	0.9737	0.6691	1.1644	0.7406	0.9573	0.9869	0.9619	0.9064
0.9761	1.3645	0.9266	0.993	0.9848	0.9552	0.9608	1.4367	0.9812	0.8705	0.8428	1.0694

- cscB data - Spec reading at 510 nm

Water blank	0	0	0
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Glucose	0.970	0.987	0.982
Starch Acetate	0.041	0.040	0.044
Starch Fructosidase	0.138	0.139	0.145
Cyano Acetate	0.002	-0.008	0
Cyano Sucrose	0.005	0.005	-0.005

Analyte	Analyte (g/L)
Starch glucose	0.022
Starch Sucrose	0.096
Cyano glucose	0.001
Cyano sucrose	0.004

pH:

Col 3 cscB - 7.36

Wild Type - 7.34

Col 2 #1 - 7.57

Col 4 - 7.37

Col 2 #2 - 7.50

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Biobrick Group (Matt/Karthik)

- Inoculation of transformed plates from yesterday