COLOR CODING KEY

Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

Week 7

July 16, 2018

Interlab (Natalie/Stephanie/Lin)

- Made 180 mL of LB Media
    - 4.5 g of LB powder
    - 180 mL of milli-Q water
    - 180 µL of chloramphenicol
- Put around 10 mL per falcon tube
- Transferred 2 colonies from each plate into liquid media
- Incubated the colonies overnight at 37 ℃ and 220 rpm

Constructs Group (Karthik/Matthew)

- Made a gel with the new promega, the bands showed up
- Tried to do a gel purification, but the gel slice wasn't melting

Plasmid Group (Stephanie/Sara)

- Miniprepped, RE Digest, DNA purification of pAM2991 and pAM1414

July 17, 2018

Interlab (Natalie/Sara/Lin)

- Made 270 mL of LB Media
    - 6.75 g of LB powder
    - 270 mL of milli-Q water
    - 270 µL of chloramphenicol
- Diluted the overnight cultures from 7/16
    - .5 mL of culture
    - 4.5 mL of LB media
    - Took absorbance readings and saw that the E. Coli were not growing enough
        - which means need to redo Day 2 incubation
        - Mary Lou said that we tightened the caps too much and suffocated the cells

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	Colony 1	Colony 2
Positive control	.044	.044
Negative Control	.041	.043
Test Device 1	.043	.044
Test Device 2	.045	.046
Test Device 3	.045	.045
Test Device 4	.045	.043
Test Device 5	.043	.044
Test Device 6	.050	.043
LB and CAM=	.039	

-

- Redid day 2
    - Added 10 mL of LB and chloramphenicol to 16 tubes
    - Transferred 2 colonies from each plates into liquid media
    - Incubated the colonies overnight at 37 ℃ and 220 rpm

Plasmid Group (Stephanie/Sara)

- Miniprepped, RE Digest, DNA purification of pAM2991 and pAM1414

July 18, 2018

Interlab (Natalie/Lin)

- Made 270 mL of LB Media
    - 6.75 g of LB powder
    - 270 mL of milli-Q water
- Measured the absorbance of the cultures

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- **Colony 1**

Sample	Abs600 Reading	Preloading culture (µL)	Volume of preloading media (µL)
Positive control	.153	2162	9838
Negative control	.164	1967	10033
Test device 1	.114	3333	8667
Test device 2	.165	1951	10049
Test device 3	.155	2124	9876
Test device 4	.129	2759	9241
Test device 5	.108	3636	8364
Test device 6	.159	2051	9949
Media + CAM	.042		

- **Colony 2**

Sample	Abs600 Reading	Preloading culture (µL)	Volume of preloading media (µL)
Positive control	.149	2182	9818
Negative control	.150	2162	9838
Test device 1	.109	3429	8571
Test device 2	.132	2581	9419
Test device 3	.147	2222	9778
Test device 4	.112	3288	8712

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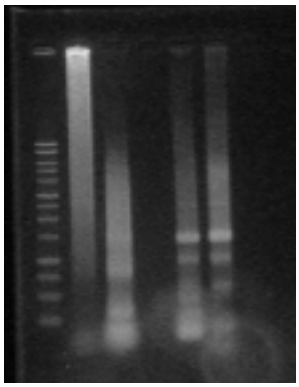
Test device 5	.096	4211	7789
Test device 6	.156	2051	9949
Media + CAM	.039		

Constructs Group (Karthik/Matthew)

- Made a gel of DNA ladder with promega agarose, soaked in fresh diamond dye
    - Purified the gel slices using Dr. Gergen's 16000 x g centrifuge
    - Very bad DNA curves on the Nanodrop
- Made a new gel of Q1 lone and combo cscB (Phire PCR) and Q3 cscB and Q3 sps (Q5 PCR) with promega agarose, soaked in fresh diamond dye (1 or 2 bands indicates that gel purification is not required and only PCR purification is necessary)
- 
    - Lane 1: Promega 1 kb ladder
    - Lane 2: Q3 sps PCR product from Q5 polymerase
    - Lane 3: Q3 cscB PCR product from Q5 polymerase
    - Lane 4: Q1 combo cscB PCR product from Phire polymerase
    - Lane 5: Q1 lone cscB PCR product from Phire polymerase
- Conclusions: Phire > Q5? (But for temps during PCR annealing temp for Phire set below calculated and annealing temp for Q5 set at calculated, so could be a factor)

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July 19, 2018

Interlab (Natalie/Manvi/Sara/Dominika/Lin)

- Made 16 agar plates with 130 mL of LB and agar
    - Added 3.25g of LB media and 1.56g of Agar
    - 130 µL of chloramphenicol
    - 130 mL of milli-Q water
    - Put in around 10 mL per agar plate
- Made 170 mL of LB Media
    - 4.25 g of LB powder
    - 170 mL of milli-Q water
    - 170 µL of chloramphenicol
    - Put 10 mL of media into 16 tubes
    - Transferred 2 colonies from each agar plate into liquid media
    - Incubated the colonies overnight at 37 ℃ and 220 rpm
- 2 tubes spilled in the incubator, so redid them with 20 mL of LB media
    - .5 g of LB powder
    - 20 mL of milli-q water
    - 20 µL of chloramphenicol
    - Put 10 mL of media into 2 tubes
    - Transferred 2 colonies from each plates into liquid media
    - Incubated the colonies overnight at 37 ℃ and 220 rpm

Plasmid Group (Stephanie/Sara)

- Miniprepped, RE Digest, DNA purification of pAM2991 (realized stock on Monday/Tuesday did not have the correct enzymes)

Constructs Group (Karthik/Matthew/Woody)

- PCR adjustments to Q3 cscB and Q3 sps:
    - 1. Raise lid temp to 99, raise annealing temp to calculated, number of cycles at 30
    - 2. Touchdown: same fixes but also start annealing at 10 above and decrease by 1 each cycle till calculated annealing temp reached

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    - Run on gel and soaked in Diamond dye: No discernable difference b/w 1 and 2, same non-specificity as before (multiple bands)
- Second PCR adjustments to same constructs:
    - 3. Same as 2 (with touchdown) but use 1.5 µL template instead of 2.5, chill on ice and put in thermocycler when machine at >95 during preheating
    - No gel run
- Gel purification of NEB ladder using EtBr gel and both kits
    - For both kits, used 500 and 1500 bp bands
    - Used faster centrifuge at 16,000g
    - None of the purifications worked well according to nanodrop curves

Cell Culture/Plating (Elon)

Unless otherwise stated, for all measurements, assume an uncertainty of +/- 10 µmoles/s/m^2

- PAR Measurements Room-light:
    - 2 lights on: 0.22-0.24 * 10^{16} photons/sec/cm^2: 36.52 µmoles/s/m^2
    - 1 light on: 0.16-0.18 * 10^{16} photons/sec/cm^2: 39.84 µmoles/s/m^2
- Shaker Tray:
    - 0.56 * 10^{16}, 0.76 * 10^{16}, 0.62 * 10^{16} photons/sec/cm^2
        - Range: 92.96 - 126.16 µmoles/s/m^2
- Effect of papers:
    - No paper: 0.56 * 10^{16} photons/sec/cm^2: 92.96 µmoles/s/m^2
    - Printer Paper: 0.18 * 10^{16} photons/sec/cm^2: 29.88 µmoles/s/m^2
        - Dimming effect: 63.08 µmoles/s/m^2
    - Parchment/Wax Paper: 0.48 * 10^{16} photons/sec/cm^2: 79.68 µmoles/s/m^2
        - Dimming effect: 13.28 µmoles/s/m^2
- Distance vs Light intensity (4 fluorescent lights):

Distance (inch)	Distance (cm)	Light Intensity (center)	Light Intensity (edge)	Moles of photons (center)	Moles of photons (edge)
1	2.54	2.30E+16	1.80E+16	381.80	298.80
2	5.08	1.90E+16	1.60E+16	315.40	265.60

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3	7.62	1.70E+16	1.45E+16	282.20	240.70
4	10.16	1.50E+16	1.20E+16	249.00	199.20
5	12.7	1.40E+16	1.00E+16	232.40	166.00
6	15.24	1.20E+16	9.00E+15	199.20	149.40
7	17.78	1.00E+16	8.00E+15	166.00	132.80
8	20.32	9.60E+15	7.60E+15	159.36	126.16
9	22.86	8.50E+15	7.00E+15	141.10	116.20
10	25.4	7.90E+15	6.80E+15	131.14	112.88
11	27.94	7.20E+15	6.30E+15	119.52	104.58
12	30.48	6.70E+15	5.70E+15	111.22	94.62
13	33.02	6.20E+15	5.60E+15	102.92	92.96
14	35.56	5.60E+15	5.20E+15	92.96	86.32
15	38.1	5.30E+15	4.80E+15	87.98	79.68
16	40.64	4.60E+15	4.60E+15	76.36	76.36

- Troubleshooting in CO2 incubator: (note: by red lights I mean warm white lights, and by blue lights I mean cool blue lights)
    - Tray 3 from bottom:
        - Red lights full power: 0.12-0.14* 10^{17} photons/sec/cm^2:199.20 - 232.40 µmoles/s/m^2
        - Red & Blue full: 0.34 * 10^{17} photons/sec/cm^2: 564.40 µmoles/s/m^2. Can range from 0.32 - 0.36 at edges: 531.20 µmoles/s/m^2 - 597.60 µmoles/s/m^2
        - Blue full: 0.21 * 10^{17} photons/sec/cm^2: 348.60 µmoles/s/m^2. Can range from 0.2-0.23 at edges: 332.00 µmoles/s/m^2 - 381.80 µmoles/s/m^2
    - Tray 3 from bottom, but with another tray on the top slot, blocking a decent amount of light:
        - Full power on both: 0.1 * 10^{17} photons/sec/cm^2: 166.00 µmoles/s/m^2. ranges from 0.9 to 1.1: 149.40 µmoles/s/m^2 - 182.60 µmoles/s/m^2

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- Same as before, but PWM for blue lights at 155 and PWM for red at 255 (full power):
    - Tray 3: 0.8 +/- 0.1 * 10^{16} photons/sec/cm^2: 132.80 +/- 16.6 µmoles/s/m^2
    - Top tray: 0.8 +/- 0.2 * 10^{17} photons/sec/cm^2: 1,328.00 +/- 332 µmoles/s/m^2

July 20, 2018

Constructs Group (Karthik/Matthew)

- 3rd PCR from previous day run on gel
    - Bands very distinct, generally pure
    - Conclusion: using less template and adjusting procedure worked
- Another PCR run and examined on gel
    - Adjustments: even less template (1 µLtemplate)
    - Gel result: bands too faint
    - Conclusion: 1 µL (10 ng) template not enough for PCR

Interlab (Natalie/Lin/Stephanie/Matthew/Karthik)

- Made 150 mL of LB Media
    - 3.75 g of LB
- Took overnight cultures out of the incubator, diluted them and completed day 2 for interlab
- Made 25 agar plates with 210 mL of LB and agar
    - Added 5.25 g of LB media and 2.52 g of Agar
    - 210 µL of chloramphenicol
    - 210 mL of milli-Q water
    - Put in around 10 mL per agar plate

Plasmid Group (Stephanie/Sara)

- Miniprepped, RE Digest, DNA purification of pAM1579