

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 11

August 12, 2018

Spectrophotometry Reading at 20:30 (Lin)

- Done at 750 nm with 1500 μ L of culture
- No bicarb room temp (room temp 5/19) A= 0.092
- 5 mM bicarb room temp (room temp 5/19) A= 0.136
- 10 mM bicarb room temp (room temp 5/19) A= 0.123
- 20 mM bicarb room temp (room temp 5/19) A= 0.090

August 13, 2018

Biobrick Group (Karthik/Matt/Natalie)

- Ran RE digest on gel
 - mixed 10 μ L loading dye in 50 μ L RE product and loaded 40 μ L into gel
 - Lone cscB, idiA not showing
 - Others showed and extracted with Monarch kit
 - Really bad
 - Cpc 2. 560-3 NOT DNA
 - Cpc 3. 560-2 POSSIBLY DNA?
- We transformed & plated HiFi from Friday
 - Opto EYFP, Orig EYFP
 - 1579 Q3 idiA, 1579 Q3 isiAB, 1579 Q3 psbA2
 - Neg Amp/Kan Ctrl

Cyanobacteria Transformation Group (Steph/Priya)

- Restreaked cpc 2 and 3, cpc-560 2 & 3
- Ordered Primers after 24 hours of stress NSI and NSII

Cell Culture/Plating (Lin/Natalie/Jenn)

- Filter sterilized the 1M sodium bicarbonate solution
- Remade the 33°C sodium bicarbonate experiments with filtered sodium bicarbonate solution

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- Made a 2% culture of cyanobacteria with sodium bicarbonate from UTEX Collier 5/19 culture sup. with 25 mL BG-11
 - No bicarb 33°C (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media
 - 5 mM bicarb 33°C (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 250 µL of sodium bicarbonate solution
 - 10 mM bicarb 33°C (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 500 µL of sodium bicarbonate solution
 - 20 mM bicarb 33°C (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 1000 µL of sodium bicarbonate solution
- Supplemented UTEX Collier 5/19 culture sup. with 25 mL BG-11 with 4 mL of BG-11 media

Spectrophotometry Reading at 18:40 (Lin/Natalie/Jenn)

- Done at 750 nm with 1500 µL of culture
- No bicarb 33°C (room temp 5/19) A= 0.035
- No bicarb room temp (room temp 5/19) A= 0.150
- 5 mM bicarb 33°C (room temp 5/19) A= 0.026
- 5 mM bicarb room temp (room temp 5/19) A= 0.194
- 10 mM bicarb 33°C (room temp 5/19) A= 0.024
- 10 mM bicarb room temp (room temp 5/19) A= 0.189
- 20 mM bicarb 33°C (room temp 5/19) A= 0.050
- 20 mM bicarb room temp (room temp 5/19) A= 0.145

August 14, 2018

Cyanobacteria Transformation Group (Natalie/Stephanie)

- Made BG-11 agar plates (high antibiotic concentration) with 220 mL of media
 - 3.30 g of agar powder
 - 220 mL BG-11 media
 - 44 µL of streptomycin and 44 µL of spectinomycin
 - 2200 µL of sodium bicarbonate solution
- Transformed *idiA*, *psbA2*, *rbc* constructs again (plates had no growth)

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Spectrophotometry Reading at 15:53 (Natalie/Sara)

- Done at 750 nm with 1500 μ L of culture
- UTEX Collier 5/19 A= 1.072
- Split from room temp Collier 7/31 A= 0.955
- 8/1 Split from UTEX Collier 7/23 Culture 1 A= 0.822
- UTEX room temp 7/23 #2 Split A= 1.162
- 8/4 from 7/31 UTEX Room temp Collier A=0.825
- 8/4 from 8/1 (UTEX Collier 7/23 Culture 1) A= 0.705
- 8/4 room temp Collier 5/19 A= 0.635

Spectrophotometry Reading at 19:00 (Lin)

- Done at 750 nm with 1500 μ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.182
- No bicarb room temp (room temp 5/19) A= 0.209
- 5 mM bicarb 33°C (room temp 5/19) A= 0.188
- 5 mM bicarb room temp (room temp 5/19) A= 0.333
- 10 mM bicarb 33°C (room temp 5/19) A= 0.201
- 10 mM bicarb room temp (room temp 5/19) A= 0.313
- 20 mM bicarb 33°C (room temp 5/19) A= 0.152
- 20 mM bicarb room temp (room temp 5/19) A= 0.225

Biobrick Group (Karthik/Matt/Priya/Stephanie)

- Miniprep from E. Coli:
 - Cpc 560 1,2, 3, 5
 - Cpc 2, 3, 5
 - idiA v2 5, v2 4
 - Lone cscB 2, 3, 4
 - psbA2 3, 4, 5
 - Rbc 3
- Inoculated 1579 isiAB, idiA, psbA2, 2991 opto eyfp and original eyfp cultures

Experimental Verification (Elon)

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Water blank	0	0	0
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

pH:

Col 3 cscB - 7.36

Wild Type - 7.34

Col 2 #1 - 7.57

Col 4 - 7.37

Col 2 #2 - 7.50

August 15, 2018

Biobrick Group (Karthik/Matt)

- Nanodrop miniprep from yesterday: everything great except cpc 560 #1
 - psbA2 #5: 304.1 ng/ μ L, 1.73 (260/280) good
 - Rbc #3: 272.0, 1.85 good
 - idiA v2 #4: 162.0, 1.84 good
 - New cpc #5: 270.2, 1.86 good
 - Lone cscB #3: 191.5, 1.73 good
 - New cpc #3: 328.7, 1.76 good
 - Lone cscB #2: 230.2, 1.71 good
 - psbA2 #3: 148.1, 1.84 good
 - Lone cscB #4: 375.4, 1.70 good

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- New cpc #2: 194.7, 1.88 good
- psbA2 #4: 107.0, 1.67 good
- Cpc560 #2: 264.7, 1.79 good
- Cpc560 #5: 369.1, 1.75 good
- Cpc560 #1: 67.6, 1.61 eh
- idiA v2 #5: 309.8, 1.72 good
- Cpc560 #3: 306.3, 1.79 good

Spectrophotometry Measurements at 18:40 (Natalie/Lin)

- Done at 750 nm with 1500 μ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.550
- No bicarb room temp (room temp 5/19) A= 0.188
- 5 mM bicarb 33°C (room temp 5/19) A= 0.446
- 5 mM bicarb room temp (room temp 5/19) A= 0.440
- 10 mM bicarb 33°C (room temp 5/19) A= 0.390
- 10 mM bicarb room temp (room temp 5/19) A= 0.413
- 20 mM bicarb 33°C (room temp 5/19) A= 0.560
- 20 mM bicarb room temp (room temp 5/19) A= 0.300

Cell Culturing/Plating (Natalie/Lin)

- Spun down the cultures, threw out the supernatant and supplemented them with 55 mL BG-11
 - No bicarb 33°C (room temp 5/19)
 - 5 mM bicarb 33°C (room temp 5/19)
 - 10 mM bicarb 33°C (room temp 5/19)
 - 20 mM bicarb 33°C (room temp 5/19)

Cyanobacteria Transformation Group (Stephanie)

- Plated idiA, psbA2, rbc liquid culture transformants

August 16, 2018

Spectrophotometry Measurements at 18:53 (Lin)

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Experimental Verification

Plasmid & Construct Design Group

- Done at 750 nm with 1500 μ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.716
- No bicarb room temp (room temp 5/19) A= 0.226
- 5 mM bicarb 33°C (room temp 5/19) A= 0.691
- 5 mM bicarb room temp (room temp 5/19) A= 0.535
- 10 mM bicarb 33°C (room temp 5/19) A= 0.690
- 10 mM bicarb room temp (room temp 5/19) A= 0.537
- 20 mM bicarb 33°C (room temp 5/19) A= 0.626
- 20 mM bicarb room temp (room temp 5/19) A= 0.401

Biobrick Group (Karthik/Matt/Woody/Manvi/Priya/Stephanie)

- Minipreped lone cscB and RE digested with EcoRI and PstI
- Minipreped Q3 isiAB, idiA, psbA2 and Q1 opto EYFP and orig EYFP

Experimental Verification (Elon)

- Mixed reagents of sucrose assay together into bottles labeled (Solution 1, 2, 3, and 4) stored in fridge and freezer
- Uploaded sucrose assay to opentrons and calibrated

August 17, 2018

Spectrophotometry Reading at 22:01 (Lin)

- Done at 750 nm with 1500 μ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.888
- No bicarb room temp (room temp 5/19) A= 0.241
- 5 mM bicarb 33°C (room temp 5/19) A= 0.895
- 5 mM bicarb room temp (room temp 5/19) A= 0.649
- 10 mM bicarb 33°C (room temp 5/19) A= 0.993
- 10 mM bicarb room temp (room temp 5/19) A= 0.635
- 20 mM bicarb 33°C (room temp 5/19) A= 0.779
- 20 mM bicarb room temp (room temp 5/19) A= 0.485

Biobrick Group (Priya/Stephanie)

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Experimental Verification

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- Miniprep of constructs for biobrick for sequencing
 - Cpc colonies 2-3
 - Cpc 560 colonies 2-3
 - cscB colonies 2-4
 - psbA2 colony 5

Plasmid Group (Priya/Steph)

- Colony PCR for cpc and cpc560

August 18, 2018

Spectrophotometry Reading at 18:45 (Lin)

- Done at 750 nm with 1500 μ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.928
- No bicarb room temp (room temp 5/19) A= 0.256
- 5 mM bicarb 33°C (room temp 5/19) A= 1.021
- 5 mM bicarb room temp (room temp 5/19) A= .719
- 10 mM bicarb 33°C (room temp 5/19) A= 1.114
- 10 mM bicarb room temp (room temp 5/19) A= 0.720
- 20 mM bicarb 33°C (room temp 5/19) A= 0.918
- 20 mM bicarb room temp (room temp 5/19) A= 0.550
- No bicarb room temp (room temp 5/19) A= 0.092
- 5 mM bicarb room temp (room temp 5/19) A= 0.136
- 10 mM bicarb room temp (room temp 5/19) A= 0.123
- 20 mM bicarb room temp (room temp 5/19) A= 0.090