

## 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 12

#### August 19, 2018

##### Spectrophotometry Reading at 20:07 (Lin)

- Done at 750 nm with 1500  $\mu$ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.986
- No bicarb room temp (room temp 5/19) A= 0.265
- 5 mM bicarb 33°C (room temp 5/19) A= 1.001
- 5 mM bicarb room temp (room temp 5/19) A= 0.763
- 10 mM bicarb 33°C (room temp 5/19) A= 1.091
- 10 mM bicarb room temp (room temp 5/19) A= 0.789
- 20 mM bicarb 33°C (room temp 5/19) A= 0.957
- 20 mM bicarb room temp (room temp 5/19) A= 0.615

#### August 20, 2018

##### Experimental Verification (Elon)

- Mixed starch solution together (can be found in iGEM fridge) - 0.25 g/L of starch
- Did a practice run for sucrose assay, seems fairly accurate but plate reader cannot be used (need a 510 nm filter). Spec will do (use triplicates)
- Results: blank: A= 0.000, D-glucose control: A=1.163, Glucose from flour A=0.051, Sucrose from flour = 0.124. Correlates to 0.022 g/L of glucose and 0.060 g/L of sucrose, which roughly matches the percentages in the 0.25g/L of starch (9.6% glucose and 28.8% sucrose by mass respectively)
- 150 mM NaCl, 8 mM of HEPES, BG-11 50 mL, 1 mL of cyano inoculum

##### Biobrick Group (Natalie)

- Miniprepped idiA V2 #4, idiA v2 #5, cpc 560 #1 , psba2 #3
- Supplemented all of the cultures with 2 mL LB + Spec

##### Spectrophotometry Reading at 15:46 (Natalie)

- Done at 750 nm with 1500  $\mu$ L of culture
- No bicarb 33°C (room temp 5/19) A= 0.910
- No bicarb room temp (room temp 5/19) A= 0.280

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- 5 mM bicarb 33°C (room temp 5/19) A= 1.068
- 5 mM bicarb room temp (room temp 5/19) A= 0.816
- 10 mM bicarb 33°C (room temp 5/19) A= 1.092
- 10 mM bicarb room temp (room temp 5/19) A= 0.831
- 20 mM bicarb 33°C (room temp 5/19) A= 1.068
- 20 mM bicarb room temp (room temp 5/19) A= 0.649

### August 21, 2018

#### Biobrick Group (Natalie)

- Nanodropped miniprep from yesterday
  - Cpc 560 #1 = 85.0 ng/μL, good
  - idiA v2 #4 = 173.6 ng/μL, good
  - idiA v2 #5 = 186.4 ng/μL, good
  - psbA2 #3 = 198.7 ng/μL, good
- RE digested Minipreps with EcoRI and PstI

#### Spectrophotometry Reading at 15:00 (Natalie)

- Done at 750 nm with 1500 μL of culture
- No bicarb 33°C (room temp 5/19) A= 0.808
- No bicarb room temp (room temp 5/19) A= 0.296
- 5 mM bicarb 33°C (room temp 5/19) A= 1.231
- 5 mM bicarb room temp (room temp 5/19) A= 0.870
- 10 mM bicarb 33°C (room temp 5/19) A= 1.435
- 10 mM bicarb room temp (room temp 5/19) A= 0.870
- 20 mM bicarb 33°C (room temp 5/19) A= 1.483
- 20 mM bicarb room temp (room temp 5/19) A= 0.678

### August 22, 2018

#### Spectrophotometry Reading at 14:32 (Natalie/Jenn)

- Done at 750 nm with 1500 μL of culture
- No bicarb room temp (room temp 5/19) A= 0.368
- 5 mM bicarb 33°C (room temp 5/19) A= 1.493

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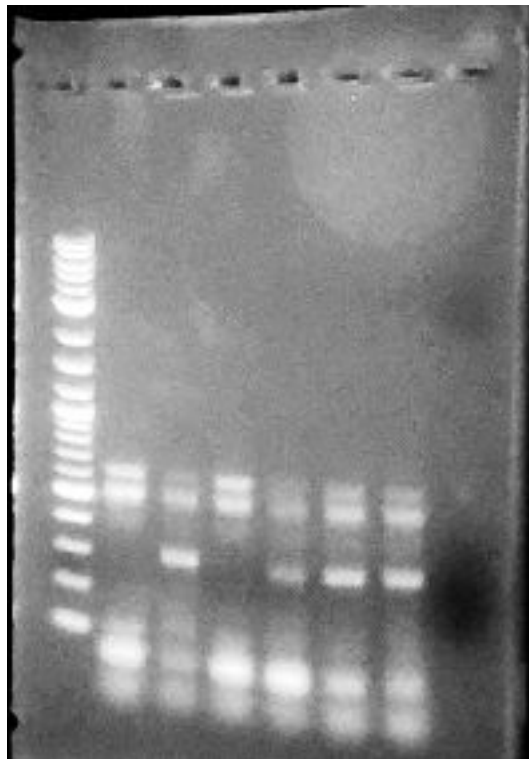
- 5 mM bicarb room temp (room temp 5/19) A= 1.067
- 10 mM bicarb 33°C (room temp 5/19) A= 1.321
- 10 mM bicarb room temp (room temp 5/19) A= 0.752
- 20 mM bicarb 33°C (room temp 5/19) A= 1.594
- 20 mM bicarb room temp (room temp 5/19) A= 1.041

### Biobrick Group (Natalie/Jenn)

- Mini-prepped cpc 560 1, cpc 560 2, cpc560 3, cpc 560 5, psbA2 3, psbA2 4, psbA2 5, lone cscB 2, lone cscB 3, new cpc 2, new cpc 5
- Reinoculated the cultures with LB and Spec

### Plasmid Group (Natalie/Jenn)

- Ran Colony PCR products on a gel
- cpc gel:



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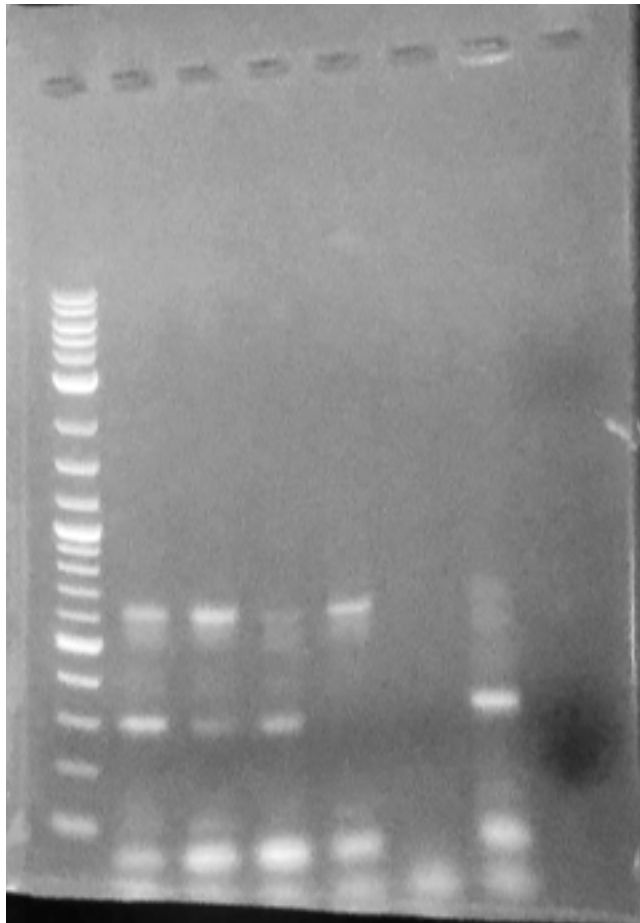
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- 1. MW 1kb ladder
  - 2. Cpc col 2 #1
  - 3. Cpc col 2 #2
  - 4. cpc col 2 #3
  - 5. Cpc Col 3 #1
  - 6. Cpc Col 3 #2
  - 7. Cpc Col 3 #3
- Cpc 560 gel:



- 
- 1. 1kb ladder
- 2. cpc 560 col 2 #1

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- 3. cpc 560 col 2 #2
- 4. cpc 560 col 2 #3
- 5. cpc 560 col 3 #1
- 6. cpc 560 col 3 #2
- 7. cpc 560 col 3 #3

### August 23, 2018

#### Biobrick Group (Natalie/Priya)

- Nanodropped constructs:
  - Cpc 560 1 = 86.7 ng/ $\mu$ L
  - Cpc 560 2 = 133.1 ng/ $\mu$ L
  - Cpc 560 3 = 109.8 ng/ $\mu$ L
  - New cpc 2 = 101.8 ng/ $\mu$ L
  - New cpc 5 = 88.8 ng/ $\mu$ L
  - Lone cscB 2 = 154.3 ng/ $\mu$ L
  - Lone cscB 3 = 105.1 ng/ $\mu$ L
  - psbA2 3 = 433.8 ng/ $\mu$ L
  - psbA2 4 = 111.0 ng/ $\mu$ L
  - psbA2 5 = 179.1 ng/ $\mu$ L
- Minipreped cpc 560 2, cpc 560 3, new cpc 2, lone cscB 2, lone cscB 3, psbA2 5

#### Cyanobacteria Transformation Group (Stephanie)

- Transformation of cyanobacteria with 8/4 from 8/1 (UTEX Collier 7/23 Culture 1) with psbA2 → accidentally left cells out, have to redo