

## 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 8

**July 23, 2018**

Interlab (Natalie/Lin/Stephanie/Jenn)

- Measured the OD of the cultures after a 1:8 dilution

|                         |      |
|-------------------------|------|
| Positive Colony 1       | .175 |
| Positive Colony 2       | .173 |
| Negative Colony 1       | .165 |
| Negative Colony 2       | .164 |
| Blank Media Replicate 1 | .049 |
| Blank Media Replicate 2 | .047 |

- Diluted the cultures to an OD of .1 and total volume of 1000  $\mu$ L
  - Positive Colony 1 = 99.2  $\mu$ L of culture and 900.8  $\mu$ L of LB and CAM Media
  - Positive Colony 2 = 99.2  $\mu$ L of culture and 900.8  $\mu$ L of LB and CAM Media
  - Negative Colony 1 = 108  $\mu$ L of culture and 892  $\mu$ L of LB and CAM Media
  - Negative Colony 2 = 107  $\mu$ L of culture and 893  $\mu$ L of LB and CAM Media
  - OD Readings of Diluted Cultures

|                                     |       |
|-------------------------------------|-------|
| Positive Colony 1 Replicate 1 (1.1) | 0.139 |
| Positive Colony 1 Replicate 2 (1.2) | 0.147 |
| Positive Colony 1 Replicate 3 (1.3) | 0.146 |
| Positive Colony 2 Replicate 1 (2.1) | 0.132 |
| Positive Colony 2 Replicate 2 (2.2) | 0.145 |
| Positive Colony 2 Replicate 3 (2.3) | 0.140 |
| Negative Colony 1 Replicate 1 (3.1) | 0.161 |

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|                                     |       |
|-------------------------------------|-------|
| Negative Colony 1 Replicate 2 (3.2) | 0.151 |
| Negative Colony 1 Replicate 3 (3.3) | 0.159 |
| Negative Colony 2 Replicate 1 (4.1) | 0.166 |
| Negative Colony 2 Replicate 2 (4.2) | 0.160 |
| Negative Colony 2 Replicate 3 (4.3) | 0.168 |

- Followed the colony forming units protocol and did serial dilutions for the triplicate starting samples
  - Plated dilution 3, dilution 4, and dilution 5 for all triplicate starting samples
  - Incubated the plates at 37 °C

### Cell Culturing/Plating (Jenn)

- Inoculated cyanobacteria from agar slab to liquid for 1 flask
- Split 3 liquid stocks in half for room temperature
  - Supplemented the flasks with BG-11 media
- Contamination experiment 2
  - DFJ were contaminated (incubator no good) discarded them
  - New plate with ACF

### July 24, 2018

#### Interlab (Lin/Priya/Stephanie)

- Counted the number of colonies on each plate
- Redid plates for positive colonies
  - Positive colony 1 repetition 3 dilution 5 (1.35)
  - Positive colony 1 repetition 2 dilution 3 (1.23)
  - Positive colony 1 repetition 2 dilution 4 (1.24)
  - Positive colony 1 repetition 2 dilution 5 (1.25)
  - Positive colony 2 repetition 3 dilution 3 (2.33)
  - Positive colony 2 repetition 3 dilution 4 (2.34)

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- Positive colony 2 repetition 3 dilution 5 (2.35)

### Cell Culturing/Plating

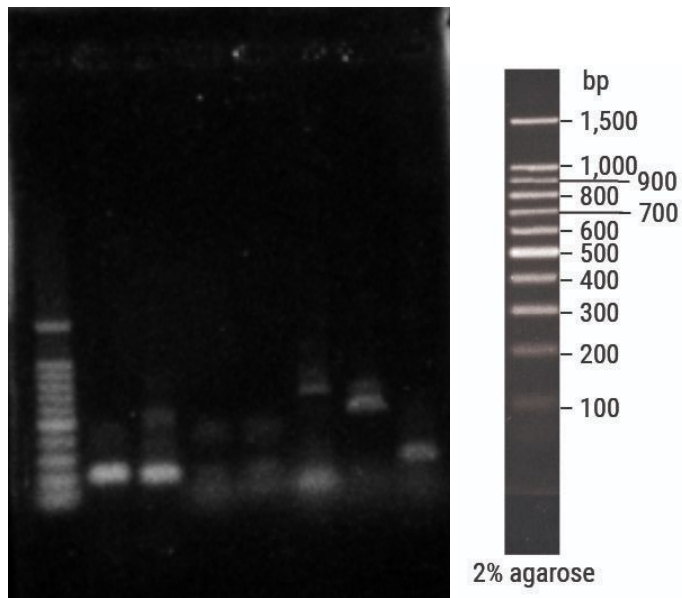
- Contaminated cyanobacteria poured out and bleached

**July 25, 2018**

### Interlab (Lin)

- Counted the number of colonies on each plate from yesterday
- Completion of Interlab Day 3

### Constructs Group (Karthik/Matthew/Woody)



- 0.7% agarose gel
  - 1. Promega Benchtop Ladder
  - 2. psbA2\_cscB (224 bp) - good
  - 3. idiA\_cscB (214 bp) - good
  - 4. psbA2\_sps (219 bp) - bad
  - 5. idiA\_sps (209 bp) - bad
  - 6. idiA\_Q2 (221 bp) - bad

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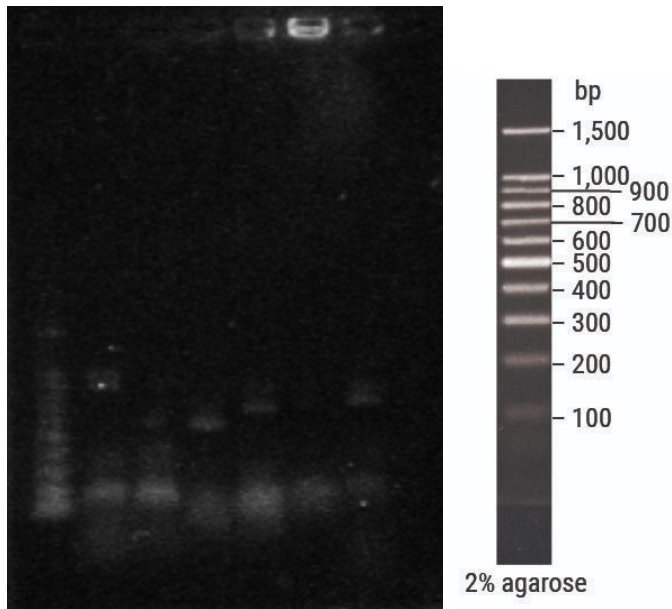
Biobrick Group

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- 7. rbc\_Q2 (511 bp) - good
- 8. psbA2\_Q2 (231 bp) - good



- 0.7% agarose gel
  - 1. Promega Benchtop Ladder 100 bp
  - 2. Opto EYFP (853 bp) - ok
  - 3. Orig EYFP (853 bp) - faint small band
  - 4. Cpc (543 bp) - ok
  - 5. Cpc560 (675 bp) - ok
  - 6. isiAB\_sps (727 bp) - faint small band
  - 7. isiAB\_cscB (722 bp) - ok

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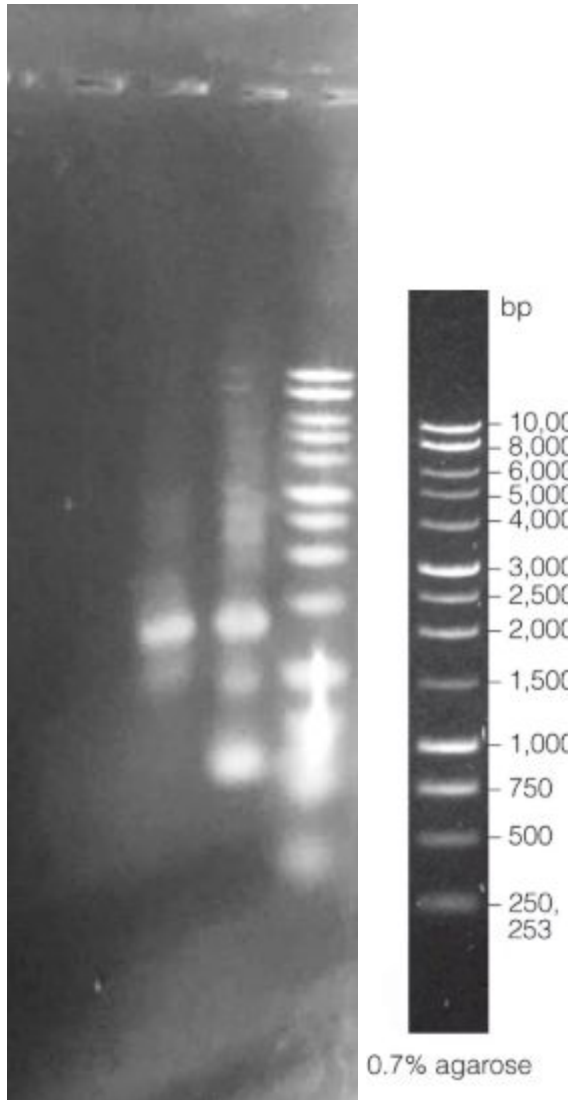
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- 0.7% agarose gel
  - 1. Promega Benchtop Ladder 1 kb
  - 2. Lone cscB Q1 (1378 bp) - good
  - 3. Combo cscB Q1 (1374 bp) - good

Cyanobacteria Transformation Group (Stephanie/Natalie/Priya)

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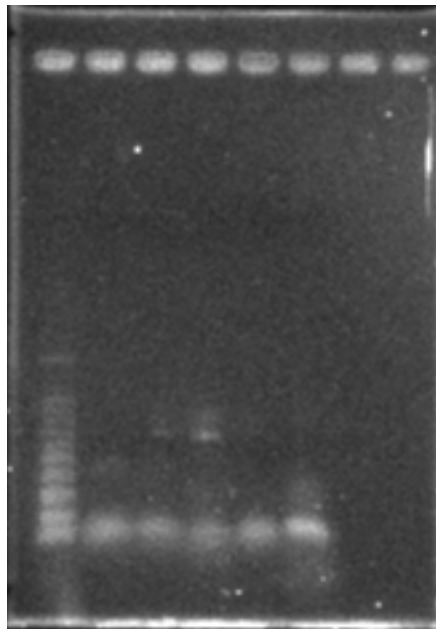
Plasmid & Construct Design Group

- Attempted HiFi protocol and transformation for old cpc
- Left plates overnight at 37 °C

**July 26, 2018**

**Constructs Group (Karthik/Matthew/Woody/Natalie/Lin)**

- PCR purified and Nanodrop all good constructs from yesterday's gels
  - idiA\_cscB: 40.2 ng/uL
  - psbA2\_cscB: 42.9 ng/uL
  - isiAB\_cscB: 61.0 ng/uL
  - Q3 cscB: 32.6 ng/uL
  - Q3 sps: 29.0 ng/uL
  - cpc: 44.8 ng/uL
- Ran PCR of psbA2\_sps, idiA\_sps, idiA\_Q2 together
- Ran PCR of original EYFP
- Ran PCR of isiAB\_sps
- Ran gel of the above 5 PCR products



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- 1. Promega Benchtop Ladder - 100 bp

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- 2. Q3 psbA2\_sps (219 bp) - bad
- 3. Q3 idiA\_sps (209 bp) - bad
- 4. Q2 idiA (221 bp) - bad
- 5. Q3 isiAB\_sps (727 bp) - ok
- 6. Orig EYFP (853 bp) - good

### Cyanobacteria Transformation Group (Priya/Stephanie)

- HiFi positive control successful!
  - negative control yielded no colonies, but cpc plated did not grow - possible failure
  - Attempted to plate another set of cpc transformants.

### July 27, 2018

#### Constructs Group (Natalie/Matthew/Karthik/Steph (PCR purify))

- Re-PCR Q3 psbA2\_sps, Q3 idiA\_sps, Q2 idiA (bad PCR gel)
- Re-PCR Q2 psbA2, Q2 rbc, Q1 opto EYFP (bad Nanodrop of PCR purified product)
- PCR purify Orig EYFP and isiAB\_sps (they turned out decent enough in gel above)
  - Bad nanodrops
- Ran gel:

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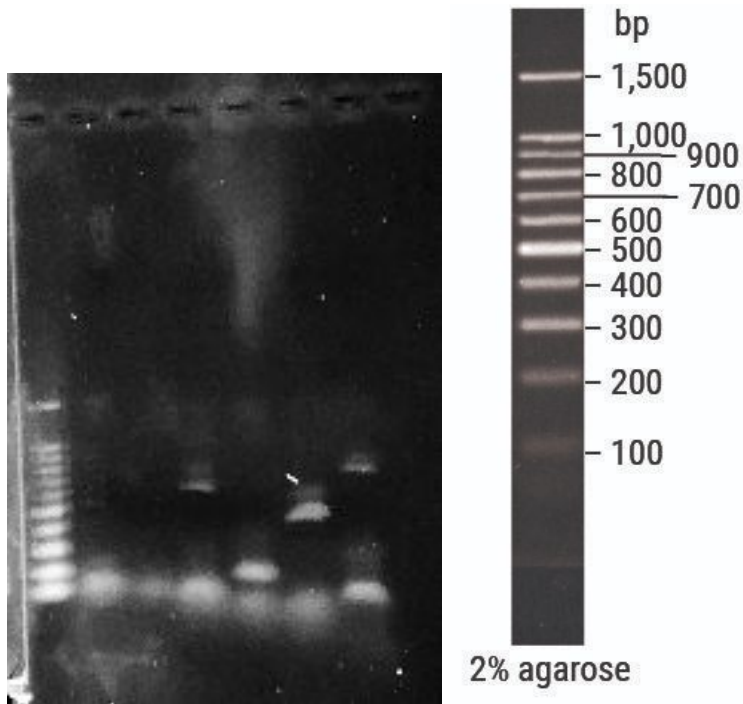
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- 1. Promega 100 bp Benchtop Ladder
- 2. Q3 psbA2\_sps (219 bp) - bad
- 3. Q3 idiA\_sps (209 bp) - bad
- 4. Q2 idiA (221 bp) -bad
- 5. Q2 psbA2 (231 bp) - good
- 6. Q2 rbc (511 bp) - good
- 7. Q1 opto EYFP (853 bp) - good

### Cyanobacteria Transformation Group (Priya/Stephanie)

- HiFi Assembly Successful!
  - 7/25 cpc plate yielded colonies
- Attempted Colony PCR with old cpc- total failure
- Inoculated the non-lysed bacteria-water solution