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 µL
  - Positive Colony 1 = 99.2 µL of culture and 900.8 µL of LB and CAM Media
  - Positive Colony 2 = 99.2 µL of culture and 900.8 µL of LB and CAM Media
  - Negative Colony 1 = 108 µL of culture and 892 µL of LB and CAM Media
  - Negative Colony 2 = 107 µL of culture and 893 µL 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 ℃

Cell Culturing/Plating (Jenn)

- Agar cyano to liquid for 1 flask
- Split 3 liquid stocks in half for room temperature
    - Supplemented the flasks with BG-11
- 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

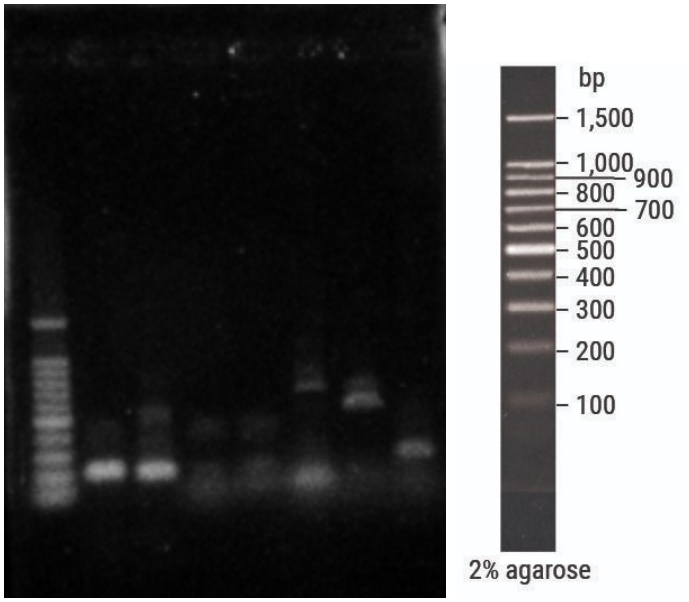
- Culture F is contaminated
    - Poured it out and bleached it

July 25, 2018

Interlab (Lin)

- Counted the number of colonies on each plate from yesterday

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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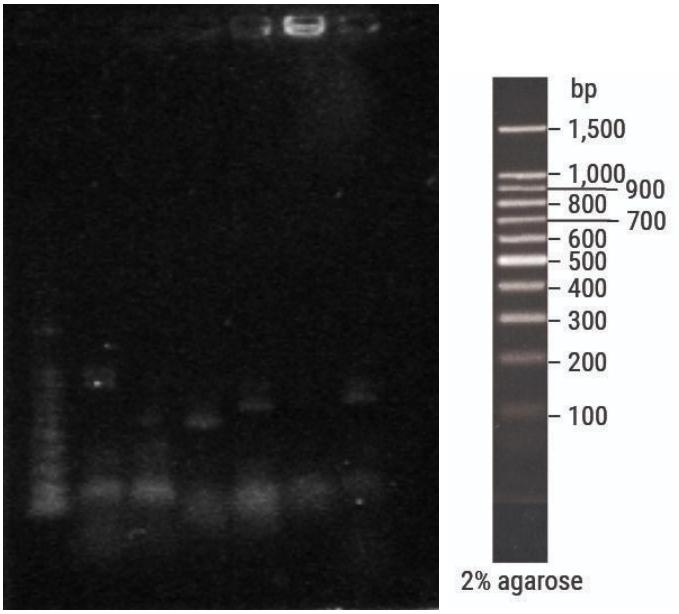
- 7. rbc_Q2 (511 bp) - good
- 8. psbA2_Q2 (231 bp) - good



- 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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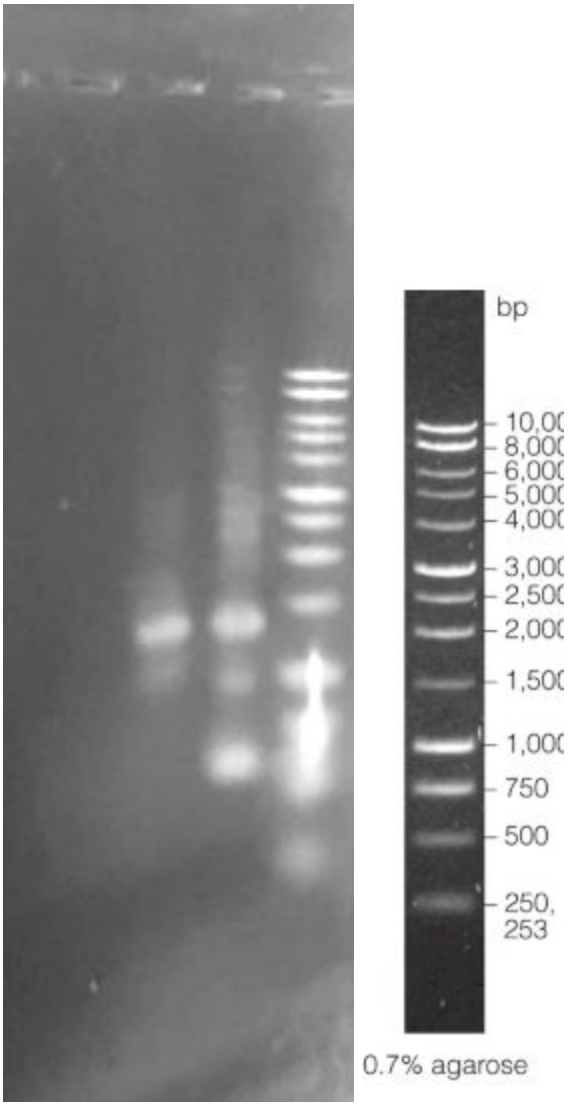

- 0.7% agarose gel
    - 1. Promega Benchtop Ladder 1 kb
    - 2. Lone cscB Q1 (1378 bp) - good
    - 3. Combo cscB Q1 (1374 bp) - good

Cyanobacteria Transformation Group (Stephanie/Natalie/Priya)

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- Attempted HiFi protocol and transformation for old cpc

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
    - 1. Promega Benchtop Ladder - 100 bp
    - 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 of:

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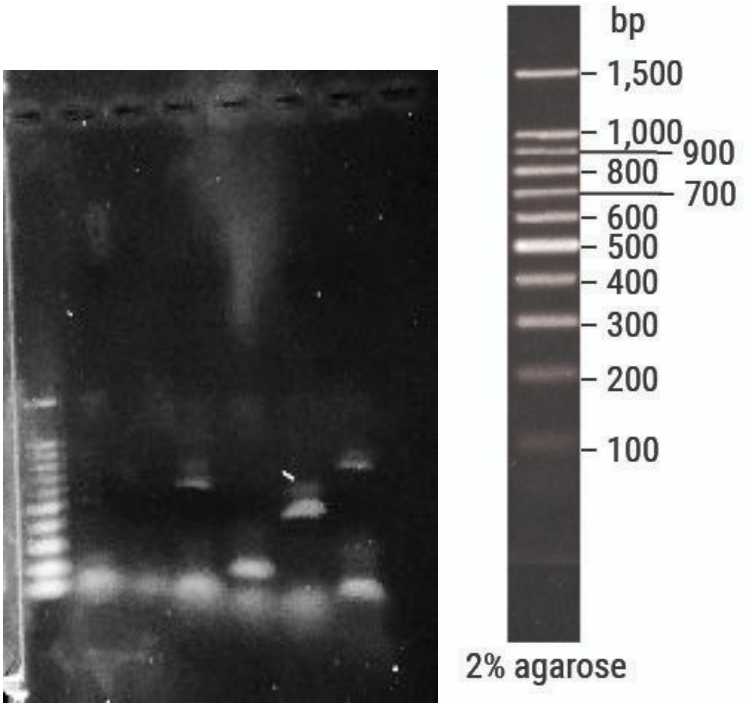

- 1. Promega 100 bp Benchtop Ladder
- 2. Q3 psbA2_sps (219 bp) - bad
- 3. Q3 idiA_sps (209 bp) - bad
- 4. Q2 idiA (221 bp) -bad
- 5. Q2 psbA2 (231 bp) - good
- 6. Q2 rbc (511 bp) - good
- 7. Q1 opto EYFP (853 bp) - good

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