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 10

August 5, 2018

Spectrophotometry Measurements at 16:32 (Natalie)

- Done at 750 nm with 1500 µL of culture
- No bicarb UTEX room temp #1 A=0.361
- 5 mM bicarb UTEX room temp #1 A= 0.573
- 10 mM bicarb UTEX room temp #1 A= 0.556
- 20 mM bicarb UTEX room temp #1 A= 0.195

Spectrophotometry Measurements at 16:50 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX Collier 7/31 Culture 1 A=0.628
- UTEX Collier room temp. 5/19 A= 0.508
- UTEX room temp. 7/23 #2 (split) A=0.457
- Split from room temp. Collier 7/31 A= 0.551
- 8/1 split from UTEX Collier 7/23 Culture 1 A=0.526
- 8/4 room temp. Collier 5/19 A= 0.351
- 8/4 from 7/31 UTEX room temp collier A=0 .306
- 8/4 from 8/1 (UTEX Collier 7/23 culture 1) A= 0.288
- UTEX room temp. 7/23 #2 (split) A= 0.468

Biobrick Group (Karthik/Natalie)

- Ran gel electrophoresis for Original EYFP, Optimized EFYP, and 2991
    - Failure → no distinct banding for colonies 1-5 for original eyfp and optimized eyfp
- Nanodrop of gel purification from yesterday

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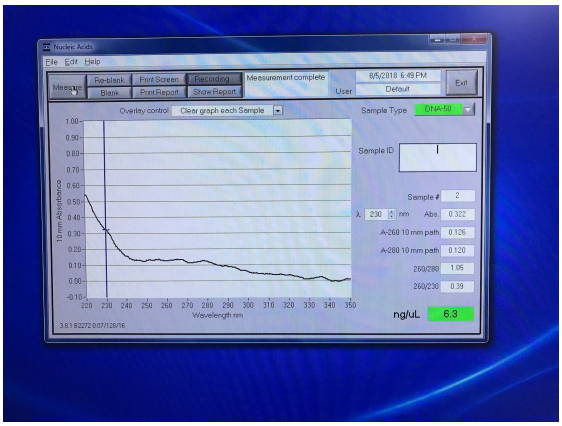
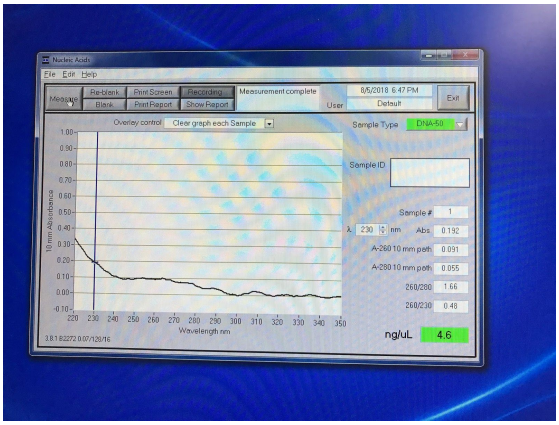
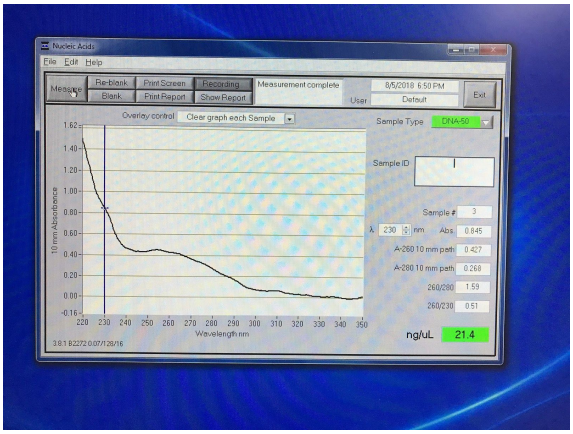
- 

following original protocol
- 

15uL elution
- 

15uL elution/incubate 37C
- Plated transformed cyanobacteria with cpc colonies 2 & 3 and cpc 560 colonies 2 & 3 and placed them in the CO_2 incubator

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- Inoculated 5 colonies from Orig EYFP and Opto EYFP each and placed them in the incubator
- Miniprep, RE digest, and ran the gel for idiA 6-9 with Kpn1 and EcoR1
    - Total failure, all negative result

August 6, 2018

Spectrophotometer Measurements at 12:00 (Sara/Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX Room temp 5/19 A= 0.650
- Split from room temp Collier 7/31 A= 0.608
- 8/1 split from collier 7/23 Culture #1 A= 0.558
- UTEX Collier 7/23 Culture 1 A= 0.741
- 8/4 room temp Collier (5/19) A= 0.447
- UTEX Room temp 7/23 (split) A= 0.560
- 8/4 split from 8/1 (UTEX Collier 7/23 culture #1) A= 0.349
- 8/4 from 7/31 UTEX room temp Collier A= 0.372

Spectrophotometer Measurements at 13:40 (Sara/Natalie)

- Done at 750 nm with 1500 µL of culture
- No bicarb UTEX room temp #1 A=0.615
- 5 mM bicarb UTEX room temp #1 A= 0.819
- 10 mM bicarb UTEX room temp #1 A= 0.678
- 20 mM bicarb #1 UTEX room temp #1 A= 0.190
- 20 mM bicarb #2 UTEX room temp #1 A= 0.016

Cell Culturing/Plating (Natalie)

- Made a 2% culture of cyanobacteria with sodium bicarbonate from UTEX Collier 7/23 Culture 1
    - 20 m bicarb #2 UTEX room temp #1 = 1 mL of culture + 50 mL of BG-11 media + 1000 µL of sodium bicarbonate solution
- Transferred 10 mL cyanobacteria from 8/1 split from collier 7/23 Culture #1 into UTEX Collier 7/23 Culture 1

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- Supplemented 8/1 split from Collier 7/23 Culture #1 with 10 mL of BG-11 media

Cyanobacteria Transformation (Stephanie)

- Used rbc colony 3, psbA2 colony 5, linear 1414
- Added 10 mL of cyanobacteria from 8/1 split from collier 7/23 Culture #1 into UTEX Collier 7/23 Culture 1
- Transformed all cyanobacteria from UTEX Collier 7/23 Culture 1

Biobrick Group (Priya/Sara/Karthik/Natalie/Stephanie)

- Miniprepped, RE digest with EcoRV and SalI, Gel Electrophoresis for opto EYFP and original EYFP colonies 6-10
    - Really confusing gel, decided to just start HiFi over for idiA, both EYFP, using positive controls
- HiFi Assembly for idiA and positive control to try to troubleshoot, realized we used the wrong ratio (5:1 for constructs with less than 200 bp)

August 7, 2018

Spectrophotometry Measurements at 16:26 (Natalie)

- Done at 750 nm with 1500 µL of culture
- No bicarb UTEX room temp #1 A=1.008
- 5 mM bicarb UTEX room temp #1 A= 0.855
- 10 mM bicarb UTEX room temp #1 A= 0.824
- 20 mM bicarb #2 UTEX room temp #1 A= 0.217

Cell Culture/Plating (Natalie/Lin/Matt)

- Threw out the 20 mM bicarb #1 UTEX room temp #1 because the solution became clear which means that the cells were dead/dying
    - Incubated the rest of the flasks at room temperature overnight
- Did a contamination experiment with the remaining flasks on a tryptic soy agar plate
    - Plated 10 µL of culture for each quadrant
    - Incubated them at 37 ℃
- Did another contamination experiment with the room temperature flasks

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Plasmid & Construct Design Group

  - A = room temp. 5/19
  - B = split from room temp. Collier 7/31
  - C= UTEX room temp. 7/23 #2 (split)
  - D= 8/4 from 7/31 UTEX room temp. Collier
  - E= 8/4 from 8/1 (UTEX Collier 7/23 Culture 1)
  - F= 8/4 room temp. Collier (5/19)
  - G= 8/1 split from UTEX Collier 7/23 culture 1
  - H = sodium bicarbonate solution
- Time 0 = 19:16 Made a 2% culture of cyanobacteria with sodium bicarbonate from UTEX Collier 5/19 culture sup. with 25 mL BG-11
  - No bicarb 33℃ (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media
  - No bicarb room temp. (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media
  - 5 mM bicarb 33℃ (room temp 5/19)= 1 mL of culture + 50 mL of BG-11 media + 250 µL of sodium bicarbonate solution
  - 5 mM bicarb room temp. (room temp 5/19)= 1 mL of culture + 50 mL of BG-11 media + 250 µL of sodium bicarbonate solution
  - 10 mM bicarb 33℃ (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 500 µL of sodium bicarbonate solution
  - 10 mM bicarb room temp. (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 500 µL of sodium bicarbonate solution
  - 20 mM bicarb 33℃ (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 1000 µL of sodium bicarbonate solution
  - 20 mM bicarb room temp. (room temp 5/19) = 1 mL of culture + 50 mL of BG-11 media + 1000 µL of sodium bicarbonate solution
  - Grew all of them at room temperature overnight, will transfer 33℃ into incubator tomorrow after getting results from contamination experiments
- Supplemented UTEX Collier 5/19 culture sup. with 25 mL BG-11 with 8 mL of BG-11 media

Spectrophotometry Measurements at 19:32 (Natalie)

- Done at 750 nm with 1500 µL of culture

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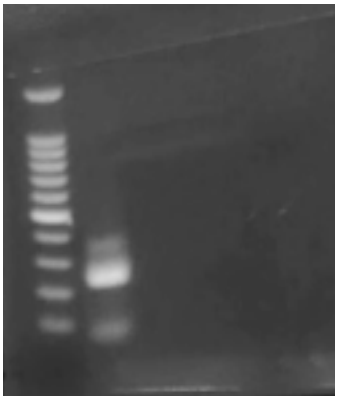
- No bicarb 33℃ (room temp 5/19) A= 0.013
- No bicarb room temp (room temp 5/19) A= 0.019
- 5 mM bicarb 33℃ (room temp 5/19) A= 0.011
- 5 mM bicarb room temp (room temp 5/19) A= 0.020
- 10 mM bicarb 33℃ (room temp 5/19) A= 0.047
- 10 mM bicarb room temp (room temp 5/19) A= 0.016
- 20 mM bicarb 33℃ (room temp 5/19) A= 0.015
- 20 mM bicarb room temp (room temp 5/19) A= 0.016

Construct Group (Karthik/Matt/Woody)

- 25µL PCR of idiA_sps
  - 12.5 µL Phire
  - 1 µL each primer
  - 0.75 µL DNA
  - 9.75 µL nuclease-free water
  - Fixes: extension time to 10s, still touchdown, 30 cycles at lowest annealing temp
- PCR run on gel and worked!
  - Need to re-PCR though because all used on gel
  - 
  - 1. Promega 100 bp Benchtop ladder
  - 2. idiA_sps PCR
- 50 µL PCR of idiA_sps and psbA2_sps using new fixes

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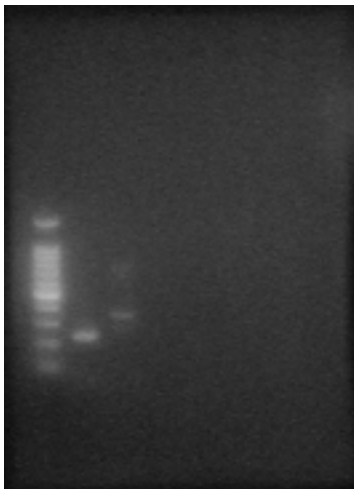
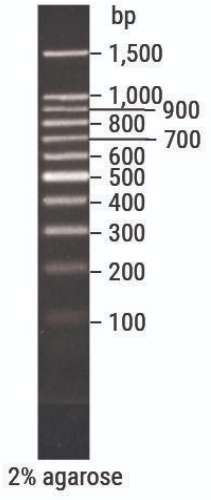
August 8, 2018

Construct Group (Karthik/Woody)

- Run yesterday's PCR on gel (isiAB_sps worked but not psbA2_sps)
  - 


  - 1. Promega 100 bp Benchtop ladder
  - 2. idiA_sps
  - 3. psbA2_sps
- PCR purify idiA_sps
  - Nanodrop good at 46.6 ng/µL → Success!!!
- Re-PCR psbA2_sps in 25 µL reaction
  - Touchdown to 3℃ below calculated annealing temp
- Gel of new psbA2_sps PCR

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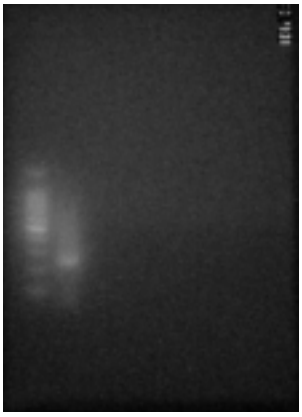
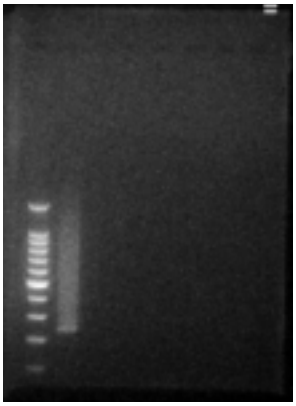


- 1. Promega 100 bp Benchtop ladder
- 2. psbA2_sps PCR

- Re-PCR psbA2_sps in 50 µL reaction using same touchdown technique
- Run PCR on gel



- 1. Promega 100 bp Benchtop ladder
- 2. psbA2_sps PCR

- PCR purify psbA2_sps

Spectrophotometry Measurements at 16:45 (Natalie/Lin)

- Done at 750 nm with 1500 µL of culture
- No bicarb 33℃ (room temp 5/19) A= 0.025
- No bicarb room temp (room temp 5/19) A= 0.030

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- 5 mM bicarb 33℃ (room temp 5/19) A= 0.027
- 5 mM bicarb room temp (room temp 5/19) A= 0.031
- 10 mM bicarb 33℃ (room temp 5/19) A= 0.028
- 10 mM bicarb room temp (room temp 5/19) A= 0.028
- 20 mM bicarb 33℃ (room temp 5/19) A= 0.035
- 20 mM bicarb room temp (room temp 5/19) A= 0.023
- No bicarb UTEX room temp #1 A= 1.011
- 20 mM bicarb #2 UTEX room temp #1 A= 0.561

Cell Culture/Plating (Natalie/Lin)

- Put the No bicarb 33℃ (room temp 5/19), 5 mM bicarb 33℃ (room temp 5/19) , 10 mM bicarb 33℃ (room temp 5/19), 20 mM bicarb 33℃ (room temp 5/19), No bicarb UTEX room temp #1, and 20 mM bicarb #2 UTEX room temp #1 in the incubator under 33℃
- Incubated the No bicarb room temp (room temp 5/19), 5 mM bicarb room temp (room temp 5/19), 10 mM bicarb room temp (room temp 5/19), and 20 mM bicarb room temp (room temp 5/19)

August 9, 2018

Construct Group (Karthik)

- Nanodrop psbA2_sps: good and 138.4 ng/µL
- Construct Group officially done

Spectrophotometry Reading at 14:23 (Natalie)

- Done at 750 nm with 1500 µL of culture
- No bicarb 33℃ (room temp 5/19) A= 0.175
- No bicarb room temp (room temp 5/19) A= 0.034
- 5 mM bicarb 33℃ (room temp 5/19) A= 0.160
- 5 mM bicarb room temp (room temp 5/19) A= 0.038
- 10 mM bicarb 33℃ (room temp 5/19) A= 0.225
- 10 mM bicarb room temp (room temp 5/19) A= 0.033
- 20 mM bicarb 33℃ (room temp 5/19) A= 0.185
- 20 mM bicarb room temp (room temp 5/19) A= 0.038

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- No bicarb UTEX room temp #1 A= 1.243
- 20 mM bicarb #2 UTEX room temp #1 A= 1.019

Biobrick Group

- Made 150 mL of LB media with Spectinomycin
- Made 5 plates of LB media and Ampicillin with 75 mL
- Made 100 mL of LB media and Kanamycin and Ampicillin

August 10, 2018

Cyanobacteria Transformation Group

- Observed 3 colonies in 2991 high concentration