COLOR CODING KEY

Spectrophotometry Measurements

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Cyanobacteria Transformation Group

Experimental Verification

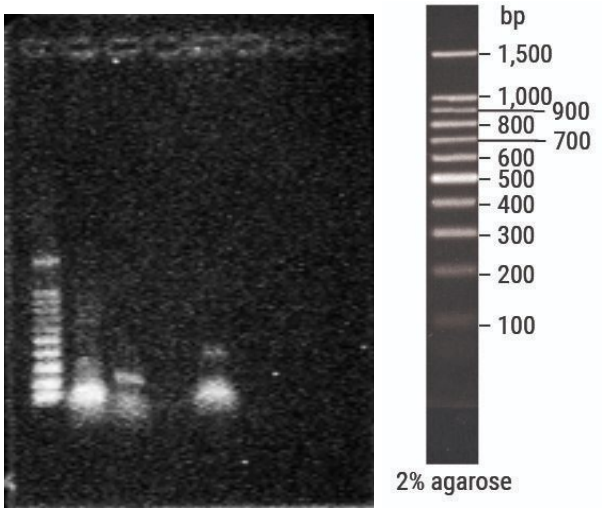
Plasmid & Construct Design Group

Week 9

July 30, 2018

Constructs Group (Natalie/Matthew/Karthik)

- Re-PCR Orig EYFP, isiAB_sps, psbA2_sps, idiA_sps, idiA



- 0.7% agarose gel
    - 1. Promega 100 bp Benchtop ladder
    - 2. idiA
    - 3. psbA2
    - 4. psbA2_sps (bad load)
    - 5. idiA_sps
    - 6. psbA2_sps (bad load)
- PCR purify Q2 psbA2, Q2 rbc, opto EYFP
    - Nanodrop: only Q2 rbc was good at 48.8 ng/µL (rest had bad curves)
    - So re-PCR opto EYFP and psbA2 (psbA2 in gel above)

Cyanobacteria Transformation Group (Priya/Stephanie)

- Performed HiFi Assembly for better PCR cpc, cpc-560, and lone cscB
    - Plated transformants
- Inoculated four more colonies from the old cpc plate
    - five colonies from the weekend died in the 50 mL conical tube

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July 31, 2018

Constructs Group (Karthik)

- Run PCR products orig EYFP. opto EYFP, isiAB_sps (bad load) from yesterday on gel
- Re-PCR idiA, psbA2_sps, idiA_sps, isiAB_sps
- PCR purify psbA2
    - Nanodrop good (21.9 ng/µL) but low concentration
- PCR purify orig EYFP and Opto EYFP
    - Opto EYFP concentration 26.7 ng/µL
    - Orig EYFP had bad nanodrop curve

Cell Culturing/Plating (Natalie)

- Split the UTEX RM Temp. 7/23 (split) in half
    - Split 7/31 from UTEX room temp 7/23 = 37.5 mL of culture with 37.5 of BG-11 media
    - Supplemented the original culture (37.5 mL) with 37.5 mL of BG-11 media
- Split the UTEX Collier 5/19 room temperature in half
    - Split from room temp. Collier 7/31 = 25 mL of culture with 25 mL of BG-11 media
    - Supplemented the original culture (25 mL) with 25 mL of BG-11 media

Biobrick Group (Priya/Stephanie)

- Inoculated five colonies from new cpc, cpc-560, and cscB plates into aerated 15 mL culture tubes
- Dumped old cpc → we had newer colonies/plates to work from and the O.D. was 0.000

August 1, 2018

Constructs Group (Woody/Matthew/Karthik)

- Purify orig EYFP
    - Nanodrop mediocre but concentration only 6.6 ng/µL, so save but try PCR again
- PCR troubleshooting

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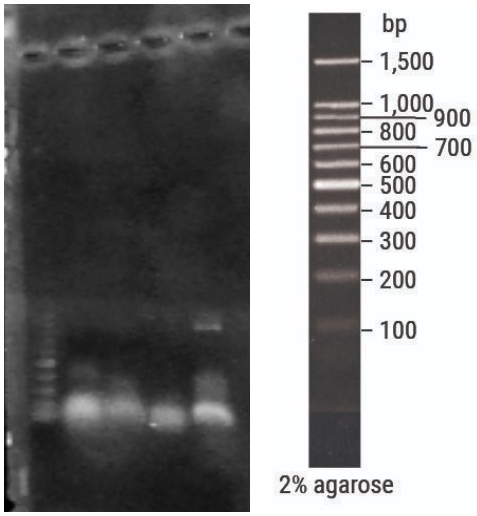
    - idiA_sps: primer concentration at ~200 ng/µL (half of expected) so increase volume to 2 µL/reaction
        - Use results to adjust psbA2_sps PCR
    - Q2 idiA: since primers working for other constructs, increased template volume to 2 µL/reaction
- Re-PCR isiAB_sps, orig EYFP using standard protocols
- idiA_sps, Q2 idiA, isiAB_sps, orig EYFP PCR products run on gel
    - 

    - 1. Promega 100 bp Benchtop ladder
    - 2. idiA_sps → eh?
    - 3. isiAB_sps → bad
    - 4. Q2 idiA → eh?
    - 5. orig EYFP → good
- PCR purify orig EYFP, idiA_sps, Q2 idiA
    - Only Q2 idiA gave good curve (concentration 37.8 ng/µL)
    - Other nanodrop curves were really bad
- PCR purify of Ladders using NEB kit with modifications: one with 6 uL MilliQ water and one with 6 uL MilliQ water and incubation at 37 ℃

Cell Culturing/Plating (Natalie)

- Split the UTEX room temperature 7/23 #2 (split) in half

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    - Split 8/1 from UTEX room temperature 7/23 #2 (split) = 37.5 mL of culture and 37.5 mL of BG-11 media
    - Supplemented original culture with 37.5 mL of BG-11 media
- Split the UTEX Collier 7/23 culture 1 in half
    - 8/1 split from UTEX collier 7/23 Culture 1 = 25 mL of culture and 25 mL of BG-11 media
    - Supplemented original culture with 25 mL of BG-11 media

Cyanobacteria Transformation Group (Natalie/Lin)

- Made sodium bicarbonate solution
    - 3.36 grams of sodium bicarbonate powder
    - 40 mL of autoclaved milli-Q water
    - Left under UV light for 30 minutes
- Made 6 BG-11 agar plates (low antibiotic concentration) with 80 mL of media
    - 1.2 g agar powder
    - 80 mL of BG-11 media
    - 3.2 µL of streptomycin and 3.2 µL of spectinomycin
    - 800 µL of sodium bicarbonate solution
- Made 1 more BG-11 agar plate (low antibiotic concentration) with 20 mL of media
    - .3 g agar powder
    - 20 ml of BG-11 media
    - .8 µL of streptomycin and .8 µL of spectinomycin
    - 200 µL of sodium bicarbonate solution
- Made 10 BG-11 agar plates (high antibiotic concentration) with 120 mL of media
    - 1.8 g of agar powder
    - 120 mL BG-11 media
    - 24 µL of streptomycin and 24 µL of spectinomycin
    - 1200 µL of sodium bicarbonate solution

Biobrick Group (Natalie/Lin)

- Made 8 LB and CAM plates with 110 mL of media
    - 2.75 g of LB powder

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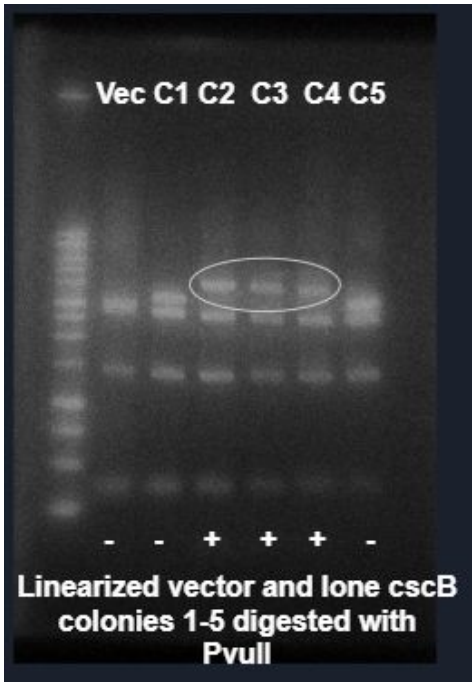
  - 1.32 g of agar powder
  - 110 mL of milli-Q water
  - 110 µL of chloramphenicol
- Made 5 LB and CAM plates with 50 mL of media
  - 1.25 g of LB powder
  - .6 g of agar powder
  - 50 mL of milli-Q water
  - 50 µL of chloramphenicol

BioBrick Group (Priya/Stephanie/Dominika/Matt L)

- Digested lone cscB, linear 2991 with PvuII and ran gel



  - 1. 1kb Promega Ladder
  - 2. linear 2991, negative control/negative result
  - 3. cscB colony 1
  - 4. cscB colony 2
  - 5. cscB colony 3

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    - 6. cscB colony 4
    - 7. cscB colony 5
    - Colonies 1, 5 had negative results for the presence of cscB (appeared as if pAM2991 recircularized)
    - Colonies 2-4 had positive results
- Digested cpc and cpc-560 with KpnI
    - Results inconclusive, need to use a different enzyme
        - Cause: re-circularization of 2991 vector in negative control has identical banding to positive result 2991
- Miniprepped cscB, cpc, cpc-560 DNA

August 2, 2018

Spectrophotometry Measurements (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX room temp. 7/23 #1 (split) A=.588
- UTEX room temp. 7/23 #2 (split) A=.380
- Split 7/31 from UTEX room temp 7/23 #1 A= .685
- Split 8/1 from UTEX room temp. 7/23 #2 A=.819
- Split from UTEX Collier 7/23 culture 1 A=.970
- UTEX Collier 7/23 culture 1 A= .633
- Split from room temp. Collier 7/31 A= .991
- UTEX Collier 5/19 culture sup. With 25 mL BG-11 A=.913
- Syn. UTEX 2434 7/23 A=1.371

Cyanobacteria Transformation Group (Elon/Stephanie)

- Combined Split 7/31 from UTEX room temp 7/23 #1 with split 8/1 from UTEX room temp. 7/23 #2 into one flask
- Started transformation of lone cscB colonies 2-4, linear 2991, and negative control (Water) following Golden Protocol

Biobrick Group (Stephanie/Natalie)

- Performed HiFi Assembly with idiA, optimized EYFP, original EYFP constructs

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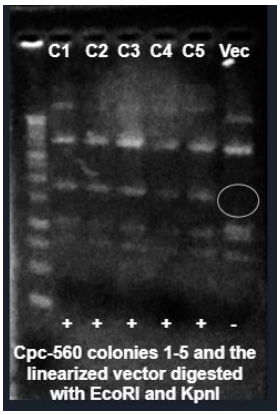
- Plated idiA, psbA2, rbc, optimized EYFP, original EYFP constructs and negative control
- Digested cpc and cpc-560 with EcoR1, Kpn1
    - Cpc-560



- 1. 1kb Promega Ladder
- 2. cpc 560 colony 1
- 3. Cpc 560 colony 2
- 4. Cpc 560 colony 3
- 5. Cpc 560 colony 4
- 6. Cpc 560 colony 5
- 7. Digest of 1414 DNA
- Cpc-560 colonies 1-5 all had a positive result for presence of insert
- 1414 had a negative result (as expected)

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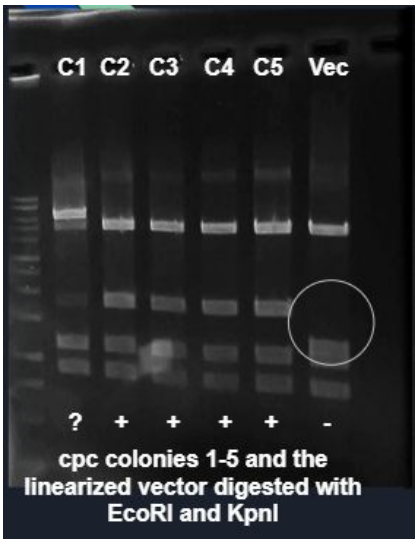
- Cpc



  - 1. 1kb Promega Ladder
  - 2. Cpc colony 1
  - 3. Cpc colony 2
  - 4. Cpc colony 3
  - 5. Cpc colony 4
  - 6. Cpc colony 5
  - 7. Digest of 1414 DNA
  - Cpc colony 1 had a negative result, 2-5 were positive for presence of insert
  - Digest of Linear 1414, negative result (as expected)
- Miniprepped cpc and cpc-560 DNA

Constructs Group (Karthik/Matt/Woody)

- PCR troubleshooting for idiA_sps/psbA2_sps, isiAB_sps
  - idiA_sps: increase template to 2 µL with increased primer at 2 µL
    - If it works, do same for psbA2_sps later
  - isiAB_sps: increase primer to 2 µL
    - If doesn't work increase template next reaction
- Re-PCR orig EYFP

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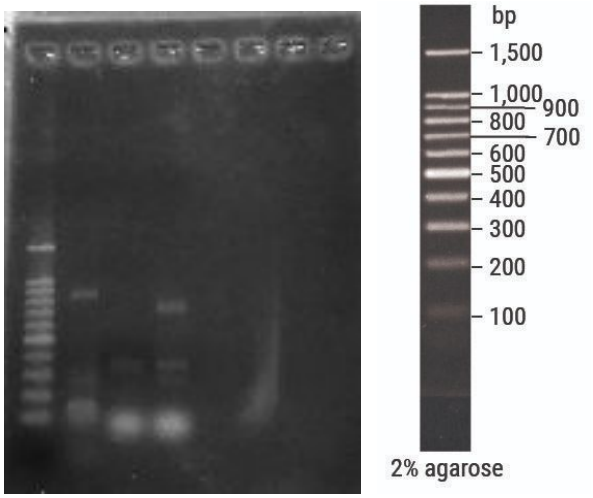
- Run gel with PCR products and see what happens
  - 

  - 1. Promega 100 bp Benchtop ladder
  - 2. isiAB_sps → good
  - 3. idiA_sps → bad
  - 4. orig EYFP → good
- PCR purify isiAB_sps and orig EYFP
  - Good nanodrop results for both
  - Concentrations: isiAB_sps 15.6 ng/µL, orig EYFP 26.2 ng/µL

August 3, 2018

Cyanobacteria Transformation Group (Natalie/Lin)

- Made 13 BG-11 Agar plates with 170 mL of media
  - 2.55 g agar
  - 170 mL BG-11
  - 34 µL streptomycin and 34 µL of spectinomycin
  - 1700 µL sodium bicarbonate solution

Cyanobacteria Transformation Group (Stephanie/Elon/Priya)

- Plated cyanobacteria transformed from day before
  - lone cscB colonies 2-4, linear 2991, negative control / water

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Biobrick Group (Stephanie/Priya/MattL)

- Inoculated five colonies from rbc, idiA, psbA2, optimized eyfp, original eyfp

Cell Culture/Plating (Elon/Natalie)

- Made a 2% culture of cyanobacteria with sodium bicarbonate from UTEX Collier 7/23 Culture 1
    - No bicarb UTEX room temp #1= 1 mL of culture + 50 mL of BG-11 media
    - 5 mM bicarb UTEX room temp #1 = 1 mL of culture + 50 mL of BG-11 media + 250 µL of sodium bicarbonate solution
    - 10 mM bicarb UTEX room temp #1 = 1 mL of culture + 50 mL of BG-11 media + 500 µL of sodium bicarbonate solution
    - 20 m bicarb UTEX room temp #1 = 1 mL of culture + 50 mL of BG-11 media + 1000 µL of sodium bicarbonate solution

Spectrophotometry Measurements at 19:09 (Lin/Natalie)

- Done at 750 nm with 1500 µL of culture
- No bicarb UTEX room temp #1 A=0.000
- 5 mM bicarb UTEX room temp #1 A= 0.000
- 10 mM bicarb UTEX room temp #1 A= 0.000
- 20 mM bicarb UTEX room temp #1 A= 0.009

August 4, 2018

Spectrophotometer Measurements at 10:35 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX room temp 7/23 #1 (split) A= 0.726
- UTEX room temp 7/23 #2 (split) A= 0.608
- 8/1 split from UTEX Collier 7/23 culture 1 A= 0.988
- UTEX Collier 7/23 Culture 1 A= 0.852
- Split from room temp. Collier 7/23 A= 1.034
- UTEX Collier room temp. 5/19 A= 1.060

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Spectrophotometry Measurements at 18:37 (Lin)

- Done at 750 nm with 1500 µL of culture
- No bicarb UTEX room temp #1 A=0.134
- 5 mM bicarb UTEX room temp #1 A= 0.136
- 10 mM bicarb UTEX room temp #1 A= 0.135
- 20 mM bicarb UTEX room temp #1 A= 0.127

Cell Culture/Plating at 11:00 (Natalie)

- Split the 8/1 split from UTEX Collier 7/23 Culture 1 in half
    - 8/4 from 8/1 (UTEX Collier 7/23 culture 1) = 25 mL of culture and 75 mL of BG-11 media
    - Supplemented original culture with 25 mL of BG-11 media
- Split the flask with split from room temp Collier 7/31 in half
    - 8/4 from 7/31 UTEX room temp. Collier = 25 mL of culture and 75 mL of BG-11 media
    - Supplemented original culture with 25 mL of BG-11 media
- Split UTEX Collier 5/19 culture (room temp.) in half
    - 8/4 room temp Collier (5/19) = 25 mL of culture and 75 mL of BG-11 media
    - Supplemented original culture with 25 mL of BG-11 media
- Supplemented UTEX rm. Temp 7/23 #2 (split)
    - Added 25 mL of BG-11 media to the culture
- Supplemented UTEX Collier 7/23 Culture 1
    - Added 15 mL of BG-11 media to the culture

Cyanobacteria Transformation Group (Lin/ Natalie/Matt/ Priya/ Stephanie)

- idiA, psbA2, rbc digested with EcoR1, Kpn1
    - Ran Gel Electrophoresis:
        - idiA colonies 1-5 all negative for presence of idiA

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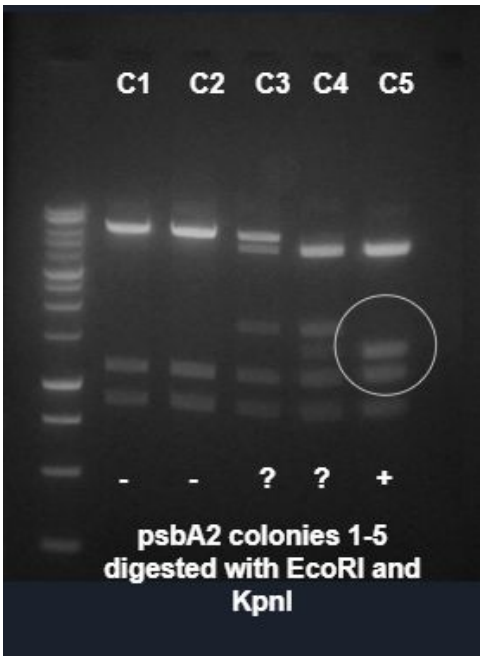

- 1. MW ladder (1kb)
- 2. psbA2 colony 1
- 3. psbA2 colony 2
- 4. psbA2 colony 3
- 5. psbA2 colony 4
- 6. psbA2 colony 5
- psbA2 colonies 1-2 negative for psbA2, 3-4 have a questionable result, colony 5 is positive for psbA2

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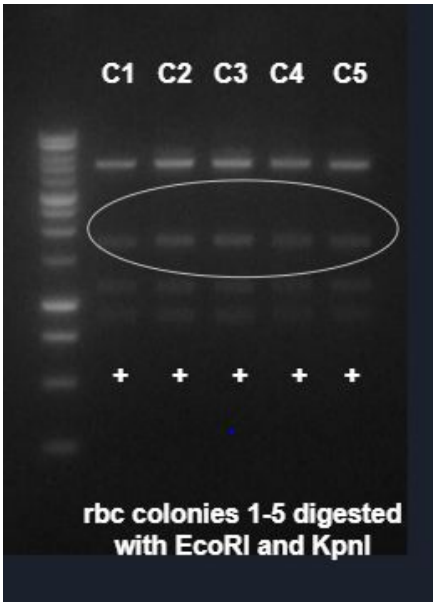
- 

    - 1. MW ladder (1 kb)
    - 2. Rbc colony 1
    - 3. Rbc colony 2
    - 4. Rbc colony 3
    - 5. Rbc colony 4
    - 6. Rbc colony 5
    - Rbc colonies 1-5 all positive for presence of rbc
- Original and optimized EYFP, 2991 digested with EcoR1, Spe1
    - Ran gel electrophoresis:
        - Failure, gels possibly punctured, will try again
    - Used the 1500 bp band from the promega ladder
- Gel Purification Troubleshooting
    - Original Protocol
    - 15 µL elution with modified protocol (lid taken off)
    - 15 µL elution and incubate at 37 for a minute instead of room temp (modified protocol)

Cyanobacteria Transformation (Stephanie)

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- Used cpc and cpc560 colonies 2-3
- Transformed all cyanobacteria from UTEX room temp 7/23 #1 (split)