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 4

June 24, 2018

Spectrophotometry Measurements at 12:00 (Stephanie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.679
- UTEX 1% room temp. 6/13 2m A= 0.885

Spectrophotometry Measurements at 23:10 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.890
- UTEX 1% room temp. 6/13 2m A= 0.872

Plasmid Group (Lin)

- New streaks of 1414, 1579, 2991 bacteria on spectinomycin plates

June 25, 2018

Spectrophotometry Measurements at 8:09 (Priya)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.870
- UTEX 1% room temp. 6/13 2m A= 0.847

Spectrophotometry Measurements at 16:00 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.480
- UTEX 1% room temp. 6/13 2m A= 0.493

Cell Culturing/Plating (Natalie)

- Supplementing measurement flasks with culture
- Added 20 mL of culture from UTEX 1% room temp. 6/13 1e into UTEX 1% room temp. 6/13 1m

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- Added 20 mL of culture from UTEX 1% room temp. 6/13 2e into UTEX 1% room temp. 6/13 2m

Construct Group (Karthik/Matthew/Natalie/Woody/Dominika)

- G-block Resuspension of PpsbA2_sps and PpsbA2_cscB (followed IDT protocol)
    - Pelleted down the dry DNA for the two tubes
    - Added 25 µL of autoclaved Milli-Q water to PpsbA2_sps and PpsbA2_cscB
    - Left the two tubes in the water bath at 53 ℃ for 15 minutes
    - Vortexed the two tubes and then centrifuged them
    - Nanodrop:
        - PpsbA2_sps= 3.7 ng/µL, 5.1 ng/µL
        - PpsbA2_cscB = 4.9 ng/µL, 8.0 ng/µL
- Primers resuspension of 1579 PcscB start, 1579 PcscB end, 1579 Psps start, 1579 Psps end (followed IDT protocol)
    - Pelleted down the dry DNA for all four tubes
    - Resuspended 1579 PcscB start with 796 µL nuclease free water
    - Resuspended 1579 PcscB end with 860 µL nuclease free water
    - Resuspended 1579 Psps start with 872 µL nuclease free water
    - Resuspended 1579 Psps end with 804 µL nuclease free water
    - Left the four tubes in the water bath at 50 ℃ for 15 minutes
    - Vortexed the four tubes and centrifuged them
- PCR of PpsbA2_sps and PpsbA2_cscB (Short Cycle)
    - PCR Mixture 50 µL
    -

Phire Mastermix	25 µL
Forward Primer	1µL
Reverse Primer	1µL
Template DNA	2.5 µL

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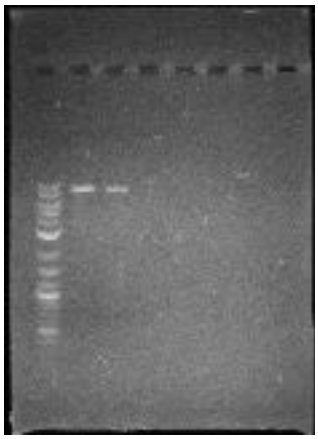
H_2O	20.5 µL

- Nanodrop:
  - PpsbA2_sps = 60.4 ng/µL
  - PpsbA2_cscB = 40.5 ng/ µL

Plasmid Group (Priya/Stephanie/Manvi/Lin)

- MiniPrep x 3
  - 1 miniprep using NEB kit
  - 2 minipreps using Qiagen kit
- Concentration of DNA = 5.4 ng/µL, 11.6 ng/µL, 58 ng/µL (not DNA curve)
- Attempted with Mary Lou and DNA curve → 26.7 ng/µL
- Split 1579
- made liquid inoculation for 1414 and 2991
- Gel extraction/gel electrophoresis for 1579
- 
  - Agarose gel (1%) used
  - EcoRV and Sal I with cutsmart buffer
  - total = 45 mL rex, incubated 1 hour at 37 ℃
  - Added 9 µL loading dye
  - 1. 1 kb MW maker

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- 2. 1579 DNA
- 3. 1579 DNA

June 26, 2018

Construct Group (Karthik/Matthew/Natalie/Woody)

- PCR purification of PpsbA2_cscB (following Qiaquick protocol)
  - Didn't add sodium acetate (skipped step 1)
  - Step 7, followed the increased DNA concentration step
  - 80 µL PB buffer
  - 16 µL used to purify
  - 30 µL of Milli-Q Water
  - 1 µL used on nanodrop
  - Nanodrop of PCR purified
    - PpsbA2_csB = 22.3 ng/µL
- Resuspension of PidiA_cscB and PidiA_sps (following IDT protocol)
  - Pelleted down the dry DNA for the two tubes
  - PidiA_cscB → 25 µL of autoclaved milli-Q water
  - PidiA_sps → 25 µL of autoclaved milli-Q water
  - Left the two tubes in the water bath at 50 ℃ for 15 minutes
  - Nanodrop:
    - PidiA_sps = 30.3 ng/µL, 6.2 ng/µL
    - PidiA_cscB = 13.8 ng/µL
- PCR of PidiA_cscB and PidiA_sps (Short Cycle)
  - Nanodrop
    - PidiA_cscB = 606.2 ng/µL(used 3 µL)
    - PidiA_sps= error (used 2.5 µL)
- PCR purification of PpsbA2_sps (following Qiaquick protocol)
  - 80 µL of PB buffer
  - 16 µL of PpsbA2_sps
  - 30 µL of autoclaved milli-Q water
  - Nanodrop:

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        - PpsbA2_sps = 8.2 ng/µL, 7.5 ng/µL
- PCR purification of PidiA_cscB and PidiA_sps (following Qiaquick protocol)
    - 80 µL of PB buffer
    - 16 µL of PidiA_cscB and PidiA_sps
    - 30 µL of autoclaved milli-Q water
    - Nanodrop:
        - PidiA_cscB = 8.4 ng/µL
        - PidiA_sps = 5.6 ng/µL

Spectrophotometry Measurements at 16:08 (Lin/Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.633
- UTEX 1% room temp. 6/13 2m A= 0.665

Plasmid Group (Priya/Stephanie/Lin)

- 1579 Miniprep success
    - Nanodrop: 102.8 ng/µL
- Split 1414/2991 cultures
- Testing restriction enzyme with digest
- Gel electrophoresis → failure as EtBr was not added to solution, but found that PstI worked.

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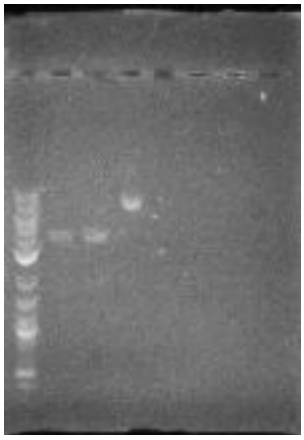
- 
- 1. MW Marker (1 kb)
- 2. PstI+EcoRV
- 3. PstI
- 4. PstI + SalI
- 5. PstI + PvuII

Cell Culturing/Plating (Natalie)

- Split the Syn. UTEX 2434 in half
- 10 mL of culture into new test tube with 10 mL of BG-11
- Supplemented the original culture with 10 mL of BG-11

Cell Culturing/Plating (Natalie)

- Split the UTEX Collier 5/19 culture sup. With 20 mL BG-11
- 25 mL of culture into new flask labeled UTEX room temp. 6/26 (split) and supplemented it with 25 mL of BG-11
- Supplemented original culture with 25 mL of BG-11

June 27, 2018

Construct Group (Karthik/Matthew/Natalie)

- Nanodrop of PCR DNA

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Plasmid & Construct Design Group

    - PidiA_sps = no good results
    - Used 2 µL
- PCR purify of PpsbA2_sps
    - 80 µL PB buffer
    - 16 µL of PpsbA2_sps
    - 30 µL of EB buffer
    - Nanodrop:
        - PpsbA2_sps = 9.9 ng/µL
        - Used 1 µL
- Made .7% agarose gel
    - .4 g of agarose and 500 µL of 100X TAE
    - Left in fridge because when pulling out comb, wells broke

Plasmid Group (Priya/Stephanie/Sara/Lin/Manvi)

- Mini-prepped 1579
    - 102.0 ng/µL DNA (Had to nanodrop 2X) → 27 µL remaining
- Gel electrophoresis for 1579 with ethidium bromide staining

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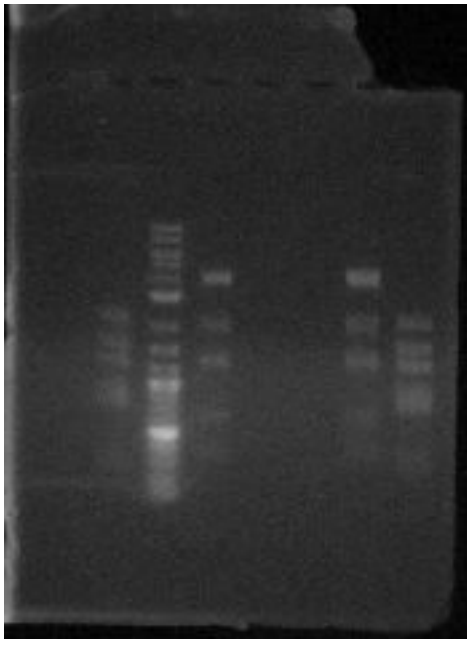
- 
- 1. PstI + PvuII
    - Psti + PvuII, confirmation that both worked
- 2. MW Marker
- 3. PstI+EcoRV
    - Both worked
- 4. PstI + SalI
    - Both worked
- 5. PstI + PvuII
    - Did this twice because we thought this lane could have been punctured

- Gel electrophoresis w/ 1% Agarose for 1579
    - .6 g of agarose and 500 µL of 100X TAE

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- 
  - 1. PstI + PvuII
  - 2. MW Marker
  - 3. PstI+EcoRV
  - 4. PstI + SalI
  - 5. PstI + PvuII
  - 6 g of agarose
  - Stained the gel with Diamond nucleic acid Dye that is 10,000 x concentrated
  - Diluted 100X TAE into 1X TAE
  - Diluted 20 µL of 10,000X Diamond Dye with 200 mL of 1X TAE
  - When transferring gel into box for shaking/staining it broke into pieces and also when transferring gel into UV box, it also broke into pieces
- Split 1579 *E. coli* in half

Spectroscopy Measurement at 17:50 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX Collier 5/19 Culture sup. with 20 mL BG-11 A=.697
- UTEX room temp. 6/13 (split) A= 1.009

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- UTEX room temp. 6/26 (split) A= .760

Spectroscopy Measurement at 17:56 (Natalie/Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.756
- UTEX 1% room temp. 6/13 2m A= 0.751

June 28, 2018

Spectroscopy Measurement at 9:56 (Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A=0.851
- UTEX 1% room temp. 6/13 2m A= 0.715

Spectroscopy Measurement at 18:20 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A=0.933
- UTEX 1% room temp. 6/13 2m A= 0.827

Construct Group (Karthik/Natalie/Manvi/Priya/Woody)

- Made a .7% agarose gel
    - Added .5 µL 10,000X Diamond Nucleic Acid Dye
    - .4 g of agarose
    - 500 µL of 100X TAE
    - Ran gel with diamond dye
        - Buffer had 18 µL of 1X diamond dye, 3.5 mL of 100X TAE, 346.32 mL of Milli-Q water
        - Added MW marker to two wells
- Resuspension of Q3cscB and Q3sps (following IDT protocol)
    - Q3cscB → 100 µL of autoclaved milli-Q water
    - Q3sps→ 100 µL of autoclaved milli-Q water
    - Left the two tubes in the water bath at 50 ℃ for 15 minutes

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    - Nanodrop (used 3µL):
        - Q3cscB= 161.0 ng/µL, 92 ng/µL (no photos, but really smooth curves), 31.5 ng/µL
        - Q3sps = 292.9 ng/µL
- Resuspension of 1414 promoters (following IDT protocol)
    - Pcpc → 50 µL of autoclaved milli-Q water
    - Pcpc560 → 50 µL of autoclaved milli-Q water
    - PidiA → 25 µL of autoclaved milli-Q water
    - PpsbA2 → 25 µL of autoclaved milli-Q water
    - Prbc → 50 µL of autoclaved milli-Q water
    - Left the 5 tubes in the water bath at 50 ℃ for 15 minutes
    - Nanodrop:
        - Pcpc560 = 4.4 ng/µL, 5.4 ng/µL (2 µL used)
        - PidiA = 9.5 ng/µL
        - PpsbA2 = 10.7 ng/µL
        - Prbc = 9.0 ng/µL, 7.7 ng/µL (2 µL used)
        - Pcpc = 3.4 ng/µL, 38.9 ng/µL (2 µL used)
- Resuspension of primers (following IDT protocol)
    - 1414 promo start → 704 µL of autoclaved milli-Q water
    - 1414 promo end → 800 µL of autoclaved milli-Q water
    - 1579 cscB start → 912 µL of autoclaved milli-Q water
    - 1579 cscB end → 824 µL of autoclaved milli-Q water
    - 1579 sps start → 904 µL of autoclaved milli-Q water
    - 1579 sps end → 908 µL of autoclaved milli-Q water
- PCR of Q3 cscB (after mixing) (CSCB cycle)
- PCR of Q3 sps (after mixing) (SPS Cycle)
- PCR of Pcpc560 (EYPF Cycle)
- PCR of PpsbA2 (SHORT Cycle)

Plasmid Group (Manvi/Priya/Stephanie)

- Miniprep with 1414 plasmid

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    - Nanodrop → 203.4 ng/µL
- Miniprep with 2991 plasmid
    - Nanodrop → 92.6 ng/µL
- Made 1% agarose gel for staining with
    - Gel ended up breaking and having to be thrown out, might be a problem with the TAE solution

Interlab (Lin/Matthew/Natalie)

- Finished pipetting and setting up 96 well plate for calibration 1 and calibration 2, but were unable to measure the plates because we didn't know the instrument information
- For Calibration 2, we changed tips when mixing for row E and used the same tips for the entire row for F, G and H
- Left the plate in room temperature overnight

June 29, 2018

Spectrophotometer Measurement at 10:45 (Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A=0.507
- UTEX 1% room temp. 6/13 2m A= 0.353

Spectrophotometer Measurement at 18:28 (Lin/Lukas)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A=1.124
- UTEX 1% room temp. 6/13 2m A=0.892

Construct Group (Woody/Matt/Natalie/Sara)

- PCR of 1414 PidiA (SHORT Cycle)
- PCR of 1414 Prbc (EYFP Cycle)
- PCR of 1414 Pcpc (EYFP Cycle)
- Resuspension of Geneblocks (following IDT protocol)
    - Q3 PisiAB_cscB → 50 µL of autoclaved milli-Q water

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    - Q3 PisiAB_sps → 50 µL of autoclaved milli-Q water
    - Left the two tubes at 49 ℃ for 15 minutes
    - Nanodrop:
        - Q3 PisiAB_cscB =13.0 ng/µL
        - Q3 PisiAB_sps = 11.2 ng/µL
- Resuspension of Geneblocks (following IDT protocol)
    - Opto EYFP → 100 µL of autoclaved milli-Q water
    - Orig EYFP → 100 µL of autoclaved milli-Q water
    - Left the two tubes at 50 ℃ for 15 minutes
    - Nanodrop:
        - Opto EYFP =15.5 ng/µL
        - Orig EYFP = 8.3 ng/µL
- Resuspension of Q1 Primers (following IDT protocol)
    - 2991 start lone → 860 µL of autoclaved milli-Q water
    - 2991 end lone → 912 µL of autoclaved milli-Q water
    - Left the two tubes at 51℃ for 15 minutes
- PCR of Q3 PisiAB_cscB (EYFP Cycle)
- PCR of Q3 PisiAB_sps (EYFP Cycle)
- PCR of Opto EYFP (EYFP Cycle)
- PCR of Orig EYFP (EYFP Cycle)

Plasmid Group (Priya/Stephanie/Manvi/Sara)

- Tried making a gel with the stationary agitation method with diamond nucleic dye
    - Broke twice
- Made a gel with diamond nucleic acid dye → SUCCESS!!
    - Incubated for 20 minutes
    - Loaded gel with RE digest of plasmid DNA
    - Banding was smeary but indicated that ECORI and BamHi were both working

Interlab (Lin)

- Made fluorescein stock for Calibration 3

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June 30, 2018

Spectrophotometer Measurement at 13:28 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.275
- UTEX 1% room temp. 6/13 2m A= 0.984