COLOR CODING KEY

Spectrophotometry Measurementss

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

Week 5

July 1, 2018

Spectrophotometer Measurements at 12:24 (Lukas)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.385
- UTEX 1% room temp. 6/13 2m A= 1.051

July 2, 2018

Spectrophotometer Measurements at 9:56 (Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.592
- UTEX 1% room temp. 6/13 2m A= 0.419

Spectrophotometer Measurements at 18:18 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.493
- UTEX 1% room temp. 6/13 2m A= 1.210

Plasmid Group (Priya/Stephanie/Sara/Manvi//Matthew/Karthik)

- Try doing a gel extraction
    - Full = .110 g = 110 mg
        - Added .11 mL of membrane binding solution
    - Partial = .100 g = 100 mg
        - Added .1 mL of membrane binding solution
    - Gel purification

Interlab (Lin/Natalie)

- Instrument Brand and Model
    - Filtermax F5 Multi-mode Microplate Reader
- Can your instrument measure both absorbance and fluorescence?
    - Yes

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- Does your instrument have pathlength correction, and if yes can it be disabled?
    - Fluorescence = weight within the well
    - Absorbance = figure it out depending on volume within the well and the distance you're measuring
    - Doesn't correct automatically? → possible but you need to know amount of volume in the wells
- What filters does your instrument have for measuring GFP?
    - BW35NM
- Does your instrument use top or bottom optics ?
    - Yes both
- Started and finished calibration 1
    -

	LUDOX CL-X	ddH_2O
Replication 1	0.047	0.033
Replication 2	0.049	0.033
Replication 3	0.052	0.033
Replication 4	0.048	0.033

July 3, 2018

Spectrophotometer Measurements at 10:01 (Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.414
- UTEX 1% room temp. 6/13 2m A= 1.096

Cell Culturing/Plating (Natalie)

- Supplementing Measurements 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

Spectrophotometer Measurements at 12:38 (Natalie/Sara)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.994
- UTEX 1% room temp. 6/13 2m A= 0.996

Spectrophotometer Measurements at 18:24 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.034
- UTEX 1% room temp. 6/13 2m A= 1.023

July 5, 2018

Spectrophotometer Measurements at 12:15 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 1.217
- UTEX 1% room temp. 6/13 2m A= 1.160

Interlab (Lin/Natalie)

- Made chloramphenicol stock solution
    - Added .10 g of chloramphenicol to 4 mL of 200% proof ethyl alcohol
- finished calibration 2 and calibration 3

Constructs Group (Karthik/Woody)

- ran a .7% gel with PCR products
    - 1. MW ladder
    - 2. Q3 cscB
    - 3. Q3 sps
    - Stained with 5 µL of gel green
- Gel purified Q3 cscB and Q3 sps (Promega Wizard Kit)

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July 6, 2018

Constructs Group (Karthik/Natalie)

- Nanodrop of PCR products from yesterday:
  - Q3 cscB/sps blue = 6.2 ng/µL , 6.3 ng/µL , 6.4 ng/µL (used 2 µL)
  - Q3 cscB/sps pink = 8.5 ng/µL
- Ran a .7% gel with PCR products
  - 1. MW ladder
  - 2. Q3 cscB
  - 3. Q3 sps
  - Stained with Nucleic Acid Diamond Dye
  - Only the Q3cscB band showed up
- Gel Purification of Q3 cscB (Promega Wizard Kit)
  - Nanodrop (used 4 µL)
    - Q3 cscB = 5.1 ng/µL
    - Q3 cscB = 6.2 ng/µL
- PCR of Q3 cscB (CSCB Cycle)
- PCR of Q3 sps (SPS Cycle)
- Resuspension of geneblocks (following IDT protocol)
  - Q1 lone cscB → 100 µL of autoclaved milli-Q water
  - Q1 combo cscB → 100 µL of autoclaved milli-Q water
  - Left the two tubes at 50℃ for 15 minutes
  - Nanodrop (1 µL used):
    - Q1 combo cscB = 8.8 ng/µL
    - Q1 lone cscB= 9.2 ng/µL

Plasmid Group (Priya/Stephanie/Lin)

- Gel Purification of 1579 Cut (Promega Wizard Kit)

Interlab (Natalie)

- remade the chloramphenicol stock because we used old powder last time
  - .10 g chloramphenicol and 4 mL of 200% proof ethyl alcohol