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 3

June 17, 2018

Spectrophotometry Measurements at 12:00 (Lukas)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.040
- UTEX 1% room temp. 6/13 2m A= 0.041

June 18, 2018

Plasmid Group (Stephanie/Priya)

- Made Streptomycin antibiotic stock (27mL at working stock (50mg/mL))
- Made five *Amp/Kan Plates,* one of which is kind of lumpy
    - Follows the 12 g/L Agar, 10g Tryptone, 10g NaCl, 5g Yeast Extract
    - 100 ug/mL Amp
    - 50 ug/mL Kan
- Made twelve *Strep Plates*
    - Follows the 12 g/L Agar, 10g Tryptone, 10g NaCl, 5g Yeast Extract
    - 50 ug/mL Strep

Spectrophotometry Measurements at 00:00 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.058
- UTEX 1% room temp. 6/13 2m A= 0.058

Spectrophotometry Measurements at 08:18 (Priya)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m
    - ~~0.077~~ redone → A= 0.068
- UTEX 1% room temp. 6/13 2m
    - ~~0.101~~ redone → A= 0.066

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Spectrophotometry Measurements at 15:54 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.040
- UTEX 1% room temp. 6/13 2m A= 0.040

Spectrophotometry Measurements at 23:45 (Lin/Woody)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.101
- UTEX 1% room temp. 6/13 2m A= 0.102

June 19, 2018

Plasmid Group (Stephanie/Priya)

- Set up streaks of Amp/Kan and Strep plates:
  - Amp/Kan: 1579, 1414 (Control)
  - Strep: 1414, 2991, 1579 (Control)
- Left overnight at 37 ℃

Spectrophotometry Measurements at 8:22 (Karthik)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.117
- UTEX 1% room temp. 6/13 2m A= 0.121

Spectrophotometry Measurements at 16:02 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.158
- UTEX 1% room temp. 6/13 2m A= 0.159

Spectrophotometry Measurements at 11:45 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.193
- UTEX 1% room temp. 6/13 2m A= 0.203

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June 20, 2018

Spectrophotometry Measurements at 8:16 (Priya)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.222
- UTEX 1% room temp. 6/13 2m A= 0.229

Spectrophotometry Measurements at 16:00 (Lukas)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.244
- UTEX 1% room temp. 6/13 2m A= 0.242

Plasmid Group (Priya/Jenn)

- Neg C 1579 + S - growth at 50 µg/mL
    - Bad sign
    - Neg C 1414 + Ak - no growth
    - 1579 → ready for culture (l)
- Experiment -- test out all three strep stocks we have in the following workup with overplating at 100 µg/mL (because other institutions use that concentration)
-

	1579	2991	1414
SP1	+	+	+
SP2	+	+	+
SP3	+	+	+

- set up liquid culture of 1579-containing cells using addgene protocol and LB containing Amp and Kan.

June 21, 2018

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Spectrophotometry Measurements at 00:00 (Woody)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.315
- UTEX 1% room temp. 6/13 2m A= 0.318

Spectrophotometry Measurements at 8:24 (Karthik)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.334
- UTEX 1% room temp. 6/13 2m A= 0.342

Spectrophotometry Measurements at 16:01 (Woody)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.393
- UTEX 1% room temp. 6/13 2m A= 0.397

Plasmid Group (Priya/Stephanie/Jennifer)

- Met with Yelena and spoke with Dr. Gergen about our Streptomycin plate issue. We realized that our bacteria were *all* Streptomycin resistant.Received Spectinomycin from Dr. French's lab, setup 50 mg/mL Spec 1000x stock solutions, and made three spectinomycin plates.
- Attempted miniprep with 1 mL of 1579 plasmid DNA. Followed Qiagen protocol.

June 22, 2018

Spectrophotometry Measurements at 00:00 (Woody)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.473
- UTEX 1% room temp. 6/13 2m A= 0.453

Spectrophotometry Measurements at 16:07 (Natalie)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.548

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- UTEX 1% room temp. 6/13 2m A= 0.555

Spectrophotometry Measurements at 23:44 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.643
- UTEX 1% room temp. 6/13 2m A= 0.657

Plasmid Group (Priya/Stephanie)

- Performed nanodrop on 1579 DNA, concentration: 0.4 ng/µL. Potential causes: not enough plasmid, centrifugal strength too strong, lysed for too long, cells did not have enough oxygen.
- Did not give the plates enough time to cool and thus punctured the plates when attempted to plate. Remade five spectinomycin plates at 50 ug/mL. Left them overnight to cool.
- Increased the liquid culture for 1579 from 2 mL total to 14. Forgot to add in enough antibiotic, will be rectified on June 24th.

June 23, 2018

Spectrophotometry Measurements at 13:47 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.715
- UTEX 1% room temp. 6/13 2m A= 0.701

Spectrophotometry Measurements at 11:40 (Lin)

- Done at 750 nm with 1500 µL of culture
- UTEX 1% room temp. 6/13 1m A= 0.739
- UTEX 1% room temp. 6/13 2m A= 0.754