

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 μ L 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 5 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 12 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 μ L 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 μ L of culture
- UTEX 1% room temp. 6/13 1m A= 0.068
- UTEX 1% room temp. 6/13 2m A= 0.066

Spectrophotometry Measurements at 15:54 (Natalie)

- Done at 750 nm with 1500 μ L of culture

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- 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 μ L 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 °C

Spectrophotometry Measurements at 8:22 (Karthik)

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

June 20, 2018

Spectrophotometry Measurements at 8:16 (Priya)

- Done at 750 nm with 1500 μ L of culture

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- 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 μ L 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)

- pAM 1579, Amp/Kan resistant, grew at 50 μ g/mL of streptomycin
 - Bad sign -- indicates that our stock might not be strong enough
- pAM1414, Streptomycin Resistant, did not grow in Amp/Kan plate
 - 1579 \rightarrow ready for culture (1/3 strains are good to go)
- Experiment -- test out all three strep stocks we have in the following workup with overplating at 100 μ g/mL (because other institutions use that concentration)
-

	1579	2991	1414
Strep 1	+	+	+
Strep 2	+	+	+
Strep 3	+	+	+

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

June 21, 2018

Spectrophotometry Measurements at 00:00 (Woody)

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

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Spectrophotometry Measurements at 8:24 (Karthik)

- Done at 750 nm with 1500 μ L 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 μ L 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
 - 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 μ L 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 μ L of culture
- UTEX 1% room temp. 6/13 1m A= 0.548
- UTEX 1% room temp. 6/13 2m A= 0.555

Spectrophotometry Measurements at 23:44 (Lin)

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

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- UTEX 1% room temp. 6/13 2m A= 0.657

Plasmid Group (Priya/Stephanie)

- Performed nanodrop on 1579 DNA, concentration: 0.4 ng/ μ L
 - 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 5 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 μ L 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 μ L of culture
- UTEX 1% room temp. 6/13 1m A= 0.739
- UTEX 1% room temp. 6/13 2m A= 0.754