

## 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 2

**June 11, 2018**

Plasmid & Construct Design Group (Karthik/Priya)

	Vector	Start/End	Gene	F/R	Sequence
2991 start lone	2991	Start of	CsCB, sps, EyFP, c/B EYFP, combo CsCB	F	5' AATTTCACACAGGAAACAGAC C 3'
2991 end lone	2991	End of	CsCB, sps, EYFP, C/b EYFP, cobmo sps	R	5' TGCATGCCTGCAGGTC 3'
2991 cscB, combo end	2991	End of	Combo cscB	R	5' TAATTGGTCAACCTCTCGTAA CGT 3'
2991 sps combo start	2991	Start of	Combo sps	F	5' CAGAGAAACGTTACGAGAGGT 3'
1414 promo start	1414	Start of	promos	F	5' AGCATACTAGAGGATCGGC 3'
1414 promo end	1414	End of	promos	R	5' AAAATCAGGCTCGATAAAGTC G 3'

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1579 cscB start	1579	Start of	cscB	F	5' GCTGATTACTGTACGACTTGTT G 3'1579
1579 cscB end	1579	End of	CscB	R	5' GATCCACGCAGTTTAGCGA 3'
1579 sps start	1579	Start of	sps	F	5' GTACGCGCGGAGCCT 3'
1579 sps end	1579	End of	sps	R	5' AGCTCGAGCCCCGGGT 3'
1579 P_cscb start	1579	Start of	P_Cscb	F	5' AGAGGGTGTAGCCCGTC 3'
1579 P_cscB end	1579	End of	P_cscB	R	5' CTACCACCAACAAGTCGTACA 3'
1579 P_sps start	1579	Start of	P_sps	F	5' TTACGTTGGTATCGCTAAACT G 3'
1579 P_sps end	1579	End of	P_sps	R	5' AGCCTGTAGAAAGGAGGTTT 3'

### Restriction Buffers

- 2991 linearization
- BamHI NEB 2.1 (3.1, cutsmart)

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- EcoRI NEB 2.1
- 1414 linearization
  - BamHI NEB 2.1 (3.1 custmart)
- 11579 linearization
  - EcoRV : NEB 3.1
  - SaII NEB 3.1

PCR Information - .05  $\mu$ M

Constructs	TM (F) (°C)	TM (R)(°C)	Annealing (°C)	Act Anneal(°C)
EYFP, cscB, sps	58.5	59.1	62.2	56 ish
Combo cscB	58.5	59.1	62.2	56 ish
Combo sps	58.8	59.1	62.4	56 ish
P2	57.5	58.1	61.3	56 ish
1579 cscB	58.8	59.4	62.4	56 ish
1579 sps	60.9	62.2	64.5	56 ish
1579 P_cscB	59.6	59.0	62.7	56 ish
1579 P_sps	57.7	58.0	61.4	56 ish

Spectrophotometry Measurements (1.5 mL diluted) (Natalie)

- Measured at 800 nm
- UTEX 5/19 culture at room temperature A= 0.325
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture
- 6/6/18 10 mL / 100 BG-11 left incubator - 1 paper A= 0.147
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture
- 6/6/18 10 mL 5/19 C right incubator - 2 paper A = 0.047
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture

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- 6/6/18 redone 5/28 C- 2 paper A= 0.002
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture

### Cell Culturing/ Plating (Priya/Stephanie)

- Plated cyanos from UTEX 5/19 culture at room temperature onto BG-11 Plates (liquid to agar)
- 6/11 5/19 UTEX (Culture Trial) room temp.
- 6/11 5/19 UTEX (Culture Trial) room temp.

### June 12, 2018

#### Cell Culturing/Plating (Matt M)

- Water bath temperature check (Matt M.)
  - Initial temperature  $\rightarrow$  20.5  $^{\circ}$ C
  - Temperature with lights and heater  $\rightarrow$  47 minutes to reach 30  $^{\circ}$ C

### Spectrophotometry Measurements (1.5 mL diluted) (Natalie)

- UTEX 5/19 culture at room temperature A= 0.420
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture
- 6/6/18 10 mL / 100 BG-11 left incubator - 1 paper A= 0.239
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture
- 6/6/18 10 mL 5/19 C right incubator - 2 paper A = 0.064
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture
- 6/6/18 redone 5/28 C- 2 paper A= 0.012
  - 1000  $\mu$ L of BG-11 and 500  $\mu$ L of culture

### Cell Culturing/Plating (Jenn)

- Plated cyanos onto BG-11 plates (Agar to Agar)
- 6/12/19 growth cyano in BG-11

### June 14, 2018

#### Cell Culturing/Plating (Natalie/Priya)

- Split the UTEX 5/19 culture at room temperature with at 16:05 (Natalie/Priya)

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- .5 mL of culture and 49 mL of BG-11
- UTEX 1% room temp. 6/13 1m
- UTEX 1% room temp. 6/13 2m
- UTEX 1% room temp. 6/13 1e
- UTEX 1% room temp. 6/13 2e

### Spectrophotometry Measurements at 16:15 (Natalie/Priya):

- Done at 750 nm with 1500  $\mu$ L of culture
- UTEX 1% room temp. 6/13 1m A= -0.013
- UTEX 1% room temp. 6/13 2m A= -0.015

### Cell Culturing/Plating (Natalie/Priya)

- Split the remainder of the UTEX 5/19 culture at room temperature in half at 16:30
- UTEX room temp. 6/13 (split) with 24 mL culture and 24 mL BG-11
- Supplemented the original culture (17.5 mL) with 32.5 mL BG-11

### Cell Culturing/Plating (Natalie/Priya)

- Split 6/6/18 10 mL/100 BG-11 (left) at 16:44 (Natalie/Priya)
- 6/13 split from the 6/6/18 10 mL/100 BG-11 (left) with 37.5 mL BG-11 and 62.5 mL of culture
- Supplemented the original culture with 37.5 mL BG-11

## June 15, 2018

### Spectrophotometry Measurements at 00:00 (Lin/Woody)

- Done at 750 nm with 1500  $\mu$ L of culture
- UTEX 1% room temp. 6/13 1m A= 0.013
- UTEX 1% room temp. 6/13 2m A= 0.022

### Spectrophotometry Measurements at 8:02 (Priya)

- Done at 750 nm with 1500 $\mu$ L of culture
- UTEX 1% room temp. 6/13 1m

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- ~~0.056~~ redone →  $A = .058$
- UTEX 1% room temp. 6/13 2m
- ~~0.016~~ redone →  $A = 0.020$

### Spectrophotometry Measurements at 16:44 (Lukas/Woody)

- Done at 750 nm with 1500  $\mu\text{L}$  of culture
- UTEX 1% room temp. 6/13 1m  $A = 0.026$
- UTEX 1% room temp. 6/13 2m  $A = 0.031$

### June 16, 2018

#### Spectrophotometry Measurements at 00:15 (Lin)

- Done at 750 nm with 1500  $\mu\text{L}$  of culture
- UTEX 1% room temp. 6/13 1m  $A = 0.029$
- UTEX 1% room temp. 6/13 2m  $A = 0.033$

#### Spectrophotometry Measurements at 12:00 (Woody)

- Done at 750 nm with 1500  $\mu\text{L}$  of culture
- UTEX 1% room temp. 6/13 1m  $A = 0.038$
- UTEX 1% room temp. 6/13 2m  $A = 0.042$