

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, original EyFP, codon optimized EYFP, combo cscB | F | 5' AATTCACACAGGAAACAGACC 3' |
| 2991 end lone | 2991 | End of | cscB, sps, original EYFP, codon optimized EYFP, combo sps | R | 5' TGCATGCCTGCAGGTC 3' |
| 2991 cscB, combo end | 2991 | End of | Combo cscB | R | 5' TAATTGGTCAACCTCTCGTAACGT 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' AAAATCAGGCTCGATAAAGTCG 3' |
| 1579 cscB start | 1579 | Start of | cscB | F | 5' GCTGATTACTGTACGACTTGTTG 3' |
| 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' AGCTCGAGCCCGGGT 3' |

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| | | | | | |
|-------------------|------|----------|--------|---|------------------------------|
| 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' TTACGTTGGTATCGCTAAACTG 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)
 - EcoRI NEB 2.1
- 1414 linearization
 - BamHI NEB 2.1 (3.1, custmart)
- 1579 linearization
 - EcoRV : NEB 3.1
 - SaII NEB 3.1

PCR Information - .05 μ M

| Constructs | TM (F) (°C) | TM (R)(°C) | Annealing (°C) | Act Anneal(°C) |
|-----------------|-------------|------------|----------------|----------------|
| EYFP, cscB, sps | 58.5 | 59.1 | 62.2 | 56 |
| Combo cscB | 58.5 | 59.1 | 62.2 | 56 |
| Combo sps | 58.8 | 59.1 | 62.4 | 56 |
| Lux Promoters | 57.5 | 58.1 | 61.3 | 56 |
| 1579 cscB | 58.8 | 59.4 | 62.4 | 56 |

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| | | | | |
|-------------|------|------|------|----|
| 1579 sps | 60.9 | 62.2 | 64.5 | 56 |
| 1579 P_cscB | 59.6 | 59.0 | 62.7 | 56 |
| 1579 P_sps | 57.7 | 58.0 | 61.4 | 56 |

Spectrophotometry Measurements (Natalie)

- Measured at 800 nm
- 1000 μ L of BG-11 and 500 μ L of culture (1.5 times diluted)
- UTEX 5/19 culture at room temperature A= 0.325
- 6/6/18 10 mL / 100 BG-11 left incubator - 1 paper A= 0.147
- 6/6/18 10 mL 5/19 C right incubator - 2 paper A = 0.047
- 6/6/18 redone 5/28 C- 2 paper A= 0.002

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
 - Initial temperature \rightarrow 20.5 $^{\circ}$ C
 - Temperature with lights and heater \rightarrow 47 minutes to reach 30 $^{\circ}$ C

Spectrophotometry Measurements (Natalie)

- Measured at 800 nm
- 1000 μ L of BG-11 and 500 μ L of culture (1.5 times diluted)
- UTEX 5/19 culture at room temperature A= 0.420
- 6/6/18 10 mL / 100 BG-11 left incubator - 1 paper A= 0.239

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- 6/6/18 10 mL 5/19 C right incubator - 2 paper A = 0.064
- 6/6/18 redone 5/28 C- 2 paper A= 0.012
- Concluded that 1 paper had more ideal growth than 2 papers

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
- .5 mL of culture and 49 mL of BG-11 (1% culture)
- 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 μ 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

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- 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 μ 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 μ L of culture
- UTEX 1% room temp. 6/13 1m A= .058
- UTEX 1% room temp. 6/13 2m A= 0.020

Spectrophotometry Measurements at 16:44 (Lukas/Woody)

- Done at 750 nm with 1500 μ 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 μ 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 μ L of culture
- UTEX 1% room temp. 6/13 1m A= 0.038
- UTEX 1% room temp. 6/13 2m A= 0.042