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 6

July 9, 2018

Constructs Group (Matt)

- Resuspension of primers (following IDT protocol)
    - 2991 cscB combo end → 964 µL of autoclaved milli-Q water
    - 2991 sps combo start → 732 µL of autoclaved milli-Q water
- PCR of Q1 combo cscB (CSCB Cycle)
- PCR of Q1 lone cscB (CSCB Cycle)

Plasmid Group (Priya/Stephanie/Jennifer)

- Nanodrop of 1579 purified (used 2 µL)
    - 1579 PUR= 4.9 ng/µL
- Gel purification again of 1579 (Monarch New England Biolab Kit)

Cell Culture/Plating (Matt L. /Elon/Karthik)

- Made glycerol stock
    - 2x in snap cap tube, 2x in screw cap tube for each 1414, 1579, 2991
    - each tube has 500 µL of 50% glycerol and 500 µL of E. coli.

July 10, 2018

Spectrophotometer Measurement (Natalie/Lukas)

- Done at 750 nm with 1500 µL of culture
- 6/13 split from 6/6 10 mL/ 100 BG-11 (left) A= .728
- 6/6/18 10 mL cyano per 100 BG-11 A=.813
- 6/6/18 redone 5/28 C A= 1.194
- 6/6/18 10 mL → 5/19 C A= .677
- UTEX Room temperature 6/13 (split) A= 1.066
- UTEX Room temperature 6/26 (split) A= 1.240
- UTE Collier 5/19 Culture sup. With 20 mL BG-11 A=1.492

Cell Culture/Plating (Matt L./Elon)

- Made 40 mL stock solution of 10% methanol and BG-11

COLOR CODING KEY

Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

    - 4 mL of methanol
    - 36 mL of BG-11
- Froze 4 tubes of cyanobacteria in the -80 ℃ freezer
    - Added 450 µLof 10% methanol and BG-11
    - Added 450 µL of UTEX room temperature 6/13 (split) into each of 4 tubes
- Plated 50 µL of 6/13 split from 6/6 10 mL/100 BG-11 (left) on tryptic soy agar plates to see if there was contamination
- Plated 50 µL of 6/6/18 10 mL → 5/19 C on tryptic soy agar plates to see if there was contamination

July 11, 2018

Interlab (Jenn/Steph)

- Made 8 LB media agar plates with 80 mL of LB and agar
    - Added 2g of LB and .96 g of agar
    - 80 µL of chloramphenicol
    - 80 mL of milli-Q water
- Made 13 LB media agar plates with 115 mL of LB and agar
    - Added 2.75 g of LB media and 1.32 g of Agar
    - 115 µL of chloramphenicol
    - 115 mL of milli-Q water

Constructs Group (Karthik/Natalie)

- Ran a .7% agarose gel
    - Buffer of 350 mL of 1X TAE = 35 mL of 10X TAE and 315 mL of milli-Q water
    - 1. MW ladder - 15 µL
    - 2. Q3 cscB - 25 µL of product and 5 µL of loading dye
    - 3. Q3 sps - 25 µL of product and 5 µL of loading dye
- Made a diamond dye dilution
    - 200 mL of 1X TAE
        - 20 mL of 1X TAE and 180 mL of milli-Q water
    - 20 µL of diamond dye
- Added 20 mL ethanol to the wash buffer to gel extraction kit

COLOR CODING KEY

Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

- Gel extraction of Q3 cscB and Q3 sps (Monarch NEB Gel Extraction Kit)
    - Q3 cscB= .07 g = 70 mg → added 280 µL of gel dissolving buffer
    - Q3 sps = .07 g = 70 mg → added 280 µL of gel dissolving buffer
    - Nanodrop (1.5 µL used)
        - Q3 sps = 6.0 ng/µL
        - Q3 cscB = 15.8 ng/µL

Plasmid Group (Stephanie/Jenn/Priya/Sara)

- Checked if DNA evaporated from stocks of plasmid DNA
- Nano dropped all of the stock DNA
    - 1579= 51.8 ng/µL
    - 1414= 564.6 ng/µL (DNA evaporated - left with about 3 µL)
    - 2991 = 178.6 ng/µL
- Made 19 plates for HIFI assembly
    - Spectinomycin plates = 125 mL of LB and Spec
    - Ampicillin/Kanamycin plates = 55 mL of LB and Amp/Kan
    - 2991 - 5 plates of Spectinomycin
    - 1414 - 8 plates of Spectinomycin
    - 1579 - 6 plates of Ampicillin/Kanamycin

Cell Culture/Plating (Jenn/Stephanie)

- 6/13 split from 6/6 10 mL/100 BG-11 (left) and 6/6/18 10 mL → 5/19 C plates showed contamination
- Plated the rest of the stock solutions to see if they were also contaminated

July 12, 2018

Constructs Group (Karthik/Natalie/Woody)

- Ran a .7% agarose gel with Diamond nucleic acid dye
    - None of the bands showed up

Plasmid Group (Stephanie)

- Labeled plates for HIFI assembly

COLOR CODING KEY

Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

Cell Culture/Plating (Stephanie)

- Out of the cyanobacteria stock solution that were plated, F is contaminated with bacteria

Interlab (Lin/Matthew/Natalie/Dominika)

- Started competent cell test (following iGEM protocol)
    - 10 pg/µL
    - 100 pg/µL
    - Incubated 2 plates at 8:00 pm at 37 ℃ and 200 rpm
- Started transformation (following iGEM protocol)
    - 8 plates with 100 µL of cells unspun
    - 8 plates with 100 µL of cells spun
    - Incubated 16 plates at 8:00 pm at 37 ℃ and 200 rpm

July 13, 2018

Constructs Group (Karthik/Matthew/Natalie)

- Made a gel and added 5 µL of diamond dye to the gels
    - 1. MW ladder
    - 2. MW ladder
    - Both ladders showed up but were very smeary
- Made another gel with a ladder and diluted diamond dye and shook it
    - Nothing showed up
- PCR of Q3 cscB and Q3 sps using NEB Q5 polymerase
    - Used 0.5 µLof each template, 1 µL of each primer

Interlab (Natalie/Stephanie/Lin)

- Made 160 mL of LB Media
    - 4 g of LB powder
    - 160 mL of milli-Q water
- Put around 10 mL per falcon tube
- Transferred 2 colonies from each plates into liquid media
- Incubated the colonies overnight at 37 ℃ and 220 rpm