Spectrophotometry Measurements

Construct Group

Plasmid Group

Interlab

Cell Culture/Plating

Biobrick Group

Cyanobacteria Transformation Group

Experimental Verification

Plasmid & Construct Design Group

Week 18

October 1, 2018

Biobrick Group (Natalie/Matt)

- RE digest of backbone
- PCR of psbA2, cpc560, idiA, cscB
- PCR purification of PCR products
- RE digest of PCR products

Experimental Verification Group (Karthik/Elon)

- Luciferase expression after 1h high light exposure of white light
 - Pipetted 95 μ L of cells onto half of the wells on the plate
 - High light exposure for an hour in the incubator
 - Afterwards, pipetted 95 μ L of cells not exposed to high light for 1 hour
 - Added 5 uL of decanal to all wells
 - Parafilmed well plates
- Rows 1-6 High light exposure, 7-12 no high light
- A:WT, B-C: cpc560 cpc2#1,3#1, D-E: cpc 2#1,3#1, F-H: psbA2 1-3

| 0 | 4 | 0 | 0 | 4 | 6 | 0 | 0 | 0 | 2 | 0 | 0 |
|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|-----|
| 203 | 935 | 1331 | 1032 | 634 | 802 | 473 | 337 | 416 | 387 | 228 | 220 |
| 119 | 521 | 768 | 519 | 207 | 157 | 496 | 142 | 130 | 107 | 170 | 107 |
| 165 | 232 | 186 | 201 | 247 | 157 | 136 | 138 | 98 | 153 | 167 | 134 |
| 274 | 952 | 561 | 287 | 396 | 454 | 314 | 391 | 310 | 308 | 251 | 228 |
| 4 | 4 | 0 | 8 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 2 |
| 0 | 2 | 0 | 2 | 4 | 2 | 4 | 2 | 2 | 0 | 2 | 0 |
| 0 | 6 | 2 | 4 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 |

October 2, 2018

Biobrick Group (Natalie/Matt/Karthik/Lin)

- Ligation of PCR products and backbone

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- Transformation and plating of products cpc560, idiA, psbA2, cscB
- RE digest of cpc560, idiA, psbA2, cscB again
- Nanodrop of RE digest
 - $cscB = 9.9 \text{ ng/}\mu\text{L}, 20.9 \text{ ng/}\mu\text{L}, 21.1 \text{ ng/}\mu\text{L}$
 - $idiA = 36.9 \text{ ng/}\mu\text{L}$
 - $psbA2 = 51.3 \text{ ng/}\mu\text{L}$
 - Cpc $560 = 39.4 \text{ ng/}\mu\text{L}$

October 3, 2018

Biobrick Group (Natalie/Matt/Karthik)

- Transformation of cpc560, idiA, psbA2, cscB

October 4, 2018

Biobrick Group (Priya/Woody/Sara)

- Inoculation of plates from yesterday
- Miniprep of inoculation and sequencing

Experimental Verification (Elon)

| Water | 0 | 0 | 0 |
|---------------------|--------|--------|--------|
| Glucose Control | 0.996 | 0.958 | 0.965 |
| Starch Acetate | 0.078 | 0.049 | 0.066 |
| Starch Fructosidase | 0.145 | 0.141 | 0.149 |
| 1 Acetate | 0.001 | 0.003 | -0.008 |
| 1 Fructosidase | -0.014 | -0.015 | -0.019 |
| 2 Acetate | -0.021 | -0.020 | -0.023 |
| 2 Fructosidase | -0.017 | -0.018 | 0.094 |
| 3 Acetate | -0.020 | -0.011 | -0.017 |

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| 3 Fructosidase | -0.009 | 0.000 | -0.009 |
|----------------|--------|--------|--------|
| 4 Acetate | -0.004 | 0.003 | -0.003 |
| 4 Fructosidase | -0.009 | -0.007 | 2.490 |
| 5 Acetate | 0.011 | 0.007 | 0.027 |
| 5 Fructosidase | 0.010 | 0.018 | 0.012 |
| 6 Acetate | 0.006 | 0.003 | 0.002 |
| 6 Fructosidase | -0.005 | -0.010 | -0.002 |
| 7 Acetate | 0.004 | -0.005 | 0.025 |
| 7 Fructosidase | N/A | N/A | N/A |
| 8 Acetate | -0.011 | 0 | -0.015 |
| 8 Fructosidase | -0.005 | -0.031 | 0.014 |
| 9 Acetate | -0.016 | -0.006 | -0.031 |
| 9 Fructosidase | 0.000 | 0.043 | -0.008 |

*Key:

#1 = cscB #3 150 mM NACL 1 mM IPTG 10 uM Spec 10 uM Strep Room Temp

#2 = cscB #2 150 mM NACL 1 mM IPTG 10 uM Spec 10 uM Strep Room Temp

#3 = cscB #2 No Salt NACL 1 mM IPTG Incubator

#4 = cscB #2 150 mM NACL 1 mM IPTG Incubator

#5 = cscB #2 150 mM NACL Incubator

#6 = cscB #4 #1 150 mM NACL 1 mM IPTG 10 uM Spec 10 uM Strep Incubator

#7 = cscB #4 150 mM NACL 1 mM IPTG 10 uM Spec 10 uM Strep Incubator

#8 = cscB #2 150 mM NACL 1 mM IPTG 10 uM Spec 10 uM Strep Incubator

#9 = cscB #4 #2 NACL 150 mM 1 mM IPTG Incubator

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| Analyte | Analyte |
|-------------------|---------|
| | (g/L) |
| | |
| Starch | 0.033 |
| Glucose | 0.033 |
| | 0.000 |
| Starch Sucrose | 0.080 |
| Sucrose | |
| 1 Glucose | 0.001 |
| 1 Sucrose | -0.002 |
| 1 Sucrose | 0.002 |
| 2 Glucose | 0.000 |
| 2 Cyaraga | 0.046 |
| 2 Sucrose | 0.046 |
| 3 Glucose | 0.000 |
| | |
| 3 Sucrose | 0.000 |
| 4 Glucose | 0.001 |
| | |
| 4 Sucrose | -0.001 |
| 5 Glucose | 0.010 |
| | |
| 5 Sucrose | -0.004 |
| 6 Glucose | 0.002 |
| | |
| 6 Sucrose | -0.004 |
| 7 Glucose | 0.006 |
| , Glucosc | 0.000 |
| 7 Sucrose | -0.012 |
| <u> </u> | |

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| 8 Glucose | 0.000 |
|-----------|-------|
| 8 Sucrose | 0.007 |
| 9 Glucose | 0.000 |
| 9 Sucrose | 0.021 |