SEPTEMBER

Date: Monday, 9/3

Objective(s):

Prepare competent BL21 cells with FNS and F3'H.

Day Summary:

6 Eppendorf tubes of samples were prepared and frozen in -80°C.

Procedure:

1. Preparation of competent cells was carried out for BL21 cells transformed with the plasmid containing FNS and F3'H as described in 'Preparation of Competent Cells' protocol.

Date: Tuesday, 9/4

Objective(s):

Perform organic extraction for the following samples: (a) Negative control, (b) F3'H, (c) FNS and (d) Experimental.

Day Summary:

Organic Extraction done and ready for HPLC

Procedure:

1. Add 1ml of 6N HCl to 10 ml of the sample in an 15ml Eppendorf tube.
2. Add 2ml of ethyl acetate to the mixture.
3. Centrifuge the mixture for 3 minutes.
4. Pipette the top layer out carefully into 2ml Eppendorf tubes. Do not pipette the bottom layer!
5. Vacuum centrifuge it for 35 mins with the caps open.
6. Add 1.5ml of ethyl acetate to the original mixture to the remaining sample.
7. Centrifuge the mixture for 3 minutes .
8. Pipette the top layer to the same 2 ml Eppendorf tube.
9. Vacuum centrifuge for 40 mins with the caps open.
10. Repeat steps 6-9.

11. Dissolve the organic solute obtained in DMSO and transfer them to HPLC vials

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Date: Wednesday, 9/5

Objective(s):

Check the protein expression for WT, FNS, F3'H & Experimental for intermediates.

Day Summary:

The protein gel was left for overnight shaking for the bands to clearly appear.

Procedure:

1. 15% separating gel was prepared in the following manner:

Content	*Volume*
40% Acrylamade	3.75 ml
H_2O	3.75 ml
4x Separating Buffer	2.5 ml
10% APS	50 µl
TEMED	9 µl

2. The mixture was quickly poured into the casting glass till the first brim and the bubbles were burst using isopropanol.

3. Separating gel was made to set for thirty minutes.

4. The stacking gel was prepared in the following manner:

Content	*Volume*
40% Acrylamade	0.6 ml
H_2O	3.1 ml
4x Stacking gel Buffer	1.25 ml
10% APS	50 µl
TEMED	12 µl

5. Once the separating gel was set, the isopropanol was poured out.

6. The stacking gel was poured into the casting glass till the second brim. The comb was inserted into the casting gel carefully and was used to burst the bubbles.

7. The gel was made to set for thirty minutes.

8. Four samples were used for this experiment: Wild Type, F3'H, FNS, Experimental. Induction was done by Wetlab Group 2 by keeping the samples under the dark.

9. The samples were placed in a centrifuge and spun down for six minutes.

10. 1X Binding buffer was produced in the following way:

Contents	*Concentration*
Imidazole	5 mM
NaCl	500 M
Tris-HCl, pH 7.9	20 mM
$MgCl_2$	5 mM

11. The cells were resuspended using 300µl of 1X binding buffer and then all the contents were then transferred into their respective Eppendorf tubes.

12. The first Eppendorf tube (wild type) was placed inside a beaker filled with ice and the cells were then lysed by sonication.

13. Sonicator settings:

Amplitude	10
Process Time	1 minute
Pulse ON Time	5 seconds
Pulse OFF Time	15 seconds

14. The sonication was repeated for the other seven Eppendorf tubes.

15. Once all cell samples were lysed, they were spun down for 10 minutes in a refrigerated centrifuge.

16. Nine Eppendorf tubes were prepared, two for each sample - one for the supernatant and one for pellet, and one for the ladder. All the nine Eppendorf tubes were filled with 10µl of 2x loading dye.

17. Once the refrigerated centrifuge was done, the supernatant was transferred to separate Eppendorf tubes. The pellets were then resuspended using 300µl 1x binding buffer.

18. 100 µl of suspended pellet and supernatants were transferred to their respective Eppendorf tubes (the ones with loading dyes).

19. All the nine tubes were placed in a heat block at 95℃ for five minutes. After five minutes the tubes underwent a short spin.

20. The samples and the ladder were injected into the wells of the gel in the following way:

21. The gel ran for seventy minutes at 150 V.

22. Once the electrophoresis was done, the gel was carefully extract the gel from the glass casting, the stacking layer was removed and then the remaining gel was submerged in water.

23. The submerged gel was then swirls using an orbital shaker at fifty rpm for five minutes. After five minutes, the water was changed. This process was repeated two more times and the final waste water was rinsed out.

24. BLS was poured into the container until the gel was submerged. It was the heated in a microwave at high for one minute.

25. Once heated, the gel was then shaken in an orbital shaker at fifty rpm for one hour.

26. BLS was then poured out and the gel was rinsed thoroughly.

27. The container was once again filled with water till the gel was submerged and was left for overnight shaken in an orbital shaker at 50 rpm.

Date: Tuesday, 9/11

Objective(s):

1. To prepare P_{htpG1}-mRFP competent cells
2. To transform P_{htpG1}-mRFP competent cells with P_{con}-GFP and plate

Day Summary:

Objectives were met.

Procedure:

1. P_{htpG1}-mRFP competent cells were prepared using 'Preparation of Competent Cells' protocol (see Protocols tab).

2. P_{htpG1}-mRFP competent cells were transformed with P_{con}-GFP and left to recover in fresh LB for 45 min at 37°C before plating 100 µL onto a LB + kan (P_{con}-GFP carries kanamycin resistance) + strep (P_{htpG1}-mRFP carries streptomycin resistance) plate.

Date: Friday, 9/14

Objective(s):

(a) Plasmid purification of the following plasmids:-

I. *De novo* A
II. *De novo* B
III. PhtpG1-GFP in PSB1C3
IV. PhtpG1-GFP in

(b) Gibson assembly of Construct E (P_{Brep}-F3'H-P_{Brep}-FNS)

Day Summary:

Purified plasmids were sent for sequencing.

Procedure:

1. Plasmids ((i) *De novo* A, (ii) *De novo* B, (iii) PhtpG1-GFP in PSB1C3 and (iv) PhtpG1 in) were purified as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol. Samples were sent for sequencing.

2. Gibson assembly of Construct E (P_{Brep}-F3'H-P_{Brep}-FNS) was carried out.

Date: Monday, 9/17

Objective(s):

Transformation of *De novo* plasmid into competent cells containing stress promoter.

Day Summary:

De novo plasmid was transformed and left overnight for cultures to grow.

Procedure:

1. 1 µL of *De novo* plasmid was added to 60 µL of cells containing the stress promoter.

2. The mixture was incubated for 40 minutes.

3. The sample was then platted and left overnight for cultures to grow.

Date: Wednesday, 9/19

Objective(s):

Perform organic extraction for the following samples: (a) De novo, (b) De novo + Coumaric and (c) De novo + Malonic.

Day Summary:

Organic extraction done and ready for HPLC.

Procedure:

1. Add 1 ml of 6M HCl to 10 ml of the sample in an 15 ml Eppendorf tube.
2. Add 2 ml of ethyl acetate to the mixture.
3. Centrifuge the mixture for 3 minutes.
4. Pipette the top layer out carefully into 2 ml Eppendorf tubes. Do not pipette the bottom layer!
5. Vacuum Centrifuge it for 35 mins with the caps open.
6. Add 1.5 ml of ethyl acetate to the original mixture to the remaining sample.
7. Centrifuge the mixture for 3 minutes.
8. Pipette the top layer to the same 2ml Eppendorf tube.
9. Vacuum centrifuge for 40 mins with the caps open.
10. Repeat steps 6-9.
11. Dissolve the organic solute obtained in DMSO and transfer them to HPLC vials.

Date: Thursday, 9/20

Day Summary:

dsDNA concentrations for Plasmid Brep+FNS+ F3'H were sent for sequencing.
Fluorescence readings for RFP, RFP+GFP, *De novo* A, B, C were recorded and plotted on the graph.

Procedure:

For DNA Purification for Plasmids Brep+FNS+ F3'H:

1. DNA purification was carried out for the samples 3 and 4 as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol.

2. Plasmid concentrations of samples 3 and 4 were measured and recorded in the table below.

Sample	*Concentration (ng/μL)*
3	61
4	119

3. At the intervals 0h, 2h, 3h, 5h, 6h and 24h. Fluorescence readings for triplicates of each of the 5 samples, RFP, RFP+GFP, *De novo* A, B, C were measured on a 96-well plate at the settings below:

	RFP	*RFP (old)*	*GFP*
Excitation	535	580	485
Emission	600	607	515
Gain	75	90	60

To note: RFP (old) is the set of RFP excitation/emission/gain settings that are used in Yan Ping's lab and were applied to verify the appropriateness of the new proposed settings (RFP) in measuring fluorescence.

4. Absorbance of each sample were measured at OD_{600} to check for OD.

Date: Tuesday, 9/25

Objective(s):

Perform organic extraction for the following samples:

(a) Control
(b) BL21 Lut 2.0
(c) Brep F3'H Brep FNS
(d) Brep FNS
(e) Brep F3'h
(f) F3'H + FNS
(g) F3'H + FNS Co
(h) F3'H + FNS + CPR

Day Summary:
Organic extraction done and ready for HPLC

Procedure:

1. Add 1 ml of 6M HCl to 10 ml of the sample in an 15 ml Eppendorf tube.
2. Add 2 ml of ethyl acetate to the mixture.
3. Centrifuge the mixture for 3 minutes.
4. Pipette the top layer out carefully into 2 ml Eppendorf tubes. Do not pipette the bottom layer!
5. Vacuum Centrifuge it for 35 mins with the caps open.
6. Add 1.5 ml of ethyl acetate to the original mixture to the remaining sample.
7. Centrifuge the mixture for 3 minutes.
8. Pipette the top layer to the same 2 ml Eppendorf tube.
9. Vacuum centrifuge for 40 mins with the caps open.
10. Repeat steps 6-9.
11. Dissolve the organic solute obtained in DMSO and transfer them to HPLC vials.

Date: Tuesday, 9/25
Objective(s):
Perform PCR for the new Brep-F3'H-Brep-FNS, htp61-RFP, PSB163 plasmid.

Day Summary:
PCR performed.

Procedure:

1. PCR for new Brep-F3'H-Brep-FNS. Temp - 72°C, Time - 56 secs

PrimeSTAR Max Premix	25 µL
New Brep-FNS F	0.75 µL
Brep-F3'H R	0.75 µL
Brep-F3'H-Brep-FNS	2.5 µL

ddH_2O	21 µL

2. PCR for htp61-RFP. Temp - 72°C, Time - 10 secs

PrimeSTAR Max Premix	25 µL
PCDF-htp61 F	0.75 µL
PCDF-RFPR	0.75 µL
PCDF RFP (188ng/µL)	1 µL
ddH_2O	22.5 µL

3. PCR for PSB163. Temp - 72°C, Time - 22 secs

PrimeSTAR Max Premix	25 µL
Term suffix F	0.75 µL
Htp61-prefix R	0.75 µL
BBG-J04450	1 µL
ddH_2O	23 µL

Date: Friday, 9/28

Objective(s):

To dye 100% wool, 99% polyester and 100% cotton to test if dyes can bind without the use of mordants. Dye used for experiments: commercial luteolin (50 mM).

Day Summary:

Waiting time (time fabric was left with dye) did not affect dyeing of cotton, polyester and wool. Among all 3 fabrics tested, dye-fastening was the most visually apparent in cotton, followed by polyester and finally, wool. Wool was observed to be highly hydrophobic- luteolin that was dissolved in DMSO (also hydrophobic) was not able to overcome this hydrophobicity to dye the wool.

Procedure:

1. Prepare 4 pieces of 1 inch by 1 inch cotton, polyester and wool.

2. Pipette 15 µL of 50 mM luteolin directly onto the fabric and wait for 5, 15, 30 and 60 min respectively before washing under running water for 10 seconds.

3. Dry fabric using a paper towel and leave on table top to dry.

4. There was no observable visual difference across the different times- 5 min was taken to be the minimal number of minutes to stain dye sufficiently.

5. An additional 4 pieces of 1 inch by 1 inch fabrics was set up for each cotton and wool.
    - Pre-wash & no pre-wash: 1 piece of fabric was washed under running water before pipetting the dye onto the fabric.
    - Detergent & no detergent: 1 piece of fabric was washed with detergent after 5 min of leaving the fabric with the dye.

Observations

- Detergent was able to help spread the dye across cotton and polyester.

Date: Sunday, 9/30

Objective(s):

To mordant common fabrics (wool) and dye to test if mordanting* enables luteolin to bind to cotton better. To dye common fabrics without mordanting to test if dyes can bind without the use of mordants. Dye used for experiments: commercial apigenin (50 mM). *Mordanting protocol for cotton was tested on wool to see if binding of dyes is enhanced.

Day Summary:

Waiting time (time fabric was left with dye) did not affect dyeing of both cotton and wool- 5 min was taken to be the minimum time needed for dye to bind to fabric.

Procedure:

Protocol 1: Mordanting wool with 4% tannic acid

Wool (100%)- Mordant

1. Prepare 4% tannic acid in 100 mL distilled water (Set-up A).

2. Prepare 100 mL distilled water for the control set-up (Set-up B).

3. Insert 6 1 inch by 1 inch cotton pieces into both set-ups and and leave for 4 hours after ensuring that each piece of fabric is fully submerged in the solution.

4. After 4 hours, dry fabric using a paper towel. Pipette 15 µL of 50 mM apigenin directly onto the fabric and wait for 30 min to dry before washing with detergent/left without washing.

Wool (100%)- No Mordant

1. In a separate set-up, prepare 4 pieces of 1 inch by 1 inch cotton.

2. Pipette 15 µL of 50 mM apigenin directly onto the fabric and wait for 5, 15, 30 and 60 min before washing under running water for 10 seconds.

3. Dry fabric using a paper towel and leave on table top to dry.

4. There was no observable visual difference across the different times- 5 min was taken to be the minimal number of minutes to stain dye sufficiently.

5. An additional 3 pieces of 1 inch by 1 inch cotton was set up. Pipette 15 μL of 50 mM apigenin directly onto 2 of the fabric and wait for 5 min before washing under running water for 10 seconds. Leave the last piece of cotton without dye.

6. After 5 min, one piece of the cotton that was dyed with apigenin was washed with detergent.

Protocol 1: Mordanting wool with 5 mM aluminium oxide

Wool (100%) - Mordant

1. Prepare 5 mM aluminium oxide in 100 mL distilled water (Set-up C).

2. Prepare 100 mL distilled water for the control set-up (Set-up D).

3. Microwave each set-up until boiling point.

4. Insert 6 1 inch by 1 inch cotton pieces into both set-ups and and leave for 1 hour after ensuring that each piece of fabric is fully submerged in the solution.

5. After 1 hour, dry fabric using a paper towel. Pipette 15 μL of 50 mM apigenin directly onto the fabric and wait for 30 min to dry before washing with detergent/left without washing.

Wool (100%) - No Mordant

1. In a separate set-up, prepare 4 pieces of 1 inch by 1 inch wool.

2. Pipette 15 μL of 50 mM apigenin directly onto the fabric and wait for 5, 15, 30 and 60 min to dry before washing under running water for 10 seconds.

3. Dry fabric using a paper towel and leave on table top to dry.

4. There was no observable visual difference across the different times- 5 min was taken to be the minimal number of minutes to stain dye sufficiently.

5. An additional 3 pieces of 1 inch by 1 inch wool was set up. Pipette 15 μL of 50 mM apigenin directly onto 2 of the fabric and wait for 5 min before washing under running water for 10 seconds. Leave the last piece of wool without dye.

6. After 5 min, one piece of the wool that was dyed with apigenin was washed with detergent.

Results

- Mordanting wool with aluminium oxide prior to dyeing appears to have made dye-fastening more efficient- color appears more apparently in mordanted fabrics.