NOTEBOOK

By Miami University's iGEM Team

Gel Electrophoresis

Gel electrophoresis is run to determine the length of various DNA parts.

Protocol:

1. For 2 gels, mix 0.65 g of Agarose gel in 50 mL of 0.5X TAE solution.
   For 1 gel, mix 0.30 g of Agarose gel in 30 mL of 0.5X TAE solution.
2. Heat the mixture until Agarose particles are dissolved.
3. Add ethidium bromide to the solution.
4. Pour the solution into a mold with well molds installed and allow it to form.
5. Place the formed gel into the electrophoresis apparatus.
6. Take the mixtures from the PCRs and mix gel loading dye into the PCR solution.
7. Pipette 6 uL of each PCR tube into each well and put the ladder into one well.
8. Allow the electrophoresis apparatus to run at 100 V until the dye is close to the bottom.
9. Remove the gels from the electrophoresis apparatus and analyze them on a UV light.

Segregation PCR

PCR is used to amplify certain parts and sequences of DNA. It uses primers to allow taq polymerase to bind to the specified sequence and add nucleotides to that sequence. The resulting solution can be used to determine different characteristics of the specified DNA strand, such as length.

Protocol:

1. Transfer colonies into PCR tubes with 9 uL ddH_2O
2. Lyse the cells in the tubes in the Thermocycler for 10 min at 99 ^oC
3. Make the Master Mix using the following table:

Added Material	**Master Mix (uL)**	**Per Tube (uL)**
UC101 Accuris Master Mix	(# of Tubes) x 10	10
Forward Primer	(# of Tubes) x 0.5	0.5
Reverse Primer	(# of Tubes) x 0.5	0.5
Total	Master Mix Total	11

4. Run the thermocycler with appropriate settings:

PCR Phase	**Temperature (^oC)**	**Time (min:sec)**	**Rounds**
Initial Denature			1X
Denature			nX
Annealing	T_a		
Extension			
Final Extension			1X
Hold		∞	

Transformation

Transformation is the process of introducing DNA into a cell. Plasmids are accepted into the competent cells of cyanobacteria and hopefully inserted into the genome of the cells.

Protocol:

1. Add 1 mL of WT from the culture flask into a 1.5 mL centrifuge tube.
2. Centrifuge the tube for 2 min at 15000 rpm to collect the cell pellet and remove the supernatant fluid.
3. Add 200 uL of BG-11 media to the tube and resuspend the cell pellet.
4. Add 2 uL of the plasmid to be transformed into the cell.
5. Wrap in foil to restrict light from entering the tube and store the tube in 30.0 ^{o}C while rotating the tube.
6. Wait 4 to 16 hours for the transformation.
7. Plate the transformants on a BG11 plate.

29 July 2021

Name: Daria Perminova

Summary:

The primers ordered from IDT were diluted to make stock primers. Then, PCR amplification and gel purification was conducted to isolate the plasmid fragments for future Gibson assembly.

Protocol:

Dilution protocol:

1. Use 10X OD nmol value on tube (amount of ddH_2O added in uL).
2. Centrifuge the mixture before adding water.
3. Vortex the solution to mix it.
4. Dilute out 10X into tubes (10uM as 20 uL primers in 180uL of water).

Part/Insert	Length (bp)	T_a (°C)
pF_fbp	1085	72
pT_tal	1244	72
pTF_fbp	1080	72
pTF_tal	1239	72
*Backbone	7968	N/A
glpX_US	831	72
glpX_DS	831	72
KanR	1253	72
Backbone	1781	66

*Backbone is the same for all over-expression plasmids

PCR reaction protocol (general):

Component	Volume (uL)	Final Concentration (uM)
5X Q5 Reaction Buffer	10	1X
10 mM dNTPs	1	200
Forward Primer	2.5	0.5
Reverse Primer	2.5	0.5
Template	Variable	Variable
Water	Variable	Variable
Total	50	

PCR for primers: pF_fbp, pTF_fbp, pTF_tal, glpX_US, glp_DS):

Component	Per Tube (uL)	Master Mix (uL)
Buffer	10	21
dNTPs	1	2.1
Forawrd Primer	2.5	5.25
Reverse Primer	2.5	5.25
Polymerase	0.5	1.05
Template (PCC 7942 WT)	1	1.00
ddH_2O	32.5	68.3
Total	50	

PCR for KanR Backbone:

Component	Per Tube (uL)	Master Mix
Buffer	10	21
dNTPs	1	2.1
Forawrd Primer	2.5	5.25
Reverse Primer	2.5	5.25
Polymerase	0.5	1.05
Template (PCC 7942 WT)	0.5	1.05
ddH_2O	33	69.3
Total	50	

Settings for: FBP, Tal, GlpX – 6 fragments

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	98	00:30	1X
Denature	98	00:10	35X
Annealing	72	00:30	
Extension	72	00:45	
Final Extension	72	2:00	1X
Hold	4	∞	

** This was a mistake that affected the reactions and yield. The backbone had a T_a of 66 °C.

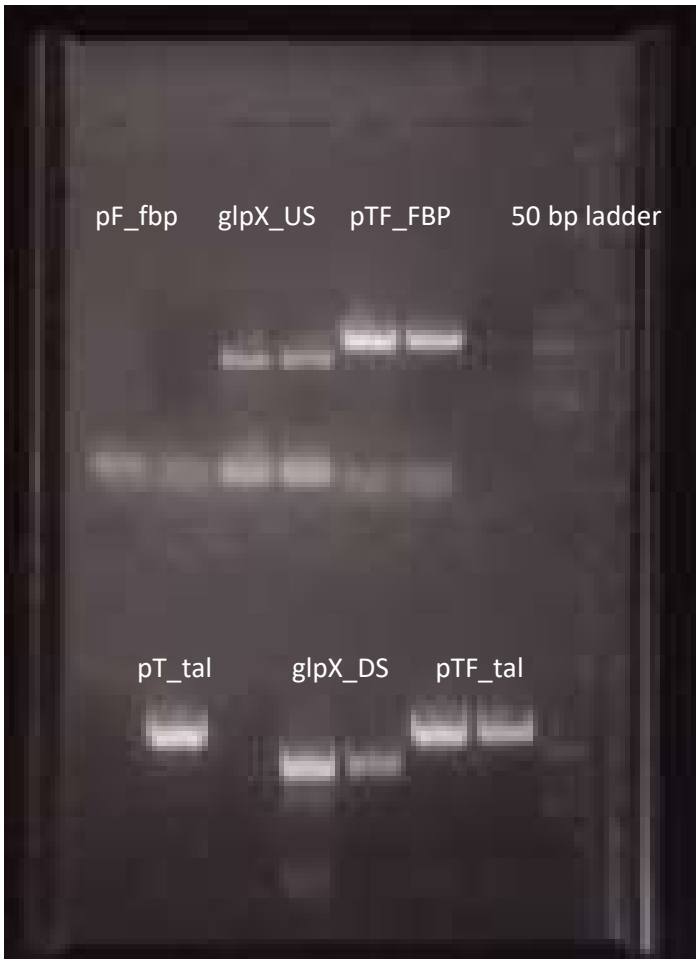
Settings for: Backbone, KanR – 2 fragments

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	98	00:30	1X
Denature	98	00:10	35X
Annealing	62 for Backbone** 72 for Kan	00:30	
Extension	72	01:00	
Final Extension	72	01:00	1X
Hold	4	∞	

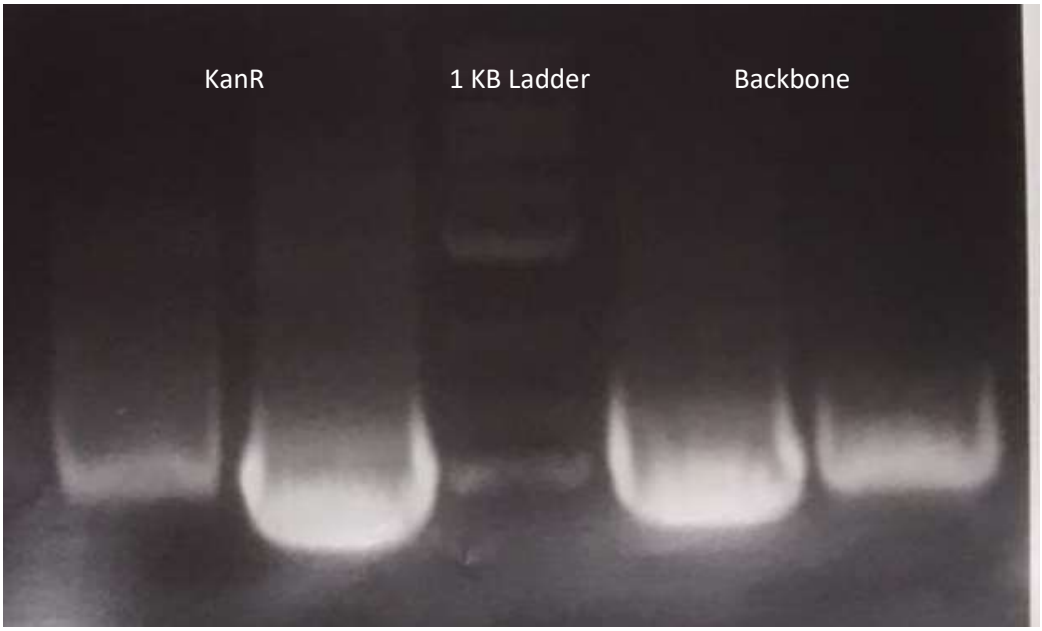
** This was a mistake that affected the reactions and yield. The backbone had a T_a of 66 °C.

Results:

Gel 1:



Gel 2:



SBPase backbone size was smaller than expected, so we chose to amplify the fragment again. pF_fbp was not amplified.

The primers ordered from IDT were diluted into stock solutions so that they could be used in experiments. After that, each primer, with the exception of pF_fbp, was amplified with amplification PCR to confirm the length of the primer. All of the primers were the expected length with the exception of the SBPase backbone, which had an input error in the thermocycler settings.

2 August 2021

Name: Avery Imes and Hope Kirby

Summary:

The pF_fbp primer and SBPase backbone were amplified because pF_fbp was not amplified the first time, and the SBPase backbone was wrongly amplified the first time. The T_a and extension temperature were verified before proceeding with the protocol.

Protocol:

1. For pF_fbp, the primer solutions were re-aliquoted for amplification.
    a. 20 uL of primer stock was diluted in 180 uL ddH_2O.
    b. The Thermocycler settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	98	02:00	1X
Denature	98	00:10	29X
Annealing	72	01:20	
Extension	72	01:20	
Final Extension	72	02:00	1X
Hold	4	∞	

2. For amplification of SBPase backbone:
    a. pΔglgC was diluted. 15 uL was added to the 10X 18 ng/uL (60 uL water)
    b. PCR reaction recipe from July 29th was followed.
    c. The PCR segregation of the SBP backbone was tried again with the T_a of 69 °C.
    d. For Primer 1, the T_m is 68 °C.
    e. For Primer 2, the T_m is 72 °C.
        i. NEB recommended T_a of 69 °C, instead of the previously attempted 66 °C, for 30 seconds and a 72
    f. The Thermocycler settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	98	00:30	1X
Denature	98	00:10	29X
Annealing	69	00:30	
Extension	72	01:00	
Final Extension	72	02:00	1X
Hold	4	∞	

3. Gel electrophoresis was run on the resulting tubes and the mass of the primers and backbones were found.

a. The average mass of the tubes used was 1.779 g.
b. The determined masses:

Product	Total Mass (g)	Product Mass (g)	Product Mass (mg)
pF-fbp	1.6462	0.4683	468.3
pTF-tal	1.8468	0.6689	668.9
pTF-fbp	1.7438	0.5659	565.9
pT-tal	1.4892	0.3113	311.3
dSBPase-backbone	1.6224	0.4445	444.5
dSBPase-glpx-US	1.6303	0.4524	452.4
dSBPase-glpx-DS	1.7605	0.5826	582.6
dSBPase-KanR	2.0279	0.85	850.0

3 August 2021

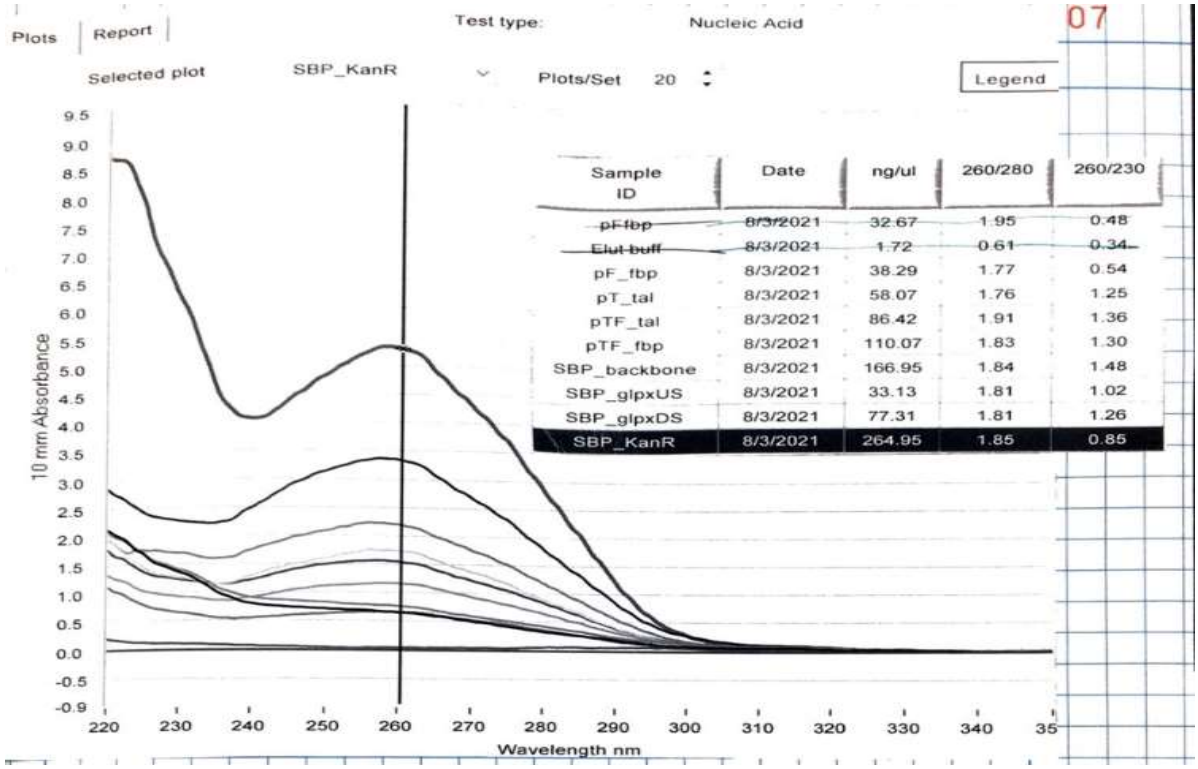
Name: Avery Imes and Hope Kirby

Summary:

Protocol:

1. The wizard Promega Gel cleanup kit protocol was followed for pF-fbp, pTF-tal, pTF-fbp, pT-tal, SBPase-backbone, SBPase-glpX-US, SBPase-glpX-DS, and SBPase-KanR.
    a. A 1:1 ratio uL of membrane binding solution was used. 60 mg of product weight was used.
    b. Because the column max volume is 350 uL and 350 mg gel and ours were significantly greater. Therefore, 700 uL was transferred in the first round, centrifuged, split into 2nd or 3rd round as needed.
    c. The tubes were flicked every 3 min during melting.
    d. The contents were eluted into 25 uL of elution buffer.
    e. Eluted samples were nano dropped using elution buffer as a blank.

Results:



Sample ID	Date	ng/ul	260/280	260/230
~~pF-fbp~~	~~8/3/2021~~	~~32.67~~	~~1.95~~	~~0.48~~
~~Elut-buff~~	~~8/3/2021~~	~~1.72~~	~~0.61~~	~~0.34~~
pF_fbp	8/3/2021	38.29	1.77	0.54
pT_tal	8/3/2021	58.07	1.76	1.25
pTF_tal	8/3/2021	86.42	1.91	1.36
pTF_fbp	8/3/2021	110.07	1.83	1.30
SBP_backbone	8/3/2021	166.95	1.84	1.48
SBP_glpxUS	8/3/2021	33.13	1.81	1.02
SBP_glpxDS	8/3/2021	77.31	1.81	1.26
SBP_KanR	8/3/2021	264.95	1.85	0.85

The nanodrop results marked unexpectedly high concentrations for backbone and KanR. Low 240/230 levels indicate a high agarose content. Good 260/230 levels ~1.8 are good for proceeding.

4 August 2021

Name: Avery Imes and Hope Kirby

Summary:

The plasmids were purified in *E. coli.*

Protocol:

1. Plasmid Prep for single colony DH5a (E. coli) pAMM2991 was grown in 3 mL LN sp50 0N for 15.5 hours.
2. For the purification of 2991 plasmid:
    a. The Purevield Plasmid Miniprep System protocol was followed for 3 mL of culture.
        i. Therefore, only 750 uL of supernatant was taken to avoid the cell pellet.
    b. We did not do the endotoxin removal wash.
    c. The sample was stored in a -20 ^{o}C freezer.
3. The nanodrop results were obtained (see above).
4. Restriction Digest for EcoR1 and Bam HI:
    a. Digest 1 ug in a 20 uL reaction with the two enzymes.

Component	**Amount**
Enzyme	1 uL (0.5 uL of each)
10X Cutsmart Buffer	2 uL
Plasmid	7 uL (1 ug of plasmid)
Water	10 uL
Total	20 uL

    b. The mixture was incubated in a 37 ^{o}C water bath for 2 hours.
    c. The mixture was run on a 1% gel with an undigested plasmid for the control.
    d. Loading dye was mixed into the digest tube and the full volume was put into the gel.
    e. 3 uL of undigested plasmid was run in 5 uL of loading dye.
        i. The material was added before putting the gel in the buffer (do not do that).
    f. The digest results:
        i. The digest had 1 bend and the undigested plasmid had 3 isoforms (1 strong band and 2 weak bands).
        ii. The empty tube was weighed at 1.0484 g and it weighed 1.1185 g with the product.
        iii. The product weighed 0.0701 g and was eluted with 20 uL water.
        iv. 70.1 uL of dissolving buffer was added.

g. The gel purification washed BamH/Eco digested pm 2991, 11.2 ng/uL.

5 August 2021

Name: Avery Imes and Hope Kirby

Summary:

We assembled the plasmids using Gibson Assembly techniques.

Protocol:

1. We performed a Gibson Assembly for the 4 constructs with the following fragments:

pGEMSBPase:

Fragment	Size	[ng/uL]	uL	ng adding	Dilutions
backbone (PCR)	1.781	167	1	55	3x
glpX_US	0.831	33.1	0.8	27	
KanR	1.253	265	1	40	1+5.6 uL (H_2O)
glpX_DS	0.831	77.31	1	27	1+1.9 uL (H_2O)
Water			1.2		

gGEM_tal_fbp

Fragment	Size	[ng/uL]	uL	ng adding	Dilutions
Backbone (digested)	7.968	11.2	4	44.8	
pTF-tal	1.239	86.42	0.5	12	25 ng/uL + 1 mL (H_2O)
pTF-fbp	1.080	110.07	0.5	12	25ng/uL

pGEM_tal

Fragment	Size	[ng/uL]	uL	ng adding	
Backbone (digested)	11	11	4	44.8	
pT_tal	1.244	58.07	1	12.5	

pGEM_fbp

Fragment	Size	[ng/uL]	uL	ng adding	
Backbone (digested)	11	11	4	44.8	

pF_fbp	1.085	38.29	1	12.5	

2. The backbone to insert ration was 1:2 and the insert to insert ratio was 1:1.
    a. The backbone mass was about 40-50 ng (in order to get the same molar ratio).
    b. Example:
        i. pGEM_tal_fbp needed about a 1:7 ratio based on its size.
        ii. For pTF_tal, 45/7 was close to 6 ng, then the uL was based on [ng/uL]. We wanted the backbone to insert ratio to be about 1:2 so it was actually 12 ng.
    c. We want a total reaction of 10 uL with 5 uL of fragments and 5 uL of master mix.
    d. Full example:
        i. pT_tal ratio = 7.968:1.244, about 6.4X.
        ii. Therefore 44.8 ng/6.4 = 7 X 2 for 1:2 backbone to insert ratio.
        iii. Then, 5 uL of 2X assembly master mix was added and mixed 15 times up and down and set for reaction at 50 ^{o}C for 1 hr, then 4 ^{o}C.
3. For the transformation into *E.Coli*:
    a. 5 uL of reaction was used to perform a chemical transformation using *E. Coli* dh 10β cells.
        i. The tubes were lightly flicked after adding 5 uL directly to the cells. Left over reactions were left at 4 ^{o}C.
        ii. Cells were left on ice for 30 minutes.
        iii. The cells were heat shocked at 42 ^{o}C for 30 seconds in a water bath.
        iv. They were left back on ice for 2 minutes.
        v. The cells were suspended in 1 mL LB.
        vi. The tubes were shaken at 37 ^{o}C horizontally for 1 hour. Plates were made and incubated at 37 ^{o}C.
        vii. The tubes were centrifuged at 4000 rpm for 2 minutes.
        viii. 200 uL of cells were plated and stored in 37 ^{o}C.
            1. For pGEM_SBPase, the cells were resuspended in 200 uL after 800 uL of supernatant was removed. They were plated with Kan50 antibiotic.
            2. For pGEM_tal, pGEM_fbp, and pGEM_tal_fbp, the cells were resuspended in 400 uL after 600 uL of supernatant was removed. These were plated with spec 50 antibiotic.
            3. 200 uL were saved on the bench.

We got over 100 colonies the next day.

9 August 2021

Name: Avery Imes and Daria Perminova

Summary:

The plasmids from *E. coli* were purified, a restriction digest was performed to confirm that *E. coli* was properly transformed, and the plasmids were used to transform *S. elongatus* PCC 7942.

Protocol:

1. Glycerol stocks were prepared:
    a. Glycerol stocks were made for cell plasmids in triplicate.
    b. 300 uL of 80% glycerol was added to 1.5 uL of culture.
2. The plasmids were extracted following the Pureyield Plasmid Miniprep System.
    a. The test for tal/fbp 1 may be contaminated with SBPase from a pipette error.
3. For running the restriction digest:
    a. Overexpression plasmids protocol:
        i. Use 300 ng
        ii. Example: fbp3 is 50 ng/uL so use 6uL. Use water to make total concentration 6 uL.

Plasmid	**Amount of Plasmid Used (uL) to get 300 ng**
fbp 1	4
fbp 2	5
fbp 3	6
tal 1	4
tal 2	6
tal 3	3
tf 1	5
tf 2	6
tf 3	4

    b. 20 uL digestion:
        i. Overexpression vectors (FBP-1-3, tal1-3, TF1-3) for 9 tubes.

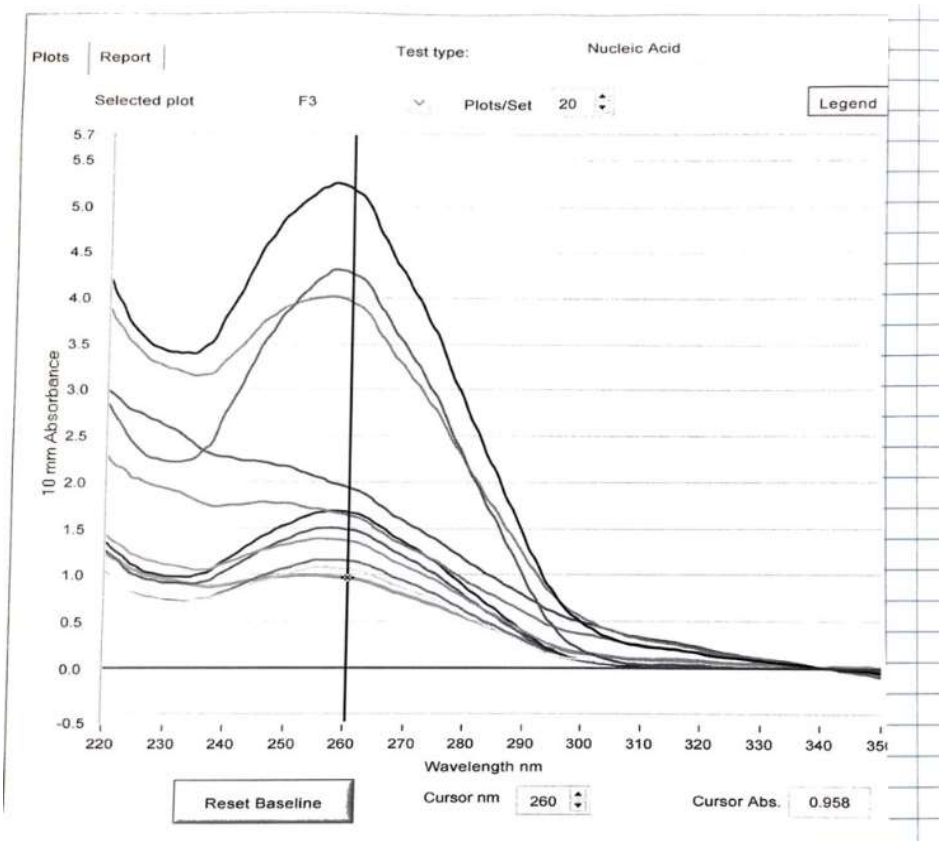
Component	**Per Tube (uL)**	**Master Mix (uL) = 9.5X(Per Tube)**
Template	6	
Cutsmart	2	19
BamH1	0.5	4.75
EcoR1	0.5	4.75
Water	11	104.5

        ii. dSBPase Vector for 3 tubes.

Component	Per Tube (uL)	Master Mix (uL) = 3.5X(Per Tube)
Template	2	
Cutsmart	2	7
BamH1	0.5	1.75
HindIII	0.5	1.75
Water	15	52.5

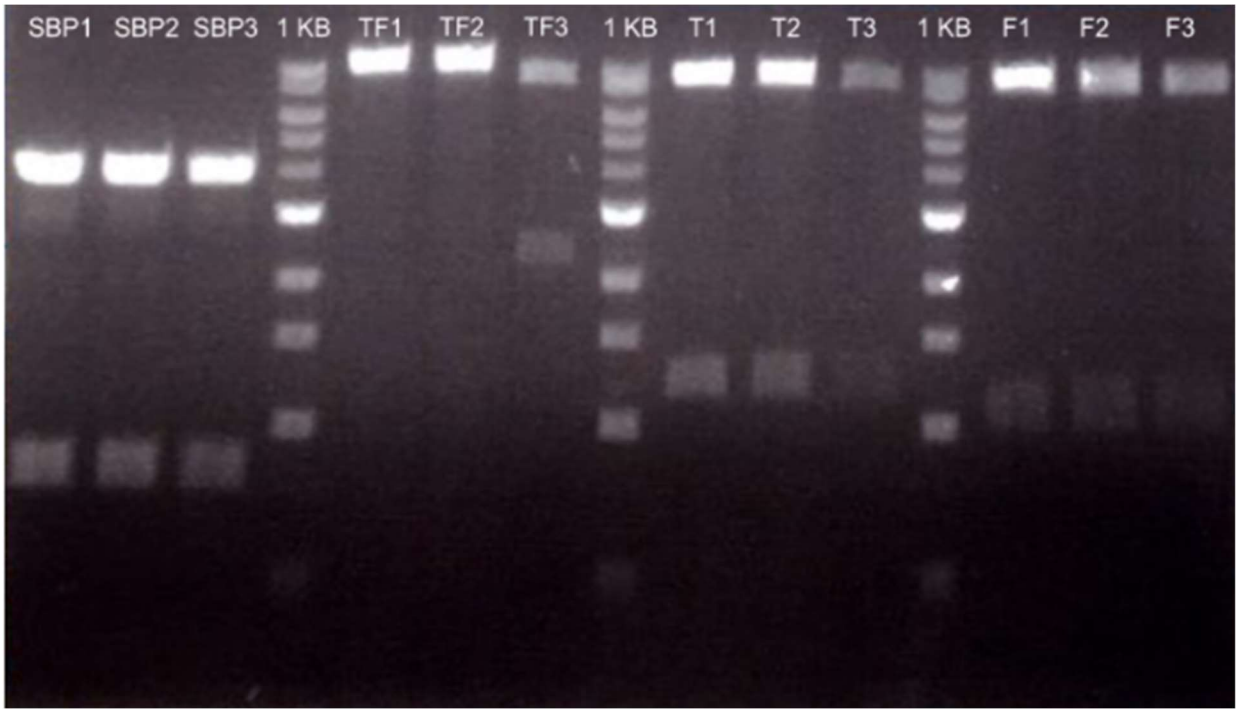
Tubes were incubated in PCR thermocycler at 37 ºC for 1 hr (lid 80 ºC).

Nanodrop Results:



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
sbp1	Default	8/9/2021	1:36 PM	260.04	5.201	2.826	1.84	1.53
sbp2	Default	8/9/2021	1:37 PM	213.87	4.277	2.223	1.92	1.93
SBP3	Default	8/9/2021	1:38 PM	198.51	3.970	2.210	1.80	1.23
TF1	Default	8/9/2021	1:40 PM	68.32	1.366	0.754	1.81	1.24
TF2	Default	8/9/2021	1:41 PM	52.89	1.058	0.561	1.88	1.42
TF3	Default	8/9/2021	1:41 PM	82.27	1.645	0.942	1.75	0.86
T1	Default	8/9/2021	1:43 PM	83.79	1.676	0.874	1.92	1.72
T2	Default	8/9/2021	1:43 PM	57.38	1.148	0.612	1.88	1.57
T3	Default	8/9/2021	1:44 PM	97.27	1.945	1.164	1.67	0.75
F1	Default	8/9/2021	1:45 PM	74.22	1.484	0.774	1.92	1.64
F2	Default	8/9/2021	1:46 PM	67.82	1.356	0.710	1.91	1.24
F3	Default	8/9/2021	1:47 PM	48.05	0.961	0.544	1.77	1.02

Electrophoresis Results:



SBPase1, TF3, T1, and F1 were selected for transformation. The transformation began at 6 pm, incubated in the dark at 30 ^{o}C. We centrifuged ~3.5 mL of *S. elongatus* cells, washed the pellet in 1 mL of 10 mM NaCl solution, and resuspended the new pellet with 200 uL of BG-11. Each culture was mixed with 200-300 ng of plasmid:

1 uL of SBPase 1

3 uL of TF3, T1 and F1 (respectively).

10 August 2021

Name: Avery Imes and Daria Perminova

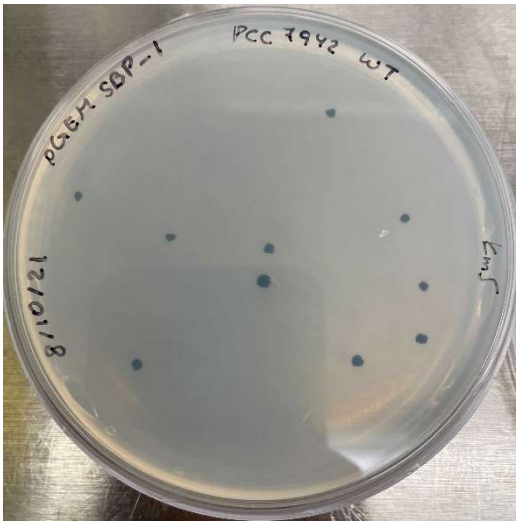
Summary:

The transformants were plated.

Protocol:

1. The PCC 7942 transformants were spread onto an antibiotic BG-11 plate for culturing colonies that integrated our SBP deletion or overexpression plasmid inserts into their genomes.
    a. SBPase colonies were plated on a km 5+ antibiotic plate.
    b. All overexpression colonies were plated on separate sp/sm 5+ antibiotic plates.
    c. All of the plates were incubated at 30°C in light for 4-7 days until transformants appeared.

SBPase Plate



13August 2021

Name: Daria Perminova

Summary:

We diluted the sequencing primers to 100 uM stocks and 10 uM working solutions. Then we performed the cycle sequencing and purification protocol outlined by the Center for Bioinformatics and Functional Genomics (within the Miami University college of Arts and Science). This was for confirmation that the amplified plasmids within the *E. coli dh10β* cells had the proper promoter and gene sequences.

Protocol:

1. The primers were diluted to 100 uM (nm X 10 = uL H_2O), and then diluted to 10 uM (20 uL primer in 180 uL H_2O).
2. 12 primers: 3 forward primers and 1 reverse primer for pTal+FBP, and 1 forward primer and 1 reverse primer for pTal, pFBP, SBPase seg, and NSI seg respectively.
3. For CBFG sequencing:
    a. ¼ Big dye reaction at a volume of 20 uL.
    b. Each tube had:
        i. 2 uL BigDye terminator v3.1 Ready Reaction Mix (BD)
        ii. 3 uL 5X sequencing dilution buffer (BDDB)
        iii. 0.5 uL primer (use 0.7 uL but inaccuracy gives us 0.5 uL)
        iv. 3 uL DNA template (either pT1, pF1, or pTF3 plasmid)
        v. 11.5 uL H_2O
    c. Master mix for 8 tubes (multiplied by 8.5 for pipetting error):
        i. 17 uL BD
        ii. 25.5 uL BDDB
        iii. 97.75 uL H_2O
    d. The thermocycler settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Denature	96	01:00	25X
Annealing	96	00:10	
Extension	50	00:05	
Final Extension	60	04:00	
Hold	4	∞	1X

    e. Purification protocol:
        i. Transferred contents to 1.5 mL tubes.
        ii. Spun the condensate down.

iii. Added 1/10 (2 uL) of the total volume of 1.5 M sodium acetate/0.25 M Na_2EDTA.
iv. Mixed the solution.
v. Centrifuged the solution at >12000 g (the max speed was used).
vi. Removed all of the supernatant with a pipette.
vii. Added 100-150 uL of 70% EtOH.
viii. Spun for 5 minutes at the top speed in the same orientation.
ix. The Supernatant was removed.
x. The pellet was allowed to air dry for 10 minutes with the tube caps open.
xi. The tubes were then stored in a -20 ^oC freezer/

16 August 2021

Name: Daria Perminova

Summary:

The transformed cultures were growing very well on the antibiotic plates- there is reason to believe that there was possible contamination. We therefore redid the transformations for PCC 7942 with the overexpression plasmids. We further transformed PCC 7942 cells that were null mutants for glucose-1-phosphate adenylyltransferase (ΔglgC). This mutant line was chosen to study the effect of overexpression of key Calvin Cycle enzymes on cells that aren't able to perform glycogen synthesis.

Protocol:

6 transformations in PCC 7942:

pGEM-fbp, pGEM-tal, and pGEM-fbp-tal in WT strains.

pGEM-fbp, pGEM-tal, and pGEM-fbp-tal in glycogen mutant (ΔglgC).

1. 1.5 mL of colonies were spun down for 2 minutes at maximum speed.
2. The colonies were washed with 1 mL of 10 mM NaCl and centrifuged for 1.5 minutes at maximum speed.
3. 1.5 uL of each plasmid (F1, T1, and TF3) were mixed into the cells.
4. The cells were incubated at 30 ^{o}C in the dark for about 16 hours.
    a. They were transformed at 3:30 pm.

17 August 2021

Name: Daria Perminova

Summary:

We spread the previous days wildtype and ΔglgC colonies onto BG-11 antibiotic plates and incubated them in light to culture colonies that inserted our overexpression plasmid inserts. These colonies, when confirmed to have the desired inserts into neutral site I (NSI) of their genomes, will be used for later growth experiments.

Protocol:

The colonies were plated on BG-11/antibiotic agar plates.

1. pGEM-fbp, pGEM-tal, and pGEM-tal+fbp (WT and ΔglgC) were plated with sp/sm2+ antibiotics.
2. The transformed WT colonies precipitated in the 1.5 mL tubes, so another transformation was done. Transformed ΔglgC colonies did not precipitate.
3. This marked the third set of plates with WT transformants (1 from August 10th and 2 from August 17th).
4. The plates will be incubated in 30 ^{o}C for 4-7 days.

18 August 2021

Name: Daria Perminova

Summary:

We analyzed the sequences returned from the CBFG to confirm that there were no mutations in the overexpression constructs within the plasmids purified from E. coli dh10$\boldsymbol{\beta}$ cells.

Protocol:

1. The sequencing results were checked.
2. It was decided that we needed to reconfirm the sequence for *tal* in pGEM-tal+fbp and *fbp* in pGEM-fbp. There was a small stretch of sequence within these genes that we could not confirm. This was most likely due to enzyme inaccuracy and inefficiency at the extreme ends of a fragment being sequenced.
3. Because we ran out of our stored purified plasmids we took frozen dh10$\boldsymbol{\beta}$ that expressed the proper plasmids and inoculated them for colony isolation- these were labeled TF3 and F1 (see 9 August).
4. Plasmid prep and sequencing will be done on Friday, August 20th (two days from now).
5. The colonies will be selected Thursday, August 19th (tomorrow).

21 August 2021

Name: Daria Perminova

Summary:

We did plasmid prep as previously described through the Pureyield Plasmid Miniprep System. We then resequenced as previously described for pGEM-tal+fbp and pGEM-fbp.

Protocol:

1. The same procedure as before was followed for TF1, TF2, F1, and F2, but 1.5 uL of the template was used.
2. We did not get all of the way to purification, only to cycle sequencing.

23 August 2021

Name: Daria Perminova

Summary:

Summary

Protocol:

1. Individual colonies from the transformed PCC 7942 WT cells were transferred onto fresh plates for eventual confirmation and growth experiments of clones.
2. pGEM-SBPase colonies were transferred onto a fresh plate to select for colonies in which a double crossover occurred for our deletion construct.

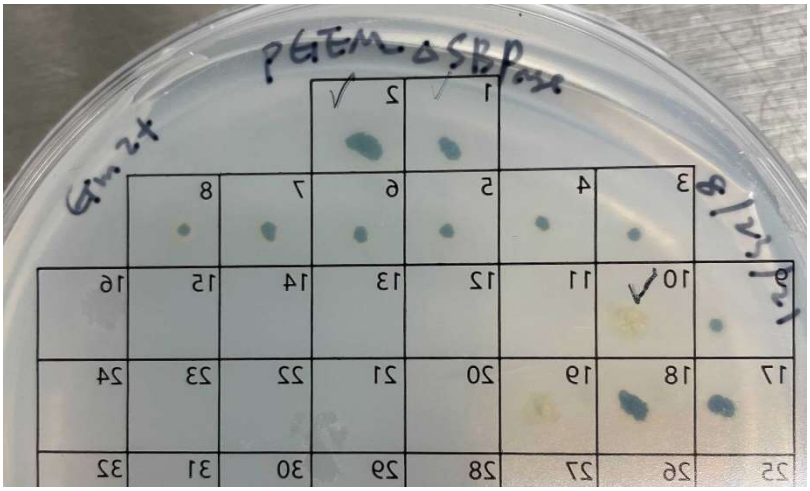
WT PCC 7942 used for sequencing may have been contaminated with a strain with sp/sm resistance already integrated into the neutral site. Sharmeeta has revived a stock from -80 °C that we will use once the OD is high enough.

Colonies were isolated and plated on BG-11 antibiotic resistance plates.

1. 10 colonies from pGEM-tal, pGEM-fbp, and pGEM-tal+fbp were plated on sp/sm 2+.
2. 3 colonies from pGEM-SBPase were plated on a km 5+ plate and a gm 2+ plate to check for double crossover.
3. Sequencing prep from last Friday was completed and the 1.5 uL tubes were turned in to CBFG.

Results:

Km5+



gm2+

Colonies that grew up on km but not gm were looked for because that means there was a double crossover and the whole plasmid was not integrated.

25 August 2021

Name: Daria Perminova

Summary:

The transformed PCC 7942 ΔglgC were transferred onto a fresh antibiotic plate. We will use clones for confirmation and later growth experiments.

Protocol:

10 colonies of each of the ΔglgC mutants with pGEM-tal, pGEM-fbp, and pGEMtal+fbp were plated on km5 and sp/sm BG-11 plates.

28 August 2021

Name: Avery Imes and Daria Perminova

Summary:

Colonies from 8/28 did not grow. We repeated the work of 8/28 to verify the same result.

We plated colonies of ΔglgC mutants with pGEM-tal, pGEM-fbp, and pGEM-tal+fbp on km5+ and sp/sm 2+ BG-11 plates

Protocol:

13 colonies of ΔglgC mutants with pGEM-tal, pGEM-fbp, and pGEM-tal+fbp were each plated on km5+ and sp/sm 2+ BG-11 plates.

30 August 2021

Name: Avery Imes and Daria Perminova

Summary:

Summary

Protocol:

Colonies from 8/23 pGEM-SBPase antibiotic plates were transferred to new plates in order to confirm growth pattern and begin segregation. Colonies 1-10 grew on Km. Colonies 1,2 and 10 did not grow on Gm2+ but grew on KM5+, indicating a single crossover and thus integration of the target sequence without integration of the vector backbone. Each colony was transferred onto a fresh identical plate (Colonies 1, 2 and 10 on Km5+ were transferred to a new Km5+ plate, in spots 1, 2, and 3. Colonies 1, 2 and 10 on Gm were transferred to spots 17,18,19 on the same Gm plate).

We selected transformed “wildtype” overexpression colonies that had previously been isolated and performed segregation PCR. This was to confirm that the oligonucleotide fragment incorporated into neutral site I of these colonies was the correct one. Knowing the size of the fragments we are amplifying helped us decide on an elongation time because Q5 adds 1 kb every 30 seconds.

We transferred 7 colonies transformed with pGEM-tal, pGEM-fbp, and pGEM-tal+fbp into PCR tubes holding 10 ul of ddH2O.

We incubated the colonies at 99°C for 10 minutes to lyse the cells and release the genomic material

We tried to prepare the following solutions for segregation PCR but had to adjust the procedure because 8.8 ul of 5X buffer was added instead of 88 ul.

Segregation PCR Mix:

Compnent	**Per Tube (uL)**	**Master Mix (uL)**
5x Bffer	4	88
dNTP	0.4	8.8
Forward Primer	1	22
Reverse Primer	1	22
Pol	0.2	4.4
H_2O	3.4	74.8
Total	10	

14 colonies (T 1-7 and F 1-7) received 10 ul from the improperly made master mix. We transferred 5 ul from T 1, 2, 5, 6, and 7 and F 1-7 into new PCR tubes. There was a

contamination issue with T 3 and T 4. We then made a new master mix adjusted for adding 15 ul to each tube. This means that instead of 3.4 ul of ddH2O per tube, the volume per tube was 8.4 ul. We added 15 ul of this new master mix to each of the 5 ul transferred solutions for T 1, 2, 5, 6, and 7 and F 1-7. We added 10 ul of this master mix to the 10 ul solutions of lysed colonies of TF 1-7. The final PCR volumes were all 20 ul.

We used a two-step PCR protocol which means that the amplification and elongation steps were at the same temperature and, therefore, combined.

Thermocycler settings:

PCR Phase	**Temperature (°C)**	**Time (min:sec)**	**Rounds**
Initial Denature	98	02:00	1X
Denature	98	00:15	40X
Annealing	72	02:45	
Extension	72	02:45	
Final Extension	72	05:00	1X
Hold	10	∞	

31 August 2021

Name: Daria Perminova

Summary:

Summary

Protocol:

We ran the gel for the segregation PCR and compared the amplicon sized to a 1 kb ladder to determine if the proper fragment was inserted into NSI of the PCC 7942 genome.

1. Gel 1 organization:

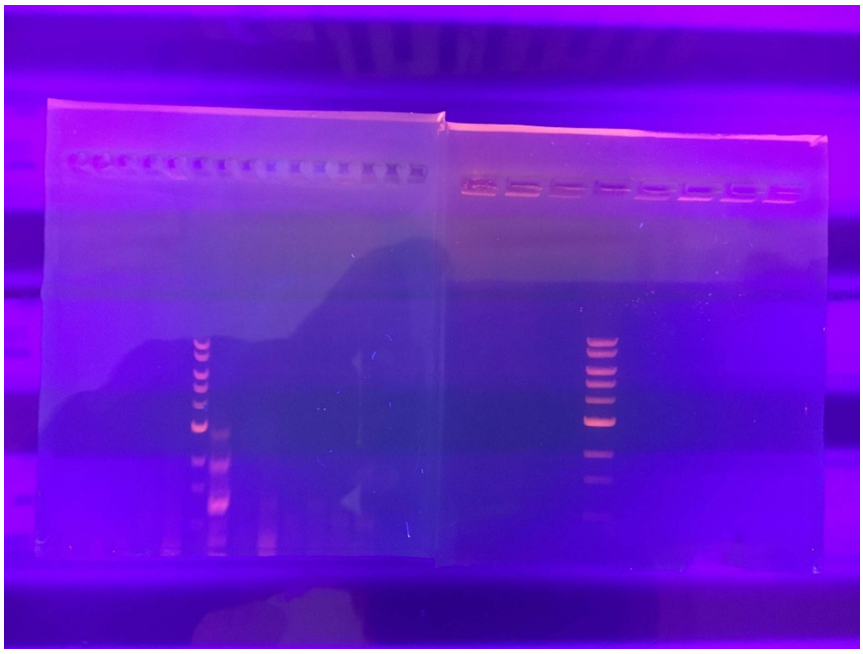
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T1	T2	T5	T6	T7	1 kb ladder	F1	F2	F3	F4	F5	F6	F7		

2. Gel 2 organization:

1	2	3	4	5	6	7	8
TF1	TF2	TF3	1 kb ladder	TF4	TF5	TF6	TF7

Results:

Gel Results:



We potentially had a WT band between 1.0 and 0.5 kb in lane 7 of gel 1 (F1). We also saw amplification (but not of desired fragment size) in lanes 5 (T7) and 9 (F3) of gel 1. There was no amplification seen in gel 2 (apart from DNA ladder).

We also transferred ΔglgC colonies transformed with the overexpression plasmids onto a fresh BG-11 antibiotic plate. These were 10 colonies each for pGEM-tal, pGEM-fbp, and pGEM-tal+fbp. The antibiotic was km 5+ and sp/sm 2+. We did this last isolation from the originally streaked plates when we first transformed the ΔglgC cells because they were drying out.

1 September 2021

Name: Avery Imes and Daria Perminova

Summary:

Summary

Protocol:

Because the gel from 8/31/21 showed that our segregation PCR did not amplify the desired fragment sizes, we redid the procedure as originally intended (and not adjusted for any mistakes). We did make the adjustment to the PCR solutions however to reduce the number of cells. After 10 ul of cells were lysed, we used only 2 ul of them for template. The master mix was then adjusted so that 18 ul would be added to each 2 ul of template. This means that the ddH2O volume was increased to 11.4 ul per PCR tube.

Component	**Per Tube (uL)**	**Master Mix (uL)**
5x Bffer	4	88
dNTP	0.4	8.8
Forward Primer	1	22
Reverse Primer	1	22
Pol	0.2	4.4
ddH_2O	11.4	250.8
Total	18	

2 September 2021

Name: Max Brenner and Daria Perminova

Summary:

Summary

Protocol:

We ran the gel for the segregation PCR from 9/1/21. We used 0.6 ul of agarose in 60 mL of TAE buffer. There was amplification but not as expected.

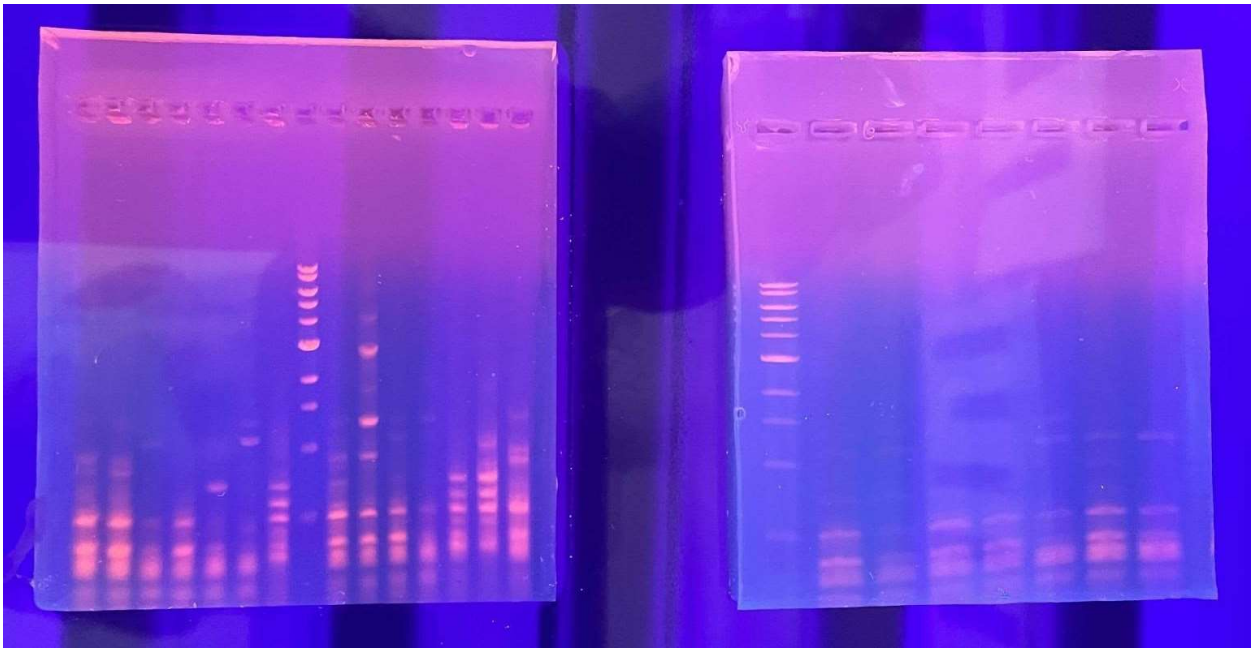
Gel 1 Organization:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T1	T2	T3	T4	T5	T6	T7	1 kb ladder	F1	F2	F3	F4	F5	F6	F7

Gel 2 Organization:

1	2	3	4	5	6	7	8
1 kb ladder	TF1	TF2	TF3	TF4	TF5	TF6	TF7

Results:



3 September 2021

Name: Avery Imes and Max Brenner

Summary:

We re-performed the standard Segregation PCR on the transformed WT colonies with different primers. We expected that this would improve the efficiency because the amplicon would consist of few nucleotide base pairs.

Protocol:

The new primers used: NSI_seq-Rev and pTal-seq742-R

pGEM-TF:

T_{ma} = 66 °C 2.6 kb

pGEM-F:

T_{ma} = 68 °C 2.6 kb

Colony #3 (#10) was plated in dilution on km5+ from August 30th.

The colony did not grow on the Gm plate but #1 and #2 did, indicating a double crossover.

Plate km colony on Gm plate?

Repeating segregation with new primers:

For pGEM-TF and pGEM-Tal, the primers NSI_seqRev with pTal_seq742-R were used with a T_{ma} of 66 ℃.

For pGEM-FBP, the primers NSI_seq_R with pFBP_seq742-R with a T_{ma} of 68 ℃.

Colonies were picked from the sp/sm2+ plate from August 23rd.

1. 1 colony was picked and put into 10 uL of water.
    a. The colonies used were T1-7, F11-17, and TF21-27.
2. The colonies were lysed at 99 °C for 10 minutes.
3. 2 uL of template was used in the 20 uL reaction.
4. The Master Mix for each pF, and the Master Mix for pT and pTF:

Component	Per Tube (uL)	pTF and pT (uL)	pF
5X Buffer	4	60	30
dNTP	0.4	6	3
Forward Primer	1	15	7.5

Reverse Primer	1	15	7.5
Pol	0.2	3	1.5
ddH_2O	3.4	171	85.5
Total	18	270	135

Name: Max Brenner and Daria Perminova

Summary:

We transformed the newly revived WT PCC 7942 cells with overexpression plasmids purified from E. coli dh10β. These were from the colonies/plasmids labeled T1, F1, and TF3. The protocol used was exactly the same as before but we had to use more initial rounds of centrifugation to reach a high enough cell density.

Protocol:

1. 9 1.5 uL aliquots of WT PCC 7942 were centrifuged for 1 minute at max speed.
2. The aliquots were combined into 3 tubes and centrifuged at max speed for 3 minutes.
3. The supernatant was discarded, and the cell pellet was washed with 1 mL of 10mM NaCl.
4. After 2 more minutes of centrifugation, the cell pellet was resuspended in BG-11.
5. In the three tubes, 1 uL of the respective DNA template (pGEM-tal, pGEM-fbp, and pGEM-fbp+tal) were added and then the tube was incubated in the dark at 30 ^oC for 4 – 16 hours (start time at 4:00 pm).

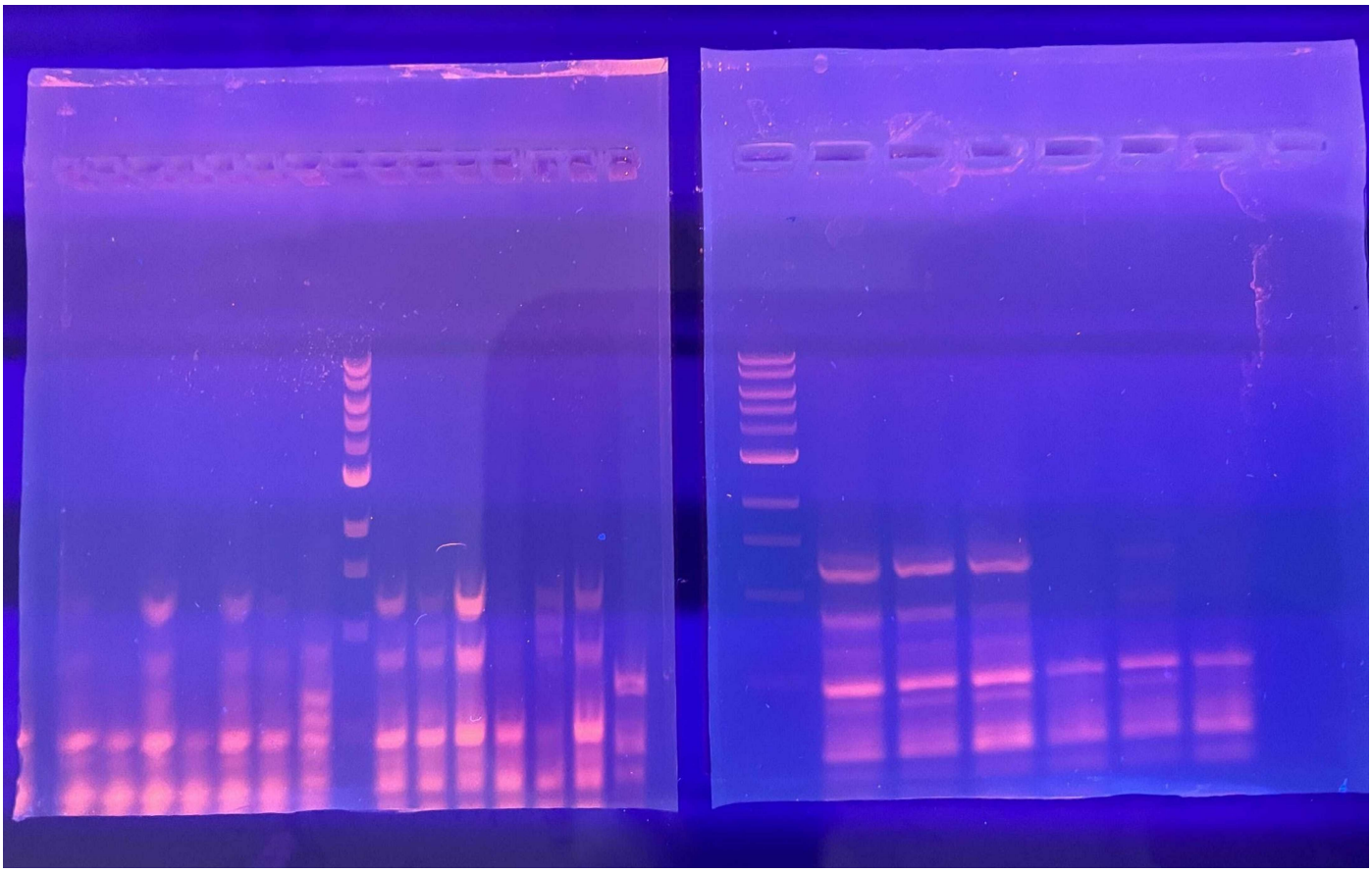
1. We also ran the gel from the segregation PCR from earlier today.
2. Gel 1 Organization:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T1	T2	T3	T4	T5	T6	T7	1 kb ladder	F1	F2	F3	F4	F5	F6	F7

3. Gel 2 Organization:

1	2	3	4	5	6	7	8
1 kb ladder	TF1	TF2	TF3	TF4	TF5	TF6	TF7

Gel Electrophoresis Results:



The band lengths were smaller than expected with more nonspecific amplication.

4 September 2021

Name: Daria Perminova

Summary and Protocol:

We spread the 200 ul solutions of overexpression transformants from 9/3 onto BG-11 antibiotic plates (sp/sm 2+). These colonies were WT PCC 7942 transformed with pGEM-tal, pGEM-fbp, and pGEM-tal+fbp. They were left in light at 30℃. Transformants are expected within 4-7 days.

7 September 2021

Name: Daria Perminova

Summary:

We redid overexpression transformants (and are going to use fresh plates) for WT and ΔglgC colonies.

Protocol:

* Yesterday (9/6), Dr. Wang transformed and plated ΔSBPase colonies on Km5+ and all three overexpression transformants on sp/sm 2+ again because the transformants from 6/4 were still growing up too fast on the plates (green "lawns"). There may be an issue with the plates because if the antibiotic was added when the solution was too hot, then it would deactivate the antibiotic.

We reattempted transformed overexpression genes into WT and ΔglgC mutant colonies.

We used 1.5 mL of WT and centrifuged the tube for 3 minutes at max speed initially.

We used 1 mL of ΔglgC and centrifuged the tube for 2 minutes at max speed initially.

The WT colonies were incubated at 11:50 am, and the ΔglgC mutant colonies were incubated at 12:54 pm.

All of the tubes were incubated at 30 ^{o}C in the dark for about 5 hours and then plated on sp/sm2+ at 30 ^{o}C in the light for 4-7 days.

8 September 2021

Name: Daria Perminova

Summary:

We did segregation PCR for potential dSBPase double crossover mutant. This colony grew on Km but not Gm. We took from the dilution series on Km5+ plate (8/30) quadrants 9-15.

Protocol:

Protocol

The primers used were SBPase_seg-F and SBPase_seg-R. The total fragment length is 1559 bp. The annealing temperature from the NEB T_m calculator was 72 ^oC. In the genome the fragment size (or wildtype/ unsegregated band) would be 825 bp.

1. The master mix:

Component	Per Tube (uL)	Master Mix (uL)
5x Buffer	4	30
dNTP	0.4	3
Forward Primer	1	7.5
Reverse Primer	1	7.5
Pol	0.2	1.5
ddH_2O	11.4	85.5
Total	18	

2. The thermocycler settings:

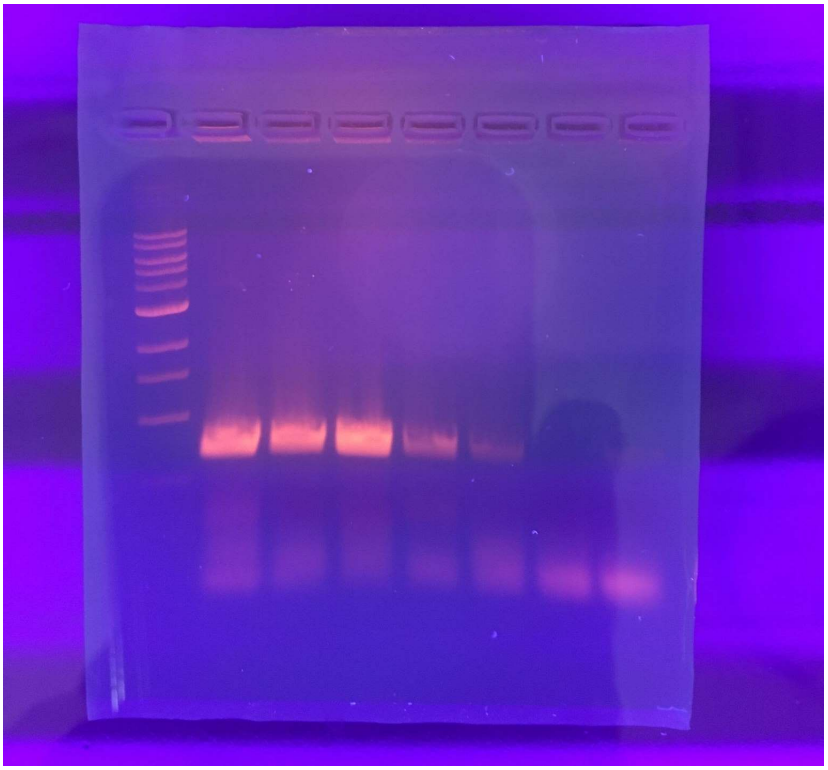
PCR Phase	Temperature (^oC)	Time (min:sec)	Rounds
Initial Denature	98	02:00	1X
Denature	98	00:15	40X
Annealing	72	00:30	
Extension	72	00:30	
Final Extension	72	05:00	1X
Hold	10	∞	

3. Gel electrophoresis was run on one gel.

Results:

The wildtype bands were below the expected length for double crossover (1559 bp) and matched more closely to that of wildtype band length (just below 1 Kb or 825 bp). This occurred

because PCC 7924 has multiple copies of its genome. SBP is an essential gene so only a few copies of the genome had the SBP removed, but this still allowed for km resistance. During PCR, the smaller fragments of the wildtype SBPase (rather than the longer interrupted fragment containing the kmR sequence) were more efficiently amplified.



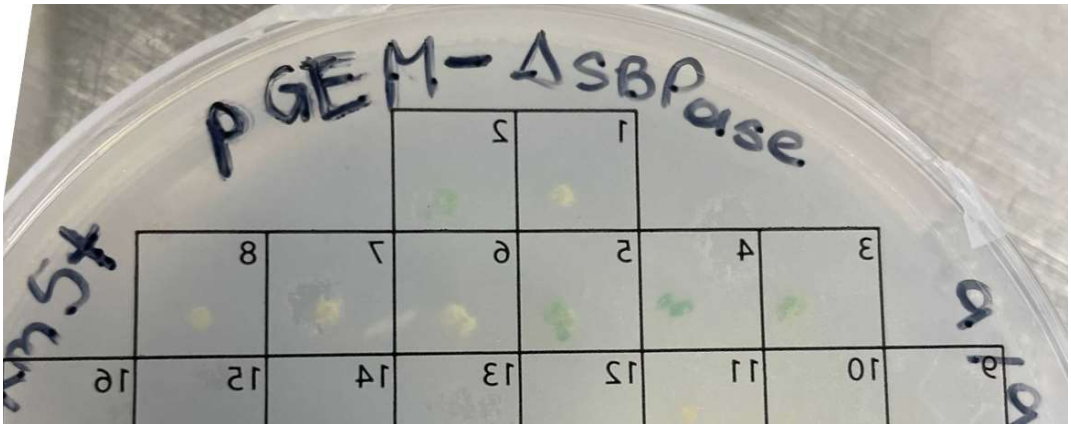
9 September 2021

Name: Daria Perminova

Summary:

We took a fresh Km5+ plate and replated colony #3 from Km5+ plate (8/30) in a dilution series from quadrants 1-11.

Results:



13 September 2021

Name: Avery Imes and Daria Perminova

Summary:

PCR segregation was performed on the pGEM-SBPase September 9th. 1-7 cells lysed

Protocol:

Protocol

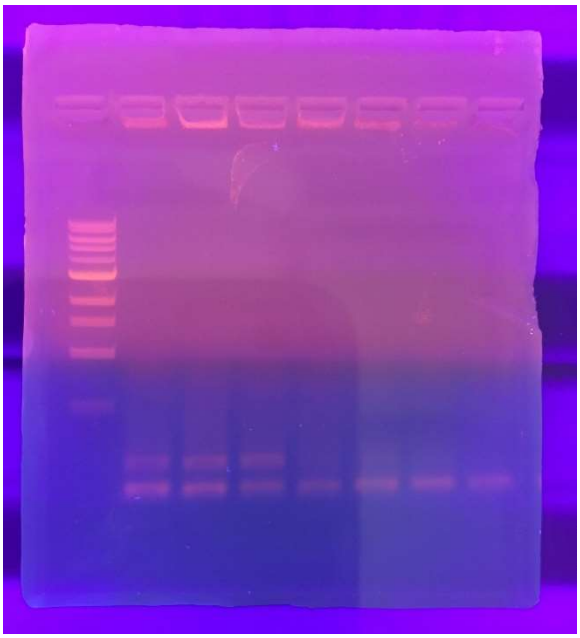
1. 1-7 cells were lysed in 9 uL of water and the total volume was used as a template. 0.5 of each primer (SBPase_seq-F and SBPase_seq-R). 10 uL of UC101 Accuris Taq Master Mix Red was added.
2. Thermocycler settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	58 (T_m for primers is 63 ^{0}C)	00:15	
Extension	72	01:15	
Final Extension	72	07:00	1X
Hold	12	∞	

3. A gel electrophoresis was run.

Results:

There wasn't much amplification- the most visible bands seemed to be far below 0.5 kb.



Protocol:

We redid the segregation PCR with colonies #25-31 from the km 5+ (8/30) plate. We lysed colonies in 10 ul of water (left at 99℃ for 10 min) and used 2 ul for template.

1. The Master Mix:

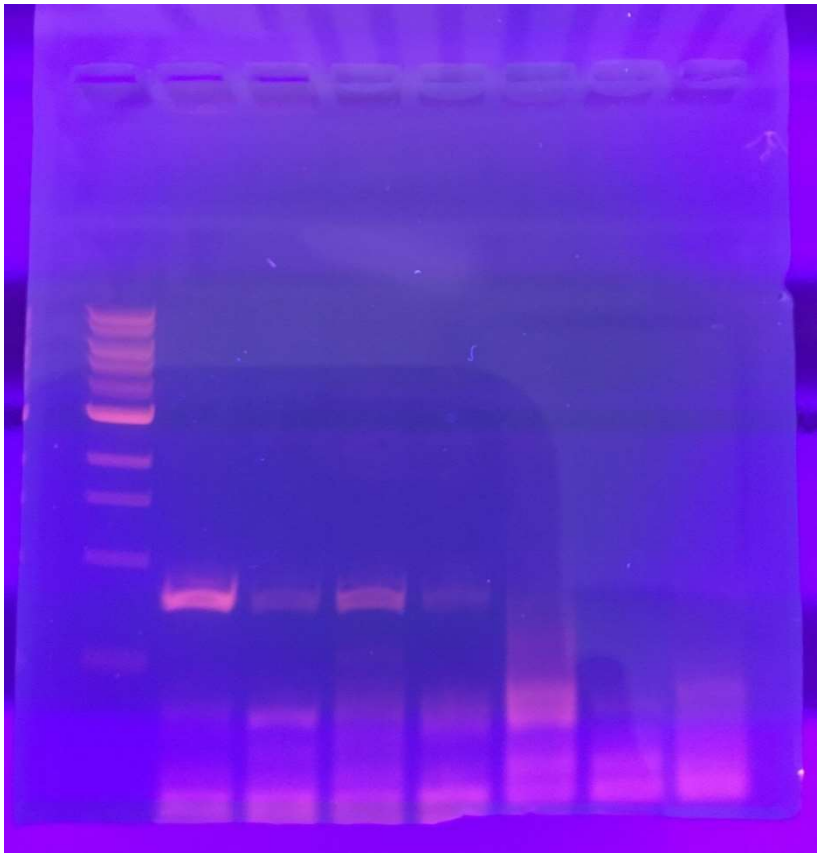
Component	Per Tube (uL)	Master Mix (uL)
5x Buffer	4	30
dNTP	0.4	3
Forward Primer	1	7.5
Reverse Primer	1	7.5
Pol	0.2	1.5
ddH_2O	11.4	85.5
Total	18	

2. Thermocycler Settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	98	02:00	1X
Denature	98	00:15	40X
Annealing	72	00:30	
Extension	72	00:30	
Final Extension	72	05:00	1X
Hold	10	∞	

3. Gel electrophoresis was run:

Results:



The bands were WT bands (~800 bp or just under 1 kb). It seems that if there were any more genome copies with the silenced/ interrupted SBP gene, it would be lethal/unfavorable. It might be difficult to get enough of the silenced version of SBP to show up on a gel (~1.5 kb).

16 September 2021

Name: Max Brenner

Summary:

We performed the transformation again for pGEM-tal+fbp for WT colonies. The transformation wasn't efficient for these colonies to grow up from the 9/7 transformation because two genes are being overexpressed in this construct.

Protocol:

Protocol

The transformation time was 2:30 pm.

1. 1 mL of WT colony was added to a 1.5 mL centrifuge tube.
2. The tube was centrifuged at 15000 rpm for 2 minutes.
3. The supernatant was removed and then the cell pellet was resuspended in 200 uL of BG-11.
4. 2 uL of pGEM-tal+fbp was added to the tube.
5. The tube was wrapped in foil and incubated in the dark at 30 ^{o}C.

17 September 2021

Name: Daria Perminova

Summary and protocol:

200 ul of the pGEM-tal+fbp transformed WT colonies from September 16th were plated onto sp/sm 2+ plate.

20 September 2021

Name: Daria Perminova

Summary:

The pGEM-tal+fbp transformed WT colonies from 9/17 grew up as a "lawn." There is a possibility of contamination from the BG-11 liquid media used to supplement them.

Protocol:

We transferred pGEM-tal, pGEM-fbp, and pGEM-tal+fbp WT colonies from September 7th onto a fresh sp/sm 2+ plate with quadrants. tal was plated on 1-15, fbp was plated on 17-31, and tal+fbp was plated on 33-onward. The ΔglgC mutants transformed with our overexpression plasmids are not growing up very well, so we aren't able to transfer.

Transformation time: 12:30 pm.

We transformed WT with pGEM-tal+fbp again (although this probably will not work).

1. We centrifuged WT colonies at max speed for 2 minutes.
2. The cell pellet was washed with 1 mL of 10 mM NaCl.
3. The tube was centrifuged at max speed for 2 minutes.
4. The cell pellet was resuspended in 200 uL of BG-11.
5. 1 uL of the pGEM-tal+fbp was added to the tube.
6. The contents were spread onto a sp/sm2+ plate.

We performed segregation PCR on SBPase colonies 1-11 from the km5+ plate from September 9th.

1. The colonies were lysed at in 9 uL of water for 10 minutes at 99 ^{o}C.
2. The Master Mix:

Component	Per Tube (uL)	Master Mix (uL)
UC101 Accuris 2X Master Mix	10	120
Forward Primer	0.5	6
Reverse Primer	0.5	6
Total	11	

3. Thermocycler Settings:

PCR Phase	Temperature (^{o}C)	Time (min:sec)	Rounds

Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	01:00	
Final Extension	72	05:00	1X
Hold	10	∞	

4. A gel electrophoresis was run.

Results:

Same conclusion as before for SBPase colonies- it is not possible to get the 1.5 kb SBP band with the km insert to show up on the gel. Colonies 2,3,4, and 5 were green- these had a prominent "WT" band at ~800 bp. The other colonies were yellow on the plate. The last two bands were both colony #11- the wells were broken in between so colony 11 leaked into the empty well next to it.

21 September 2021

Name: Daria Perminova and Max Brenner

Summary:

The segregation PCR and gel electrophoresis for the km5+ 9/9 plate colonies #1-11 with SBPase_KanR-rev and SBPase_sef-F was performed.

Protocol:

The total fragment length is 1.428 kb.

Primer	**T_m (°C)**
dSBPase_KanR-rev	66.7
dSBPase_seg-F	63.2

1. SBPase colonies #1-11 from the km5+ plate from 9/9 were lysed in 9 uL of ddH_2O for 10 min at 99 °C.
2. The Master Mix was made in the following way:

Added Material	**Per Tube (uL)**	**Master Mix (uL)**
UC101 Accuris Master Mix	10	120
7942NS1_Seg-F	0.5	6
7942NS1_Seg-R	0.5	6
Total	11	132

3. Thermocycler Settings:

PCR Phase	**Temperature (°C)**	**Time (min:sec)**	**Rounds**
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	01:15	
Final Extension	72	7:00	1X
Hold	12	∞	

Results:

The gel was over run so there were no viable results.

22 September 2021

Name: Daria Perminova and Max Brenner

Summary:

The segregation PCR and gel electrophoresis for the km5+ 9/9 plate colonies #1-11 was repeated with SBPase_KanR-rev and SBPase_sef-F.

Protocol:

The total fragment length is 1.428 kb.

Primer	**T_m (°C)**
dSBPase_KanR-rev	66.7
dSBPase_seg-F	63.2

4. SBPase colonies #1-11 from the km5+ plate from 9/9 were lysed in 9 uL of ddH_2O for 10 min at 99 °C.
5. The Master Mix was made in the following way:

Added Material	**Per Tube (uL)**	**Master Mix (uL)**
UC101 Accuris Master Mix	10	120
7942NS1_Seg-F	0.5	6
7942NS1_Seg-R	0.5	6
Total	11	132

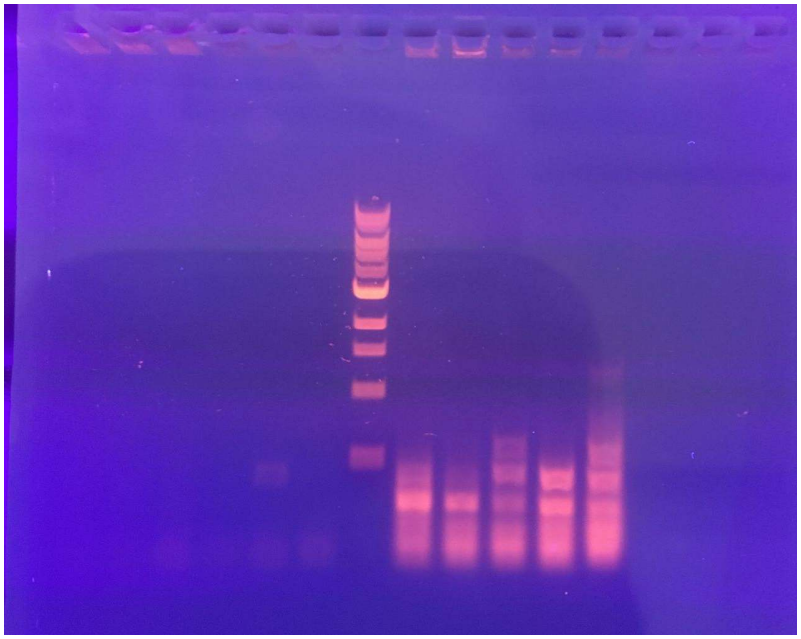
6. Thermocycler Settings:

PCR Phase	**Temperature (°C)**	**Time (min:sec)**	**Rounds**
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	01:15	
Final Extension	72	7:00	1X
Hold	12	∞	

Transferring colonies to new plates:

1. We redid colony transfers for transformants from 9/7 due to slow growth in the transfers from 9/20. We transferred WT colonies transformed with pGEM-tal, pGEM-fbp, and pGEM-tal+fbp (9/7) onto a fresh sp/sm 2+ plate with quadrants. tal was plated on 1-15, fbp was plated on 17-31, and tal+fbp was plated on 33-onward. The tal-fbp 9/7 plate doesn't have many colonies so less transfers can be done.

Results:



There was little to no amplification for colonies #1-6 (most likely very few copies of km- interrupted SBPase). Colony #11 may have potentially had some amplification at ~1.4 kb. Overall, it is very difficult to have amplification of km-interrupted/ ΔSBPase due to only a few copies of the genome having this insert.

The pGEM-tal+fbp-transformed WT colonies from 9/20 are growing up as a lawn as expected.

27 September 2021

Name: Daria Perminova and Max Brenner

Summary:

Segregation PCR was performed on the overexpression colonies transferred to sp/sm2+ plates on September 20th. Reordered neutral site primers 7942NS1_Seg-F and 7942NS1_Seg-R from Dr. Wang's lab were used for the segregation PCR. (**WHY?**)

Protocol:

Primer	**T_m (°C)**
7942NS1_Seg-F	64
7942NS1_Seg-R	65.9

T_{ma} is 72 °C

Expected fragment length for pGEM-tal is 6380 bp, and for pGEM-tal+fbp is 7435.

1. 7 colonies for each overexpression plasmid was transferred and lysed in 9 uL of ddH_2O for 10 min at 99 °C.
2. The following colonies were used from the 9/20 sp/sm2+ plate:
    a. gGEM-tal: #8, #10-15
    b. pGEM-fbp: #17-21, #23, #27
    c. pGEM-tal+fbp: #34-40
3. The master mix was made in the following way:
    a.

Added Material	**Per Tube (uL)**	**Master Mix (uL)**
UC101 Accuris Master Mix	10	210
7942NS1_Seg-F	0.5	10.5
7942NS1_Seg-R	0.5	10.5
Total	11	231

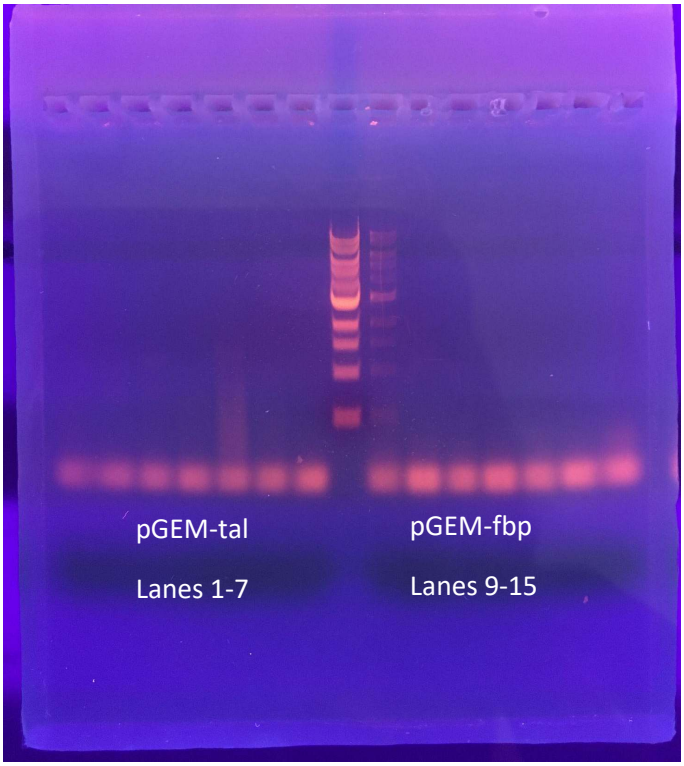
    b. More Master Mix was made because there was not enough for each tube. The additional Master Mix contained 1 uL of each primer and 20 uL of UC101 Accuris Master Mix.
4. Thermocycler settings:

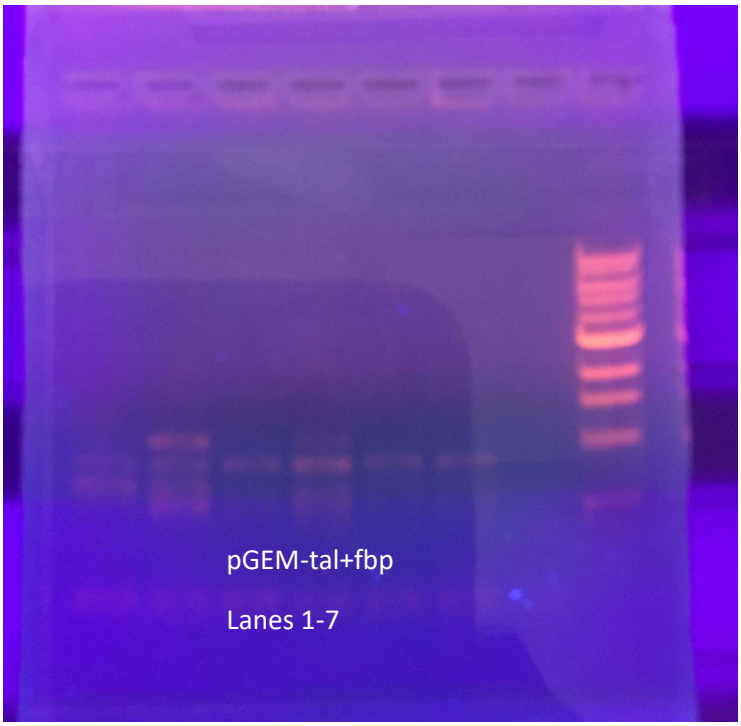
PCR Phase	**Temperature (°C)**	**Time (min:sec)**	**Rounds**
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	61	00:15	
Extension	72	08:00	

Final Extension	72	7:00	
Hold	12	∞	1X

5. Gel electrophoresis was run with 0.3 g agarose in 30 mL of 0.5x TAE buffer.

Results:





The desired fragment lengths of about 6 and 7 kb were not found. There is a possibility that we can find colonies that do not produce WT bands and/or the large ~6 to ~7 kb fragments once the transfers grow more.

Final Summary:

After running a PCR with a longer extension time the gel electrophoresis did not show results that contained the over expression genes.

29 September 2021

Name: Daria Perminova

Summary:

We prepared 500 mL of liquid BG-11 media and inoculated it with our overexpression colonies. At a future date we will re perform the segregation PCR with these liquid cultures and begin growth experiments in a multi cultivator.

Protocol:

Protocol

The media contained:

1. 10 mL 50X BG-11 pH 4.4 (6-22-21)
2. 5 mL 100X TES pH 8.2 (3-19-21)

We prepared ~500 mL of media. 50 mL of media was taken and treated with 5 ul of 20 mM sp/sm (1000X dilution), and ~3 mL were allotted per well. There were a total of 18 wells and 6 were designated for each overexpression treatment. We inoculated each well with green colonies (colony numbers recorded below).

We used transfers from September 20th and September 22nd (1=9/20, 2=9/22):

1. tal: 1-8, 1-10, 1-11, 1-12, 1-13, and 1-14
2. fbp: 1-17, 1-18, 1-19, 1-20, 1-21, and 1-26
3. tal+fbp: 1-33, 1-36, 1-39, 1-40, 2-33, and 2-34

4 October 2021

Name: Daria Perminova

Summary:

We performed segregation PCR on the liquid cultures prepared on September 29th.

Protocol:

Protocol

1. We used 10 ul of each culture and lysed the cells at 99℃ for 10 min. There were a total of 18 cultures (each made from a single colony). There were 6 cultures of transformants for each overexpression plasmid (pGEM-tal, pGEM-fbp, and pGEM-tal+fbp).
2. The Master Mix:

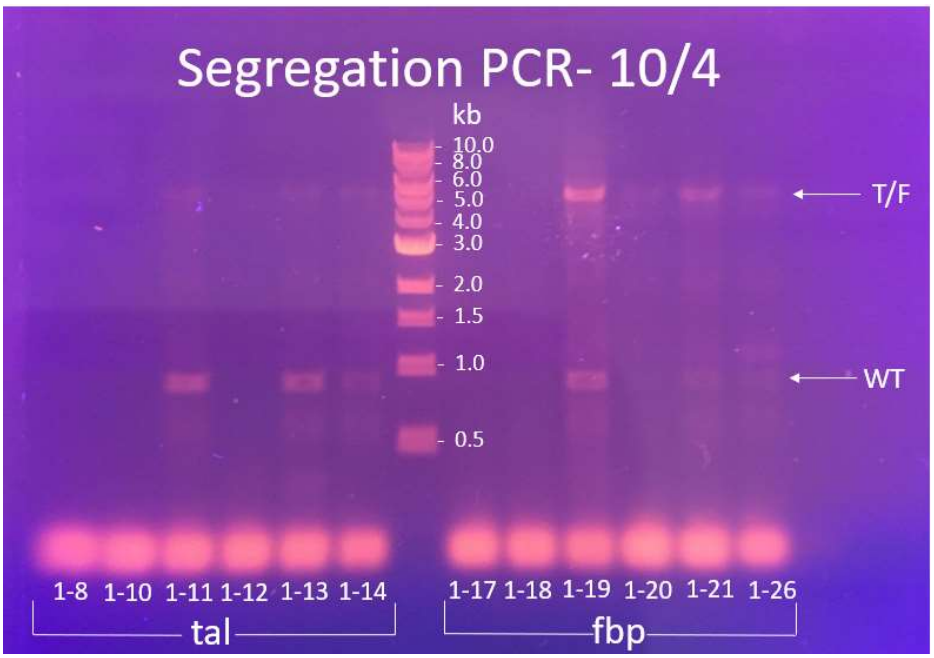
Added Material	Per Tube (uL)	Master Mix (uL)
UC101 Accuris Master Mix	10	185
7942NS1_Seg-F	0.5	9.25
7942NS1_Seg-R	0.5	9.25
ddH_2O	7	129.5
Total	18	

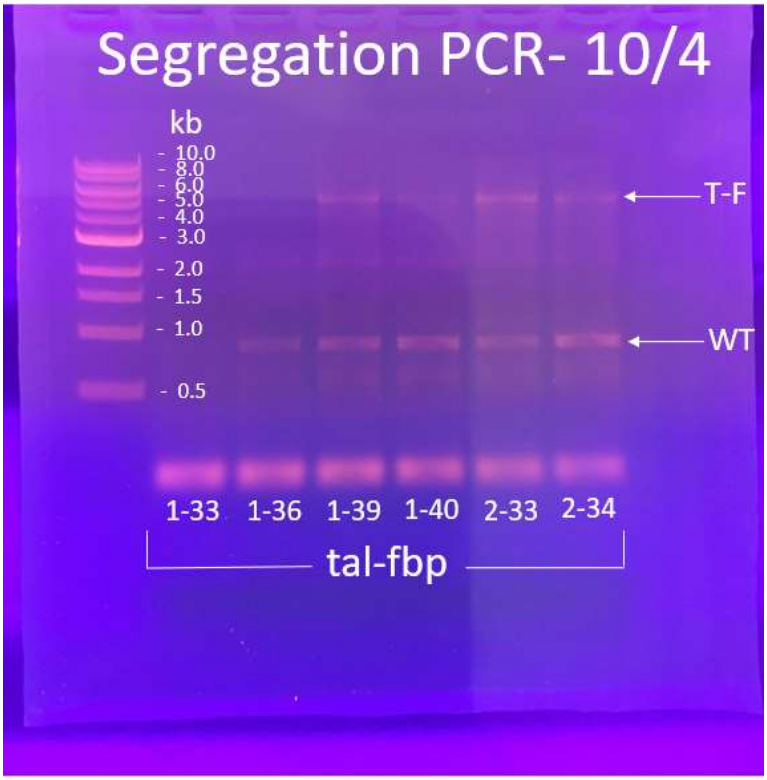
3. Thermocycler Settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	04:30	
Final Extension	72	7:00	1X
Hold	12	∞	

4. A gel electrophoresis was run.

Results:





For tal, colonies 1-11, 1-13, and 1-14 showed partial segregation. For fbp, colonies 1-19, 1-21, and 1-26 showed partial segregation. For tal-fbp colonies 1-39, 1-40, 2-33, and 2-34 showed segregation.

5 October 2021

Name: Daria Perminova

Summary and Protocol:

Based on yesterday's PCR results, we transferred tal 1-14, fbp 1-19, and tal+fbp 2-23 to a larger volume of media. We chose the colony numbers based on a higher relative amplification of a ~6-7 kb fragment and/or smaller amplification of the ~900 bp WT band. We added 500 ul of each culture in separate 250 mL flasks and added the BG-11 media (prepared on 9/29) up to 20 ml (~19 ml). In addition, we added 2 ul of 20 mM sp/sm to each flask (1000X dilution).

We will use the sine function in the MC-1000 OD protocol to simulate the natural pattern of changing light levels during the day. Light usually peaks at ~noon.

6 October 2021

Name: Daria Perminova

Summary:

We reperformed segregation PCR for the liquid cultures prepared on 10/5

Protocol:

Protocol

1. 10 uL of tal 14, fbp 19, and tal+fbp 2-23 were lysed at 99°C for 10 minutes.
2. For three reactions 1 uL of template was used, and 2 uL of template was used for the other three reactions.
3. Master Mixes:

Added Material	Per Tube (uL)	Master Mix (uL)
UC101 Accuris Master Mix	10	35
7942NS1_Seg-F	0.5	1.75
7942NS1_Seg-R	0.5	1.75
ddH_2O	8	28
Total	19	

Added Material	Per Tube (uL)	Master Mix (uL)
UC101 Accuris Master Mix	10	35
7942NS1_Seg-F	0.5	1.75
7942NS1_Seg-R	0.5	1.75
ddH_2O	7	24.5
Total	18	

4. Thermocycler Settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	04:30	
Final Extension	72	7:00	1X
Hold	12	∞	

5. The tubes were stored in -20 °C overnight until a gel electrophoresis could be run the next day.

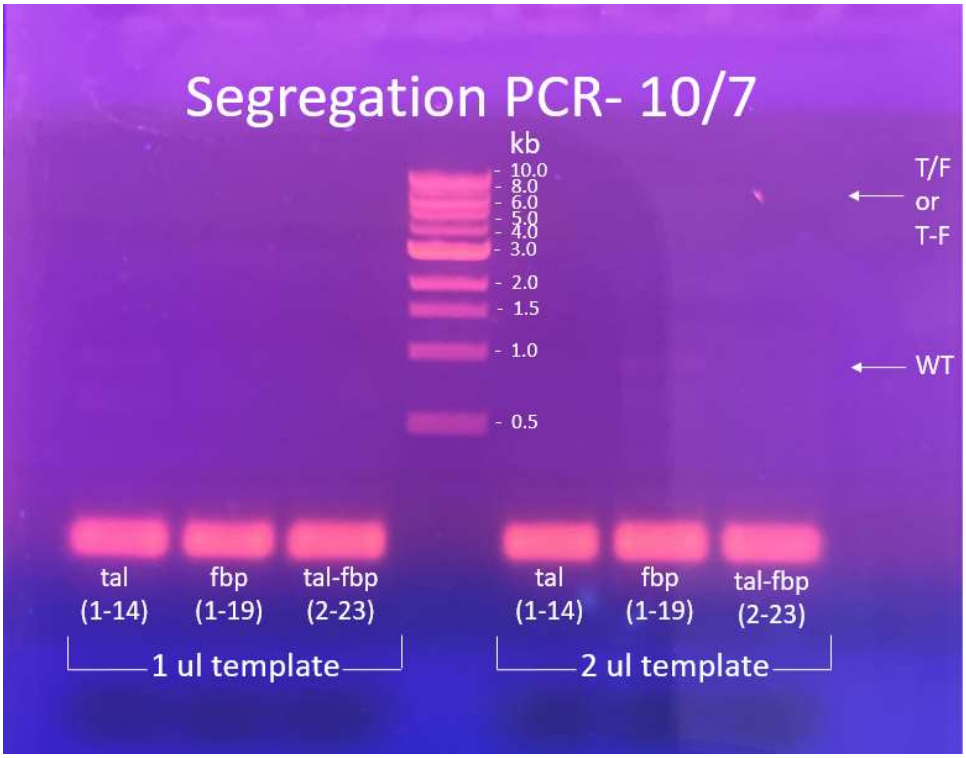
7 October 2021

Name: Max Brenner and Daria Perminova

Summary:

A gel electrophoresis but this time the voltage was set for 80 V instead of 100 V to increase accuracy. 2 L of BG-11 was made as well.

Results:



There was no amplification possibly du to not enough template.

Protocol:

1. 2 L of BG-11 was made. The following recipe was followed twice.
    a. 970 mL of ddH_2O was filtered into the solution.
    b. 10 mL of 8.2 pH TES buffer was added.
    c. 20 mL of 50X BG-11 4.4 pH solution was added

6 tubes were prepared for the multicultivator. 3 tubes have T 11, 13, and 14. The other 3 tubes were TF 1-40, 2-33, and 2-34.

1. Each tube has 50 mL of prepared BG-11.
2. For tal colonies, 1 mL of culture was used. For tal+fbp colonies, 750 uL was used to get to approximately 0.05 OD.
3. 5 uL of sp/sm 20 was put into each tube (10000X).

4. 50 uL of IPTG 1M stock was used (1000X),
5. The day night cycle was originally set for a sine function to start at 8:00 am on October 8th. Peak light would be 500 uE.
6. CO_2 was set at 1% and 18 psi. The cultures sat in dark until 8:00 am October 8th.

8 October 2021

Name: Max Brenner and Daria Perminova

Summary and Protocol:

The sine function wasn't working. Growth settings were changed to continuous light during the light period with 10 hr/ 14 hr L/D cycles. The temperature settings were 30℃ and 25℃ during the light and dark periods respectively. We will collect OD data every ten minutes until Wednesday night (~5 days).

11 October 2021

Name: Max Brenner and Daria Perminova

Summary:

We repeated the segregation PCR with liquid cultures prepared on October 5th.

Protocol:

1. We lysed 10 ul of culture at 99℃ from each of the 250 mL flasks containing either tal, fbp, or tal-fbp.
2. We used 1 ul from each lysate as template for three reactions and 2 ul from each lysate as template for the other three reactions. For reactions with 1 ul template we added 1 ul of ddH2O in addition to the template and 18 ul of master mix.
3. Master Mix:

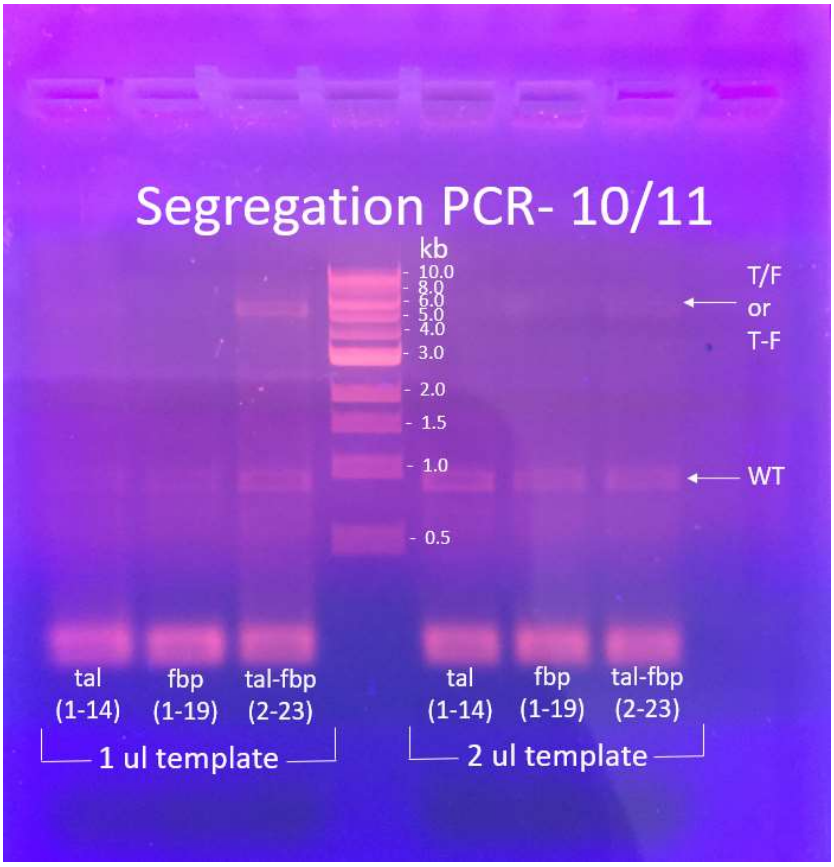
Added Material	Per Tube (uL)	Master Mix (uL)
UC101 Accuris Master Mix	10	65
7942NS1_Seg-F	0.5	3.5
7942NS1_Seg-R	0.5	3.5
ddH_2O	7	45.5
Total	18	

4. Thermocycler Settings:

PCR Phase	Temperature (°C)	Time (min:sec)	Rounds
Initial Denature	95	01:00	1X
Denature	95	00:15	40X
Annealing	60	00:15	
Extension	72	04:30	
Final Extension	72	7:00	1X
Hold	12	∞	

5. A gel electrophoresis was run.

Results:



We were able to get some larger (~6-7 kb) bands indicating the presence of our insert at NSI for tal-fbp (2-23) using 1 ul of template. It is usually difficult to amplify such a large fragment, so this might not be extremely concerning. However, the most prominent amplification product was the WT band at 896 bp for all colonies with either 1 or 2 ul of template (indicating no insert at NSI). Complete segregation is still not achieved.

13 October 2021

Name: Max Brenner and Daria Perminova

Summary:

We set up the multi cultivator and scaled up solutions for WT1, WT3, WT4, F19, F21, and F26 from their respective six well plates.

Protocol:

Protocol

The WT wells were made on October 7th and the Fbp wells were made on October 10th We made a new well with Fbp to make sure the solutions reach a similar OD with WT.

We had to get the end OD in the 50 mL solutions to be 0.5. We found the amounts of each component we needed using OD measurements from 10X dilutions of our well cultures. The 10X dilutions were made using 900 ul of BG-11 and 100 ul of liquid culture.

1. The OD readings in the wells at 730 nm (X10 from the dilution OD readings):

Strain (# Well Plate)	**OD in 6-Well Plate**	**Culture Added to BG-11 to make 50 mL Total (mL)**
fbp (19)	1.439	1.75
fbp (21)	1.634	1.53
fbp (26)	1.494	1.67
WT (1)	3.040	0.85
WT (3)	2.144	1.17
WT (4)	3.967	0.63
fbp strains had 50 uL of IPTG and 5 uL of sp/sm20 in addition to 50 mL of culture and BG-11. WT strains only had 50 uL of IPTG in addition to 50 mL of culture and BG-11.		

Tube	**BG-11 (mL)**	**Culture (mL)**	**IPTG (uL)**	**sp/sm 20 (uL)**
fbp 19	48.25	1.75	50	5
fbp 21	48.47	1.53	50	5
fbp 26	48.33	1.67	50	5
WT 1	49.15	0.85	50	0
WT 3	48.83	1.17	50	0
WT 4	49.37	0.63	50	0

2. The multicultivator protocol settings were the same as on October 8th.
    a. We used 10hr/14hr light/dark cycles. The temperature was 30℃ and 25℃ for the light and dark periods respectively. We used continuous light during the light period at 100 uE. We will collect OD data every 10 minutes for 5 days.