Name: Laura Das Neves, Mo,

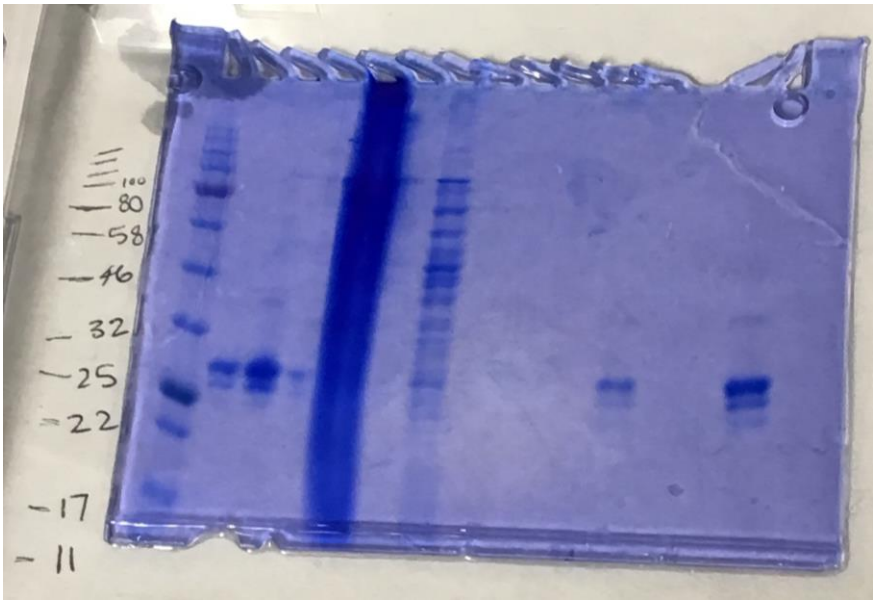
Date**:** 8/4/18

Goal:

Materials

Protocol

Results



Conclusion

Name: Laura Das Neves, Lynda

Date: 09/05/2018

Goal: We hope to have isolated the recombinant HCG beta subunit and prove/disprove this by running a Western Blot.

Materials:
100 mL of SDS sample buffer dye
100 mL of samples 1-8

Procedure:

The samples previously made on 08/31/18 were thawed on ice. Then they were boiled for 5 minutes. Labeled eppendorf tubes were then filled with 100 mL of the sample as well as 100 mL of the SDS dye. 25 microliters of each sample were loaded into their corresponding lane.

The samples were loaded into two identical gels as follows:
Lane 1- A elution 1
Lane 2- A elution 2
Lane 3- A pellet
Lane 4- A supernatant
Lane 5 B elution 1
Lane 6- B elution 2
Lane 7- B pellet
Lane 8- B supernatant
Lane 9- 10 microliters of Cell extract (positive control)
Lane 10- 10 microliters of supersignal protein ladder

The gels were ran @100 volts for approximately 1 hour.

Results:
The gels were inconclusive. The bands were light when the transfer was done, this was due to the the gel not running all the way to the bottom

Name: Rehmat, Mo

Date: 9/17/18

Goal: To do a transformation using electrically competent DH5a E. Coli cells using the DNA that was ligated yesterday: Beta 3 Loop in pGEX.

Materials

Ligated Beta 3 Loop in pGEX

SOC Medium

Invitrogen ElectroMAX DH5a Electrocompetent Cells Lot 1788252

Bio-Rad MicroPulser

Protocol

Electroporation Transformation

1. 40 µL of the electrically competent cells and 1 µL of ligated DNA was added to an Eppendorf tube.
2. The contents of the Eppendorf tube was transferred to a cuvette and the cuvette was lightly tapped on the table to evenly distribute the contents.
3. The cuvette was placed into the Bio-Rad MicroPulser and an electric shock was delivered.
4. Immediately after, SOC medium was added to the cuvette and the solution was micropipette mixed.
5. The solution in the cuvette was added to a shaker tube and placed in a shaker at 37° at 200 rpm for 1 hour.
6. After shaking for 1 hour, 150 µL of the solution was streaked onto an agar plate with the respective antibiotics (ampicillin for pGEX).

Results:

No growth on any plates

Conclusion

Redo transformation

Name: Laura das Neves, Christina

Date: 9/18/18

Goal: To do a transformation using electrically competent DH5a E. Coli cells using the DNA that was ligated yesterday: Beta 3 Loop in pGEX.

Materials

Ligated Beta 3 Loop in pGEX

SOC Medium

Invitrogen ElectroMAX DH5a Electrocompetent Cells Lot 1788252

Bio-Rad MicroPulser

Protocol

Electroporation Transformation

1. 40 µL of the electrically competent cells and 1 µL of ligated DNA was added to an Eppendorf tube.
2. The contents of the Eppendorf tube was transferred to a cuvette and the cuvette was lightly tapped on the table to evenly distribute the contents.
3. The cuvette was placed into the Bio-Rad MicroPulser and an electric shock was delivered.
4. Immediately after, SOC medium was added to the cuvette and the solution was micropipette mixed.
5. The solution in the cuvette was added to a shaker tube and placed in a shaker at 37° at 200 rpm for 1 hour.
6. After shaking for 1 hour, 150 µL of the solution was streaked onto an agar plate with the respective antibiotics (ampicillin for pGEX).

Streaking Plates

There are 4 total Plates for Laura's trial. One for RFP positive control at 150 microliters. And the next 3 were b3 in pGEX in increments of 50microL starting with 150microL. (150,200,250)

1. Add agar to center of plate
2. Heat up streaker and make sure it is not hot to spread ethanol on plate
3. Put the lid back on
4. Let it sit for about 10,15,20 minutes because of the increase of sample onto the plate.
5. Once you put lid back on let it soak into the agar
6. Then flip the plate over with the lid so the part with agar is on the top
7. Put in the incubator at 37 ℃

8. Leave overnight

Results:

Conclusion:

Name: Christina Clodomir

Date: 9/18/18

Goal: To do a transformation using electrically competent DH5a E. Coli cells using the DNA that was ligated yesterday: Beta 3 Loop in pGEX. TRIAL2 Had to do another trial today because there may have been some mistakes done in the first trial.

Materials

Ligated Beta 3 Loop in pGEX

SOC Medium

Invitrogen ElectroMAX DH5a Electrocompetent Cells Lot 1788252

Bio-Rad MicroPulser

Protocol

Electroporation Transformation

7. 40 µL of the electrically competent cells and 1 µL of ligated DNA was added to an Eppendorf tube.
8. The contents of the Eppendorf tube was transferred to a cuvette and the cuvette was lightly tapped on the table to evenly distribute the contents.
9. The cuvette was placed into the Bio-Rad MicroPulser and an electric shock was delivered.
10. Immediately after, SOC medium was added to the cuvette and the solution was micropipette mixed.
11. The solution in the cuvette was added to a shaker tube and placed in a shaker at 37° at 200 rpm for 1 hour.
12. After shaking for 1 hour, 150 µL of the solution was streaked onto an agar plate with the respective antibiotics (ampicillin for pGEX).

Name:Christina and Prachi

Date: 9-21-18

Protocol:
Colony PCR

4 colonies each from 1 plates containing B3 in pGEX.

The PCR tubes will be 20 µL.

a. HCG Beta 3 Loop in pGEX (4 TUBES): 10 µL PCR Master Mix (2X) , 7 µL IDT Nuclease-Free Water, 1 µL B3 for pGEX Fwd, and 1 µL B3 for pGEX reverse.
b. Then a p10 tip was dipped into the picked colonies and was swirled around in 10 µL of IDT Nuclease-Free Water so that there are 4 different tubes containing water and the colonies..

2. (We used cocktail containing 50 microliters of PCR Master Mix, 35 microliters Nuclease-Free Water, 5 microliters of B3 for PGEX Fwd, 5 microliters of B3 for PGEX reverse)
3. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes(1-4)
4. All of the tubes containing HCG Beta 3 loop had the following PCR settings:

1. 95° C for 3:00 minutes

2. 95° C for 1:00 minutes

3. 56° C for 1:00 minutes

4. 72° C for 1:00 minutes

5. 30X Cycles

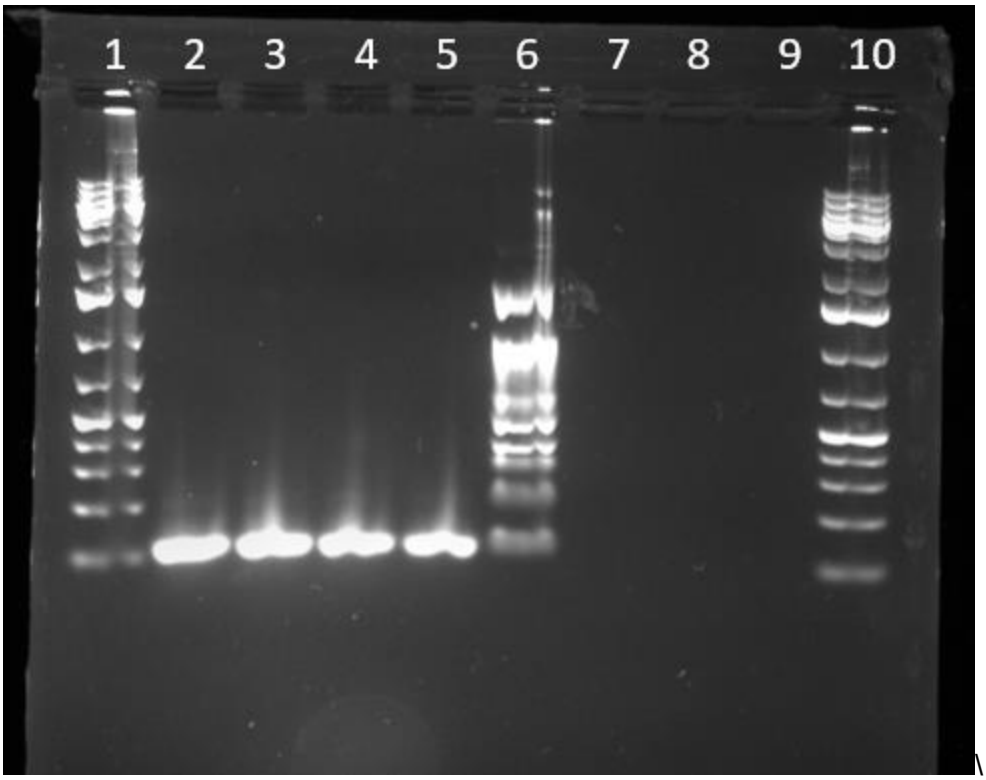
72° C for 5:00 minutes

Lid temperature: 105° C

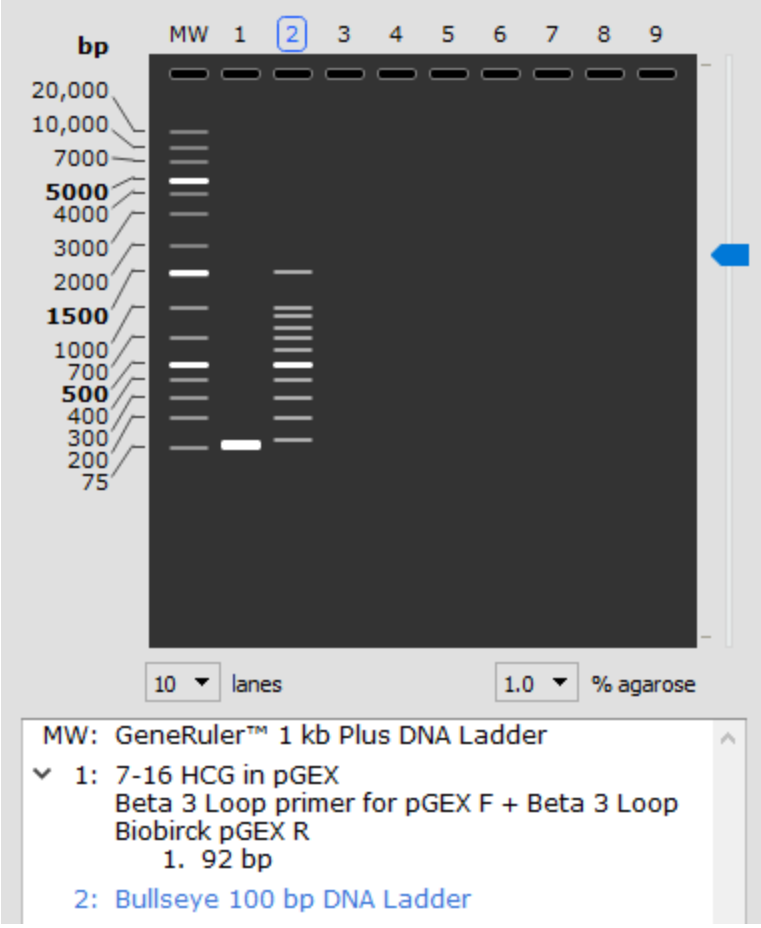
Infinite hold: 4° C

Images of picked colonies

Results



1. **GeneRuler 1kb plus ladder**
2. **PCR of Colony 1**
3. **PCR of Colony 2**
4. **PCR of Colony 3**
5. **PCR of Colony 4**
6. **Bullseye 100 bp DNA Ladder**
7.
8.
9.
10. **GeneRuler 1kb Plus Ladder**



Conclusion:
The resultant bands are around 100 bp long as predicted, so this sample will continue to be used for HCG expression.

Name: Julia Kelly

Date: 9-26-2018

Goal: Transform several possible promoters for the HCG Detection system.

Materials:

One Shot TOP10 Chemically Competent cells

BBa_K346002 from 2018 Kit Plate well 1O (PmerT)

BBa_K540001 from 2018 Kit Plate Well 13I (rcn)

BBa_K541503 from 2018 Kit Plate Well 11I (strong constitutive promoter)

BBa_J04450 RFP Construct.

SOC Medium

LB agar + Chloramphenicol plates

Protocol:

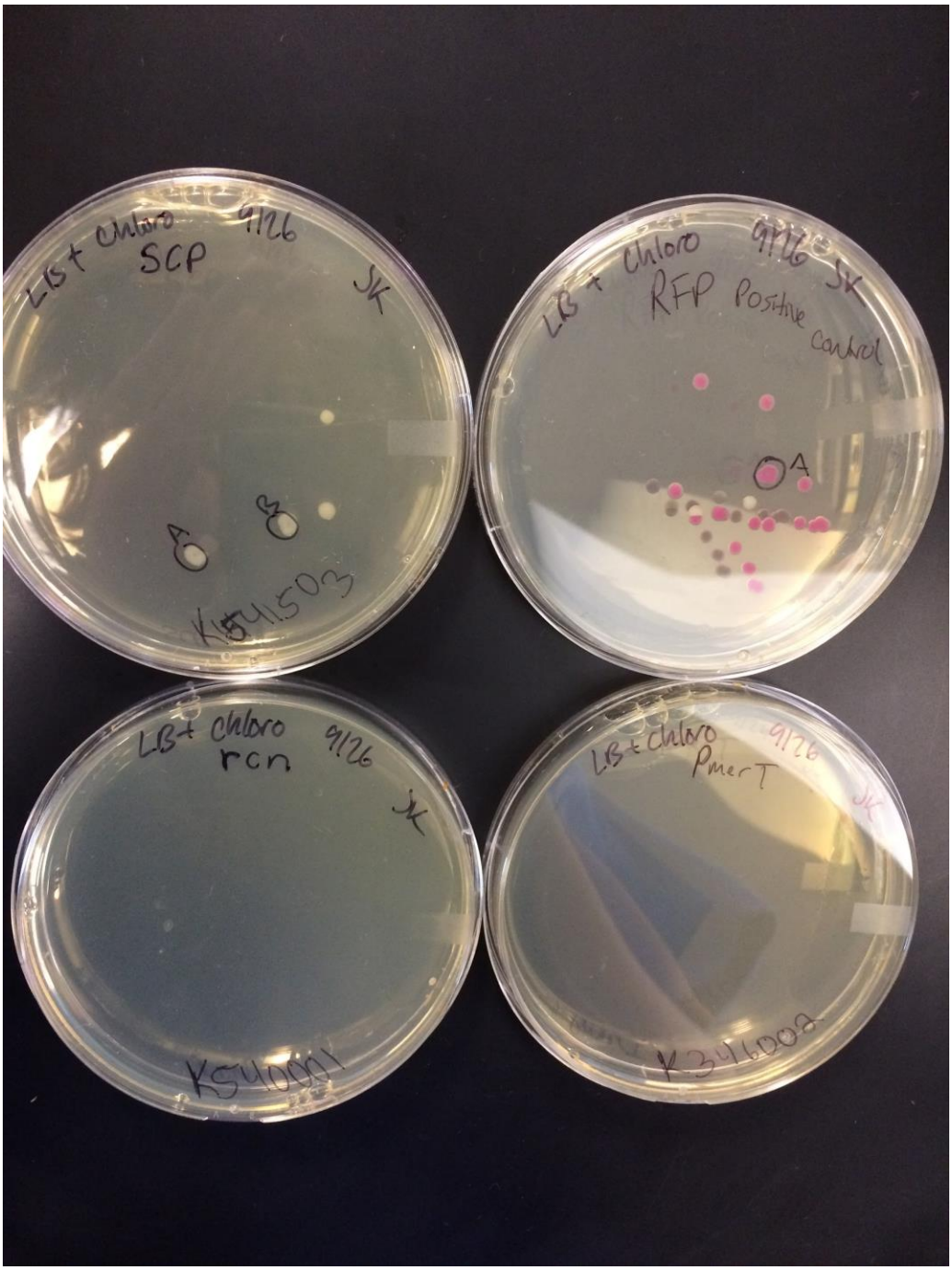
Heat Shock

1. The One Shot TOP10 chemically competent cells were thawed on ice.
2. Two microliters of each promoter DNA from the Kit Plate (BBa_K346002, BBa_K540001,BBa_K541503, BBa_J04450) was added to four competent cell vials. They was mixed by tapping.
3. The cells were incubated on ice for 35 minutes.
4. After the ice incubation, the samples were placed into a 42° C water bath for 30 seconds.
5.They were quickly taken out and 250µL of SOC medium was immediately added to each tube.
6.The samples were placed into a 37° C shaking water incubator for 1 hour at 200 rpm. After the hour, the shaking was turned off and the sample sat in the water until the plates were ready.

Plating

1. Plates were made with 500mL of LB agar and 500µL chloramphenicol. The solution was autoclaved and poured into plates when it had slightly cooled down. Each plate held 25mL of the agar.
2. Once the plates were solidified, 150µL of each transformation was spread on the plates and left to soak.
3. After 25 minutes, the plates were put into the incubator at 37° C and left overnight.

Results:



The plates with the RFP positive control and the strong constitutive promoter showed minimal growth but the heavy metal induced promoters had zero growth.

Conclusion: Overnight cultures need to be done for the strong constitutive promoter. Transformation need to be redone for rcn and PmerT.

Name:Julia Kelly
Date: 9-26-18

Goal: Grow up culture for minipreps on the HCG B3 Loop in pGEX

Materials:

Luria Broth made on 9-17

HCG B3 Loop in pGEX colonies from plates 9-21-18

Ampicillin

Protocol:

10 mL of LB and 10 µL of Ampicillin was added into 8 different falcon tubes under a flame. Colonies were picked using a p10 tip and dropped into falcon tubes. The overnights were put into a shaking water bath for 18 hours at 37℃ and 200 rpm.

Results: The overnight cultures on colonies 1a, 1b, 2, 3, and 4 showed growth.

Conclusion: A miniprep needs to be performed on the five cloudy cultures so they can undergo a restriction digest and be sent for sequencing.

Name: Julia Kelly

Date: 9-27-18

Goal: Isolate plasmid DNA from the HCG B3 Loop in pGEX overnight cultures so it can be used in the HCG construct.

Materials:

QIAprep Spin Miniprep Kit (lot#157025733)

Overnight Cultures from 9-26 (Tubes 1a, 1b, 2, 3, 4)

Protocol:

Mini Preps

a. 1.5 mL (750 µL twice) of each overnight culture was added to a clean and labeled eppendorf tube. This was done twice for a total of 3 mL of each overnight culture separated into two different eppendorf tubes
b. 3 mL of bacterial overnight culture was centrifuged at 8,000 rpm for 3 minutes at room temperature
c. The supernatant was discarded and the pelleted bacterial cells were resuspended in 250 µL Buffer P1 by adding 250 µL of Buffer P1 to the tube without a sticker, the solution was pipette mixed, and this solution was transferred to the other tube with the same label. The hand-labeled tube was discarded.
d. 250 µL of Buffer P2 was added and each tube was inverted 5 times
e. 350 µL of Buffer N3 was added and immediately mixed by inverting the tubes 5 times.
f. All the tubes were centrifuged for 10 minutes at 13,000 rpm.
g. 800 µL of the clear supernatant was micropipetted into a spin column and was centrifuged for 60 seconds and the excess liquid was discarded
h. 500 µL of PB was added and the spin columns were centrifuged for 60 seconds and the flow through was discarded
i. 750 µL of PE was added and to the spin columns, they were centrifuged for 60 seconds, and the flow through was discarded
j. The spin columns were centrifuged again for 60 seconds to remove residual wash buffer and the flow through was discarded
k. The spin columns were transferred to a clean eppendorf tube and 50 µL of EB was added to the center of the spin column to elute the DNA
l. The spin column was allowed to stand for one minute and was centrifuged for one minute

m. The concentrations for each sample were recorded

Results:

Tube	DNA Concentration (ng/µL)	A260/A280
1a	120.0	1.846
1b	32.5	2.167
2	95.0	1.900
3	Too low	-
4	118.0	1.880

Conclusion: The concentrations for tubes 1a, 2, and 4 were high so they can be used for further parts in HCG expression process.

Name: Julia Kelly

Date:9/27/18

Goal: Save the overnight cultures on B3 Loop in pGEX for later use.

Materials:

Overnight cultures on colonies 1a, 1b, 2, 3, 4 from 9/26/18

50% glycerol

Protocol: Under a flame, 1000µL of glycerol and 1000µL of overnight culture sample were added to a cryogenic tube. A glycerol stock was made for each of the five colony samples. They were stored at -80℃.

Conclusion: If the restriction digest and sequencing data look like the beta 3 loop in pGEX, these glycerol stocks will be used for protein expression.

Name:Julia Kelly
Date: 9-27-18

Goal: Grow up culture for minipreps on RFP in pSB1C3 and the strong constiutive promoter in pSB1C3

Materials:

Luria Broth made on 9-17

BBa_K541503 (scp) from 9/29/18 plate

BBa_J04450 (RFP) from 9/26/18 plates

Chloramphenicol

Protocol:

10 mL of LB and 10 µL of Chloramphenicol were added into 4 different falcon tubes under a flame. Two colonies were picked from each plate using a p10 tip and dropped into separate falcon tubes. The overnights were put into a shaking water bath for 18 hours at 37℃ and 200 rpm.

Results: Each overnight showed growth and the RFP overnight was red.

Conclusion: A miniprep needs to be performed on the cloudy cultures so they can undergo restriction digest and PCR reactions.

Name: Julia Kelly

Date:9/28/18

Goal: Redo the transformations of the three promoter parts from 9/26/18 using both chemically competent cells and electrocompetent cells.

Materials:

BBa_K346002 (PmerT)

BBa_k540001 (rcn)

BBa_K541503 (strong constitutive promoter)

BBa_J04450 (RFP positive control)

One Shot TOP10 chemically competent cells

SOC Medium

ElectroMAX™ DH5α-E™ Competent Cells

Protocol:

Heat Shock

1. The One Shot TOP10 chemically competent cells were thawed on ice.
2. Two microliters of each promoter DNA from the Kit Plate (BBa_K346002, BBa_K540001,BBa_K541503, BBa_J04450) was added to four competent cell vials. They was mixed by tapping.
3. The cells were incubated on ice for 35 minutes.
4. After the ice incubation, the samples were placed into a 42° C water bath for 30 seconds. 5.They were quickly taken out and 250µL of SOC medium was immediately added to each tube. 6.The samples were placed into a 37° C shaking water incubator for 1 hour at 200 rpm. After the hour, the shaking was turned off and the sample sat in the water until the plates were ready.

Plating

1. Plates were made with 500mL of LB agar and 500µL chloramphenicol. The solution was autoclaved and poured into plates when it had slightly cooled down. Each plate held 25mL of the agar.
2. Once the plates were solidified, 150µL of each transformation was spread on the plates and left to soak.
3. After 25 minutes, the plates were put into the incubator at 37° C and left overnight.

Electroporation

1. Combine 40 µL of electrically competent DH5a cells and 1 µL promoter DNA from the Kit Plates (BBa_K346002, BBa_K540001,BBa_K541503, BBa_J04450).
2. Transfer the contents of the Eppendorf tube to a cuvette and lightly tap the cuvette on the table to evenly distribute the contents and get rid of air bubbles.
3. Place the cuvette into the Bio-Rad MicroPulser and deliver the electric shock.

4. Immediately after, add 900 µL SOC medium to the cuvette and micropipette mix the solution.
5. Transfer the solution from the cuvette to a shaker tube and place in a shaker at 37°C at 200 rpm for 1 hour.
6. After shaking for 1 hour, streak 150 µL of the solution onto an agar plate with the respective antibiotics.
7. Incubate plates at 37°C for 24 hours.

Results:

The heat shock plates showed no growth, even the positive control. The competent cells might have been the issue because they looked a little dried up. The electroporation plates had growth.

Rcn- 50 colonies

Strong Constitutive promoter- 70 colonies

PmerT- 30 colonies

Conclusion:Overnight cultures cultures need to be made next so the promoters can eventually be isolated for use in the HCG system.

Name: Julia Kelly

Date: 9-28-18

Goal: Isolate plasmid DNA from the cells that contain the BBa_K541503 (scp) part.

Materials:

QIAprep Spin Miniprep Kit (lot#157025733)

Overnight Cultures from 9-27 (SCP colonies A and B)

Protocol:

Mini Preps

a. 1.5 mL (750 µL twice) of each overnight culture was added to a clean and labeled eppendorf tube. This was done twice for a total of 3 mL of each overnight culture separated into two different eppendorf tubes
b. 3 mL of bacterial overnight culture was centrifuged at 8,000 rpm for 3 minutes at room temperature

c. The supernatant was discarded and the pelleted bacterial cells were resuspended in 250 µL Buffer P1 by adding 250 µL of Buffer P1 to the tube without a sticker, the solution was pipette mixed, and this solution was transferred to the other tube with the same label. The hand-labeled tube was discarded.
d. 250 µL of Buffer P2 was added and each tube was inverted 5 times
e. 350 µL of Buffer N3 was added and immediately mixed by inverting the tubes 5 times.
f. All the tubes were centrifuged for 10 minutes at 13,000 rpm.
g. 800 µL of the clear supernatant was micropipetted into a spin column and was centrifuged for 60 seconds and the excess liquid was discarded
h. 500 µL of PB was added and the spin columns were centrifuged for 60 seconds and the flow through was discarded
i. 750 µL of PE was added and to the spin columns, they were centrifuged for 60 seconds, and the flow through was discarded
j. The spin columns were centrifuged again for 60 seconds to remove residual wash buffer and the flow through was discarded
k. The spin columns were transferred to a clean eppendorf tube and 50 µL of EB was added to the center of the spin column to elute the DNA
l. The spin column was allowed to stand for one minute and was centrifuged for one minute
m. The concentrations for each sample were recorded

Results:

Colony	[DNA] ng/µL	A260/A280
A	95.0	1.906
B	82.5	1.737

Conclusion:

The concentrations of the DNA are adequate so they can continue to be used for further steps.

Name: Julia Kelly

Date:9/30/18

Goal: Run Multiple restriction Digest Reactions to evaluate what how well the ligations and transformations worked.

Materials:

B3 Loop in pGEX Minipreps (1a, 1b, 2, and 4) from 9/27/18

BamHI (lot # 00193092)

NotI (lot # 00606681)

EcoRI (lot #00611985)

PstI (lot # 00472716)

FD Buffer (lot # 00642060)

Thermofischer 6x Loading Dye (lot # 00188661)

GeneRuler 1 kb Plus DNA Ladder (lot # 00519263)

Protocol:

EcoRI + PstI

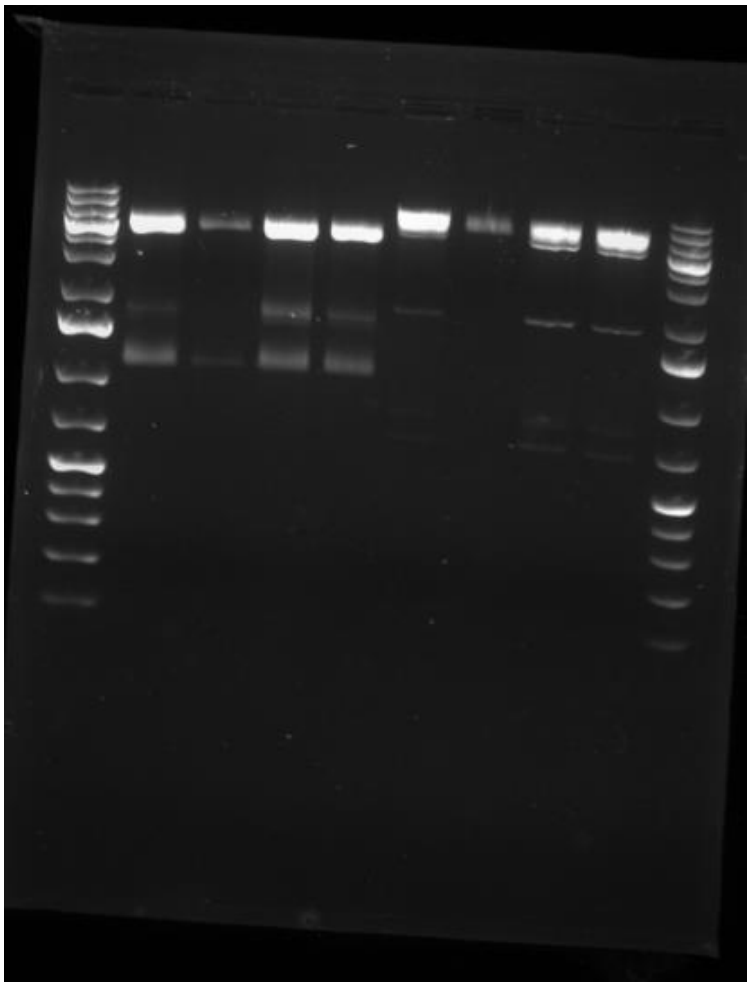
1. A 20 µL reaction was prepared using a 6x cocktail. 6 µL of PstI, 6 µL of EcoRI, 18 µL of Fastdigest Buffer and 90 µL of water were mixed together.
2. 15 µLof the cocktail was put into the four eppendorf tubes.
3. 5 µL of each miniprep DNA (B3 Loop in pGEX) from 9/27/18 was added to the tubes.
4. The samples were incubated at 37 degrees for 30 minutes.
5. After incubation, 4 µL of 6x Loading dye was added to the samples.

BamHI + NotI

1. A 20 µL reaction was prepared using a 6x cocktail. 6 µL of BamHI, 6 µL of NotI, 18 µL of Fastdigest Buffer and 90 µL of water were mixed together.
2. 15 µLof the cocktail was put into the four eppendorf tubes.
3. 5 µL of each miniprep DNA (B3 Loop in pGEX) from 9/27/18 was added to the tubes.
4. The samples were incubated at 37 degrees for 30 minutes.
5. After incubation, 4 µL of 6x Loading dye was added to the samples.

The eight samples were loaded on to a 1% agarose gel with 10 µL of GelRed in it.

Results:



Well
1. GeneRuler 1 Kb Plus DNA Ladder
2 . 1a digested with EcoRI and PstI
3. 1b digested with EcoRI and PstI
4. 2 digested with EcoRI and PstI
5. 4 digested with EcoRI and PstI
6. 1a digested with BamHI and NotI
7. 1b digested with BamHI and NotI
8. 2 digested with BamHI and NotI
9. 4 digested with BamHI and NotI
10. GeneRuler 1 Kb Plus DNA Ladder

Conclusion: The bands seem a bit larger than predicted but these minipreps look like they should. These samples should be sequenced and their glycerol stocks should be grown up to be used in protein expression.

Name:Julia Kelly

Date: 9-30-18

Goal: Grow up culture for minipreps on three promoter parts (rcn, PmerT, scp)

Materials:

Luria Broth made on 9-17

BBa_K541503 (scp) from 9/28/18 plate

BBa_K346002 (PmerT) from 9/28/18 plate

BBa_k540001 (rcn) from 9/28/18 plate

Chloramphenicol

Protocol:

10 mL of LB and 10 µL of Chloramphenicol were added into 9 different falcon tubes under a flame. Three colonies were picked from each of the three plates using a p10 tip and dropped into separate falcon tubes. The overnights were put into a shaking water bath for 18 hours at 37℃ and 200 rpm.

Results: All overnight cultures were cloudy from cell growth.

Conclusion: A miniprep needs to be performed on all the promoter parts so they can undergo restriction digest and PCR reactions.