Name: Rehmat Babar, Julia Kelly, Laura Das Neves

Date: 8/1/18

Goal: HCG recombinant subunit protein expression and isolation

Materials

1X PBS

1M DTT

500 mL mass culture from 7/31/18

Sigma CellLytic Express Lot SLBN1169V

Roche cOmplete Tablets, Mini EDTA-free, EASYpack Protease Inhibitor Cocktail Tablets Lot 04693159001

Pierce Gluthathione Agarose Lot TD262116

Protocol

1. The 500 mL mass culture was divided evenly into 10 different falcon tubes and this was centrifuged at 5,000 rpm for 15 minutes to pellet the DH5a cells.
2. The supernatant was discarded from each of the tubes and the cells were resuspended in 25 mL of 1X PBS with 1 mM DTT.
    a. The PBS was prepared from a 10X stock solution: 5 mL of 10X PBS and 45 mL of diH2O
    b. The 1M DTT was prepared by dissolving 0.15424 grams of DTT in 1 mL of diH2O
    c. Then 25 mL of 1X PBS and 25 µL of 1M DTT were mixed together and used as the resuspension media above.
3. Once all of the pelleted cells are fully dissolved in 25 mL of 1X PBS with 1 mM DTT, a Protease Inhibitor Cocktail Tablet was added to the solution and fully dissolved as well. To help with this, it was vortexed repeatedly.
4. Once it is fully dissolved, 2 volumes of a scoopula tip full of Sigma CellLytic Express powder was added to the mixture and vortexed.
5. The solution was allowed to sit benchside for 1 hour.
6. At this point the cells should be lysed and all of the proteins should have been released so we did a quick test to see if HCG was present and our results under the results header.
    a. Two trials were done to check the presence of HCG and both of them tested negative. This was expected due to so much other cell matter being present and the HCG being severely underconcentrated.
7. The lysed cell solution was centrifuged at 14,000 rpm for 15 minutes
8. While that ran, 1 mL of Pierce Gluthathione Agarose was centrifuged at 13,000 rpm for 1 minute and the ethanol was removed.
    a. The resin was washed with 1X PBS with 1 mM DTT, spun down at 13,000 rpm for 1 minute, and the supernatant was removed. This step was repeated 3 times

9. Then once the 1X PBS with 1 mM DTT was removed, the resin was resuspended in the supernatant that came from the lysed cells being centrifuged which now contains all of the proteins released from the cells
10. This was allowed to shake at 215 rpm in the fridge at 4° overnight.

Results

protein was released. We kept a sample of the cell debris that resulted from step 7 to run on the Western blot to check the presence of any protein that was not released. We will continue with the next steps of protein isolation after the solution has been shaking in the fridge overnight.

Name: Rehmat Babar

Date: 8/2/18

Goal: Want to continue protein isolation which has been a continuous effort for the past two days. We hope to isolate the recombinant HCG beta subunit by running a Western Blot.

Materials

Glutathione (Reduced) Lot TC263876

Protocol

1. Continuing from yesterday, the protein solution was shaking overnight at 215 rpm in the fridge at 4°. A column was prepared by running some diH2O through it, and the chilled solution was ran through the column and the flow through was collected and labeled.
2. Then about 3 mL 1X PBS with 1 mM DTT was run through the spin column as a wash step and the flow through was collected and labeled. This was done 3 times for 3 different wash stages and the absorbance at 280 was measured using a UVette and recorded.
3. A 10 mM solution of Glutathione (reduced) was prepared by dissolving 0.0307 grams in 10 mL of 1X PBS with 1 mM DTT. This will be used for elution.
4. 3 mL of the solution prepared in step 3 was run through the column and the flow through was collected and labeled elution 1. This was repeated two more times for a total of 3 separate elutions.
5. The absorbance of the first elution was recorded using a UVette at an absorbance of 280. All of the elutions were combined and concentrated using a molecular weight concentrator and the absorbance of this concentrated combined elution was measured.
6. The samples were prepared to load by taking 48 µL of each of the samples and adding 12 µL of the 6X loading dye to a clean eppendorf tube. These tubes were then boiled for 5 minutes.
7. 10X SDS running buffer was prepared by dissolving 72 grams of Glycine and 15.1 grams of Tris Base in 400 mL of diH2O. Then once this was completely dissolved, 5 grams of SDS was added and dissolved.
8. SDS plates were loaded with 4 µL of standard and 10 µL of the samples. This was run on polyacrylamide gel electrophoresis at 200 volts in the SDS running buffer prepared in the step before until the ladder was formed and the loading dye reached the bottom.
9. Then the protein was transferred onto nitrocellulose paper for the western blot.

Results

Sample	Absorbance at 280
Wash 1	0.019 A
Wash 2	-0.006 A
Wash 3	-0.042 A
Elution 1	0.108 A
Combined Concentrated Elution	0.238 A

The gel was loaded as follows:

Key

Lane 1: Color Protein Standard Broad Range

Lane 2: Left empty

Lane 3: Resin

Lane 4: Left empty

Lane 5: Cell debris from yesterday

Lane 6: Left empty

Lane 7: Flow through

Lane 8: Wash 1

Lane 9: Wash 2

Lane 10: Wash 3

Lane 11: Elution 1

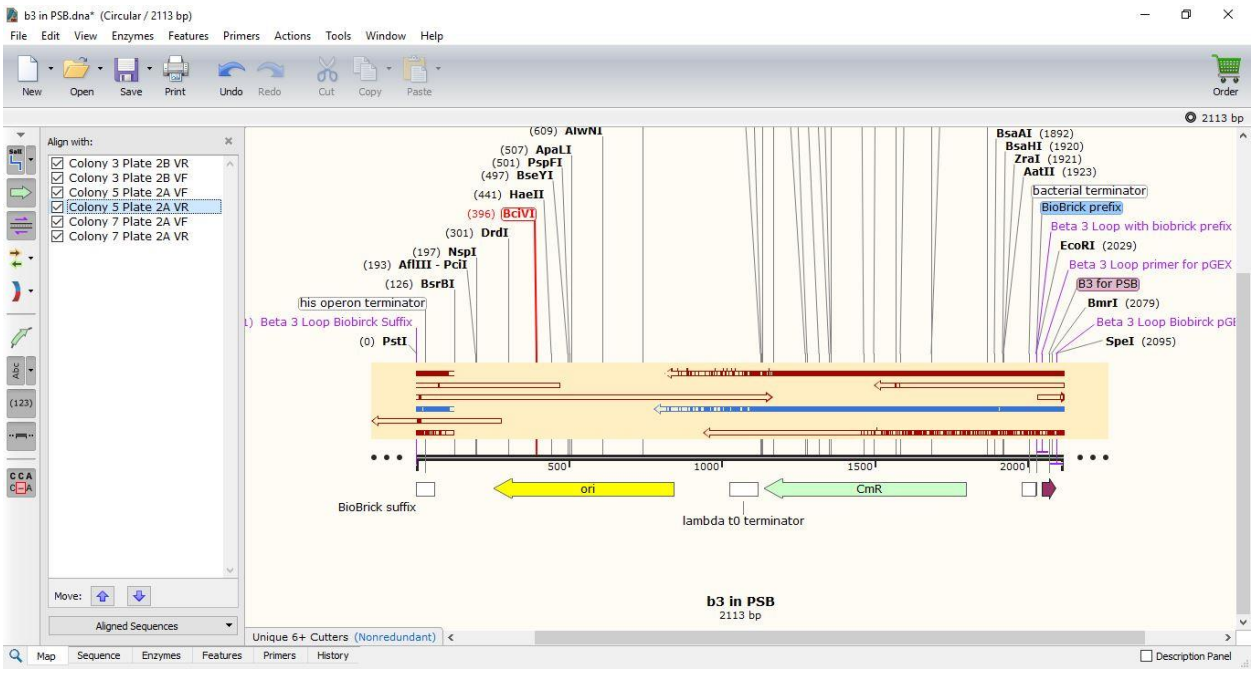
Lane 12:

Conclusion

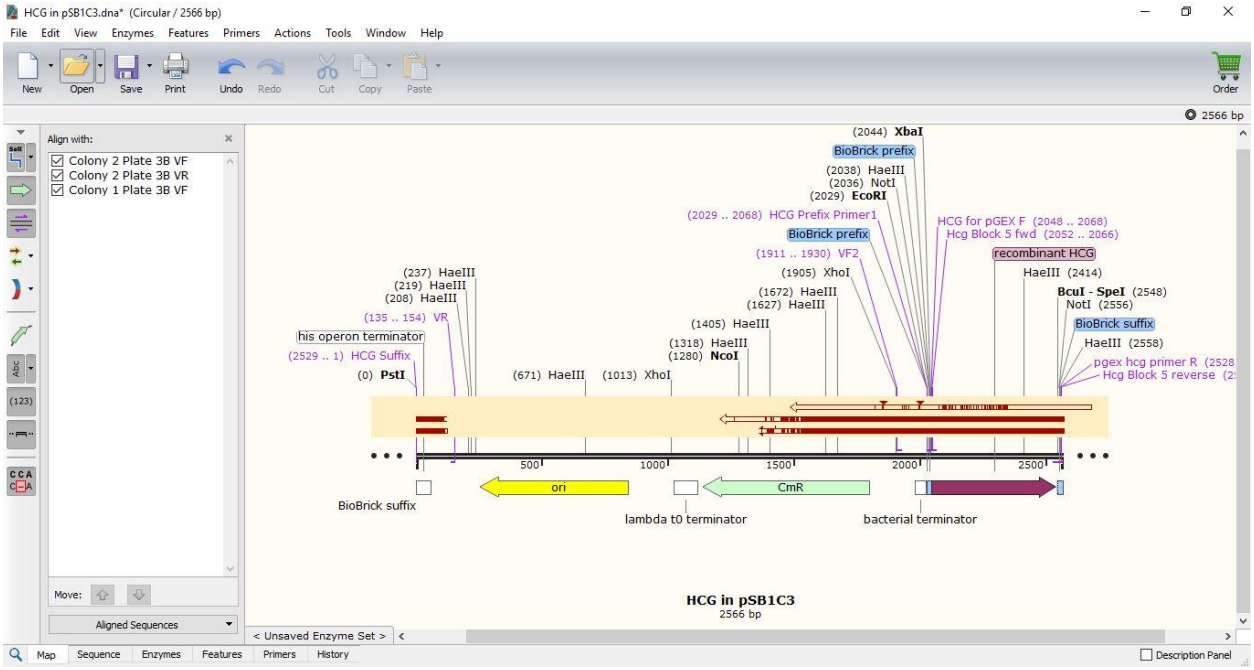
Inconclusive results today, we will find out whether or not we were able to isolate HCG bound to GST tomorrow based on the size of the protein.

Sequecning Data from 8/2/18

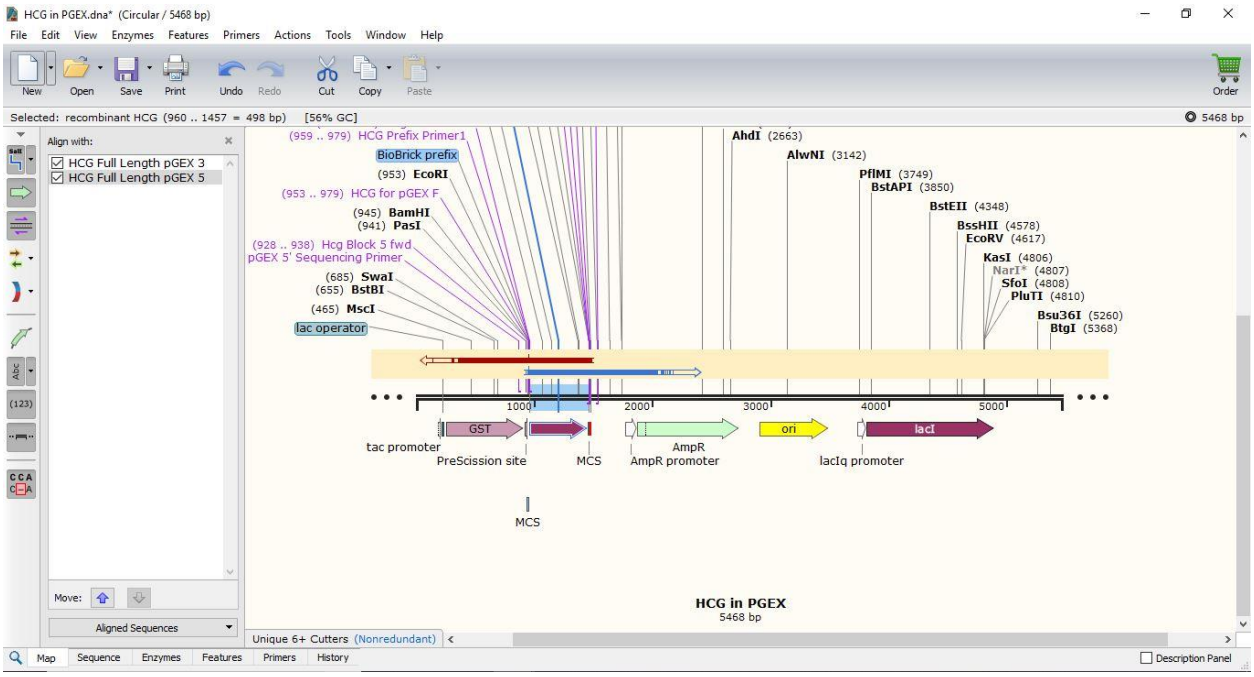
Sent the Mini Preps from 7/26/18 (Beta 3 Loop in pGEX and Beta 3 Loop in pSB1C3), the Midi Prep from 7/19/18 (HCG Beta Subunit in pGEX) and Midi Preps from 7/27/18 (HCG Beta Subunit in pSB1C3) off for sequencing, the data is as follows:



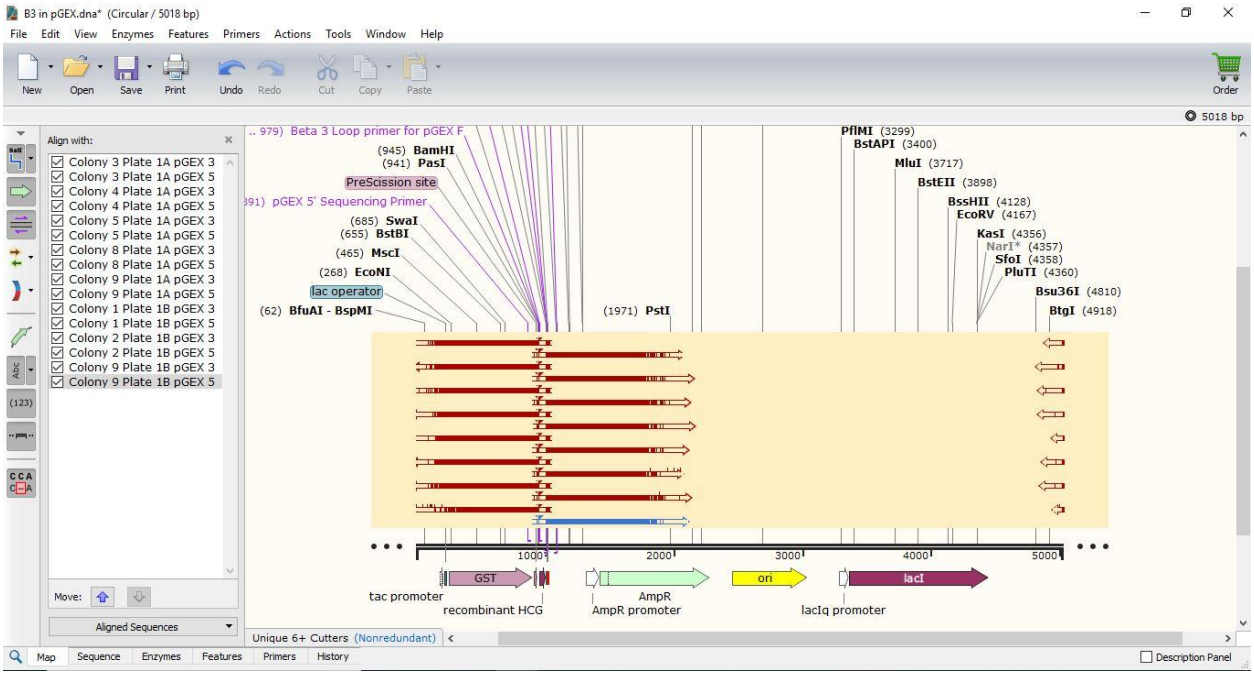
Beta 3 Loop in pSB1C3 was successfully ligated.



HCG Beta Subunit was successfully ligated with pSB1C3.



HCG Beta Subunit was successfully ligated with the pGEX vector is currently being used in protein expression and protein isolation.



Beta 3 Loop was not successfully ligated with pGEX, instead we have data for an empty vector with no insert. This ligation will be repeated again.

Name: Laura Das Neves, Mo,

Date**:** 8/3/18

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

Materials

10x fast wash

Antibody

Mouse optimized HRP reagent

Saran wrap

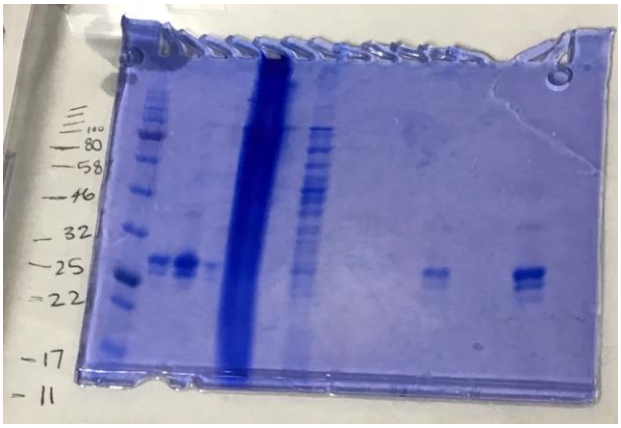
Supersignal Enhancer solution

Supersignal Peroxide buffer

Protocol

1. Continuing from the previous day, the membrane from the western blot was washed with 10 ml of 1x fast wash solution that was prepared in a final volume of 50 mL (5 mL of 10x fast wash & 45 mL of water)
2. A 1 in 2,000 dilution of the antibody was done preparing a final volume of 4 mL containing 4 mL of … and 2 microliters of the antibody.
3. In a weigh boat the membrane was placed on the shaker for 10 minutes @ 158 rpm allowing the antibody to saturate the membrane completely.
4. The membrane was then washed two more times with 20 mL of the fast wash prepared earlier.
5. A 10 mL solution was prepared with 1 mL of the mouse optimized hrp reagent and 9 mL of the antibody. The solution was mixed and poured onto the membrane. It was placed on the shaker for a couple minutes.
6. The membrane was washed two more times.
7. The membrane was transferred onto saran wrap and a prepared solution containing 3 mL of supersignal solution enhancer and 3 mL of supersignal peroxide buffer was poured onto the blot and allowed to sit for 10 minutes. The blot was then covered because of its sensitivity to light.
8. The blot was then imaged.

Results



Conclusion

The overall results were inconclusive. The imaged gel showed a band at around 22-23 kDa, showing the presence of some protein it appears low compared to an expected band around 46 kDa.We should expect to see higher bands, so we would re-do an expression for the transformation in HCG-pGEX.

Name: Saleh Alhassan

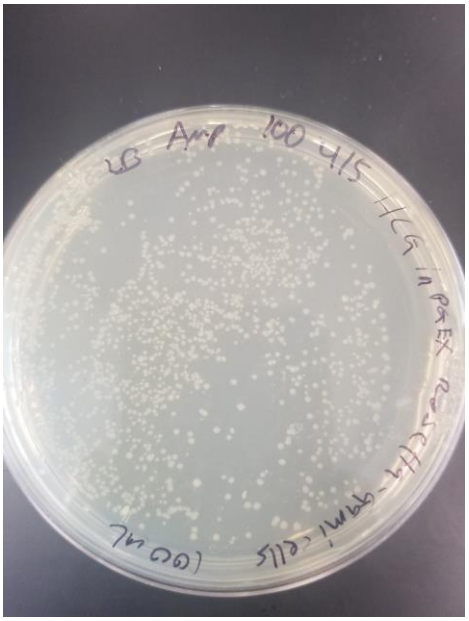
Date: 8/6/18

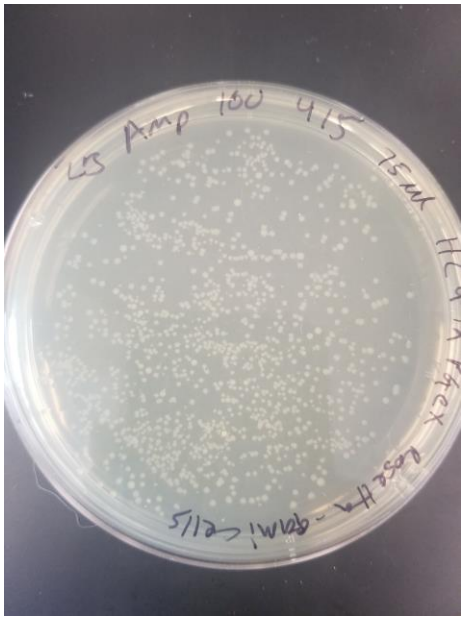
Goal: Results from our western blot indicated that there could have been a problem with the transformation of HCG in pGEX in the E.Coli DHalpha cell line, so we will attempt it again. We will transform HCG-pGEX construct in Rosetta-gami 2(DE3) competent cells using their provided protocol.

Protocol

1. Two LB-Ampicillin plates were pulled out from the fridge and placed in the incubator at 37℃ to thaw
2. Rosetta-gami 2(DE3) competent cells was taken out from the -80 freezer and thawed out on ice
3. 50 µl of the competent cells was pipetted into a new eppendorf tube and 1 µl of HCG in pGEX construct was added to the eppendorf tube. The tube was gently filcked a few times to mix.
4. The tube sat on ice for 5 minutes then was heated for exactly 30 seconds at 42℃ in a water bath
5. It was subsequently placed on ice for 2 minutes then 250 µl of SOC medium was added to the mixture.
6. The LB plates were taken out of the incubator, and under aseptic conditions, 100 µl of the transformed cell mixture was inoculated onto one plate while 75 µl more was inoculated on another plate

Results





Conclusion

There were ample number of cell colonies after transformation on both plates as depicted in the results, so the next step would be to perform a colony PCR to discern the success of the transformation and grow overnight cultures to start the process of protein expression.

Name: Christina Clodomir, Mo Adeleke, Rehmat Babar

Date: 8/8/18

Goal: Do a PCR on the HCG in pGEX into Rosetta Cells from transformation done on 8/6/18 before we do overnight culture.

Materials

HCG in pGEX Rosetta cells miniprep samples colonies 1-6 from 8/6/18

1:10 Dilution of pGEX 5'

1:10 Dilution of pGEX 3'

1:10 Dilution of HCG for pGEX F

1:10 Dilution of HCG for pGEX R

PCR MM (2X) Lot number: 00391316

diH2O

Protocol

PCR Using two sets of different primers

Cocktail A w/ pGEX primers

66 µL of PCRMM

7 µL of pGEX5'

7 µL of pGEX3'

1. 95° C for 3:00 minutes

2. 95° C for 1:00 minutes

3. 64° C for 0:30 minutes

4. 72° C for 2:00 minutes

5. 30X Cycles

72° C for 5:00 minutes

Lid temperature: 105° C

Infinite hold: 4° C

Cocktail B w/ HCG primers

66 µL of PCRMM

7 µL of HCG for pGEXF

7 µL of HCG for pGEXR

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

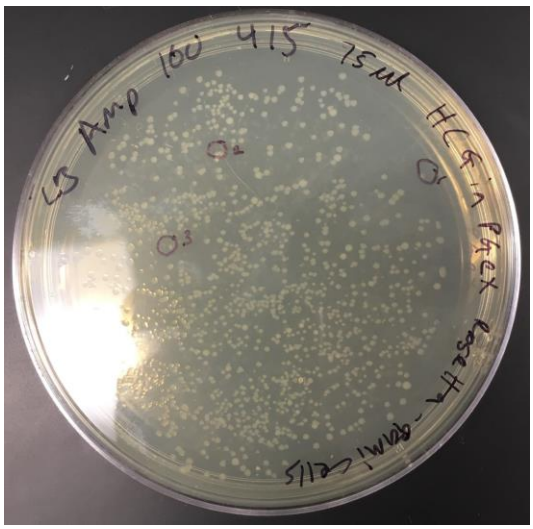
    Infinite hold: 4° C

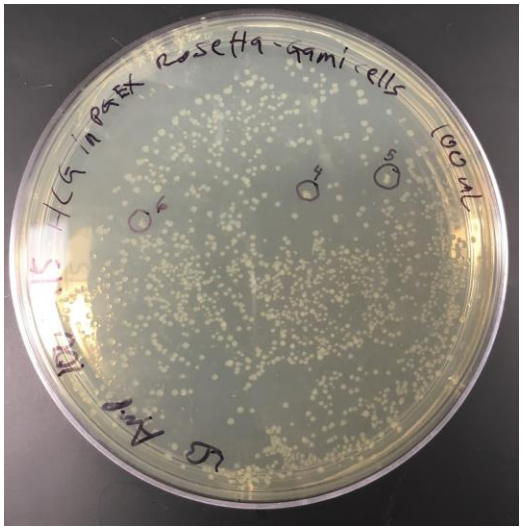
There were 6 tubes that contained 7 µL of water. So that way when we touched the colonies from the plate, we could touch the water and then instead of discarding the tube we would place the tip into the overnight tube.

Overnight Cultures

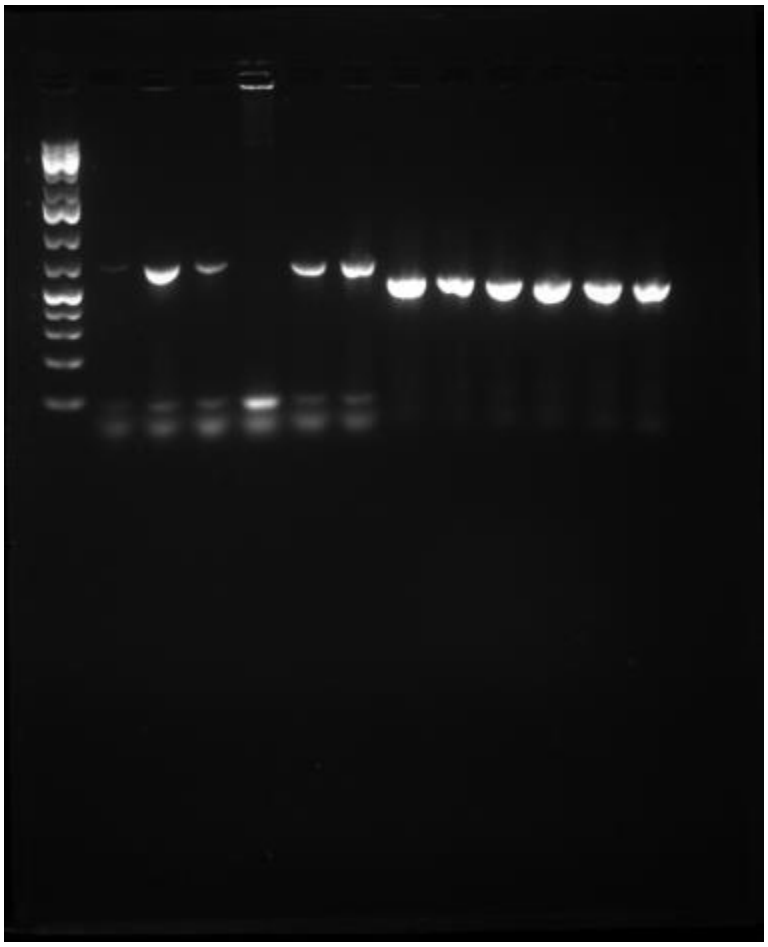
10 mL of LB + Amp overnight culture started for each of the six colonies

Picked Colonies





Results



Key

Lane 1: GeneRuler 1kb Plus DNA Ladder

Lane 2: Colony 1 Cocktail A

Lane 3: Colony 2 Cocktail A

Lane 4: Colony 3 Cocktail A

Lane 5: Colony 4 Cocktail A

Lane 6: Colony 5 Cocktail A

Lane 7: Colony 6 Cocktail A

Lane 8: Colony 1 Cocktail B

Lane 9: Colony 2 Cocktail B

Lane 10: Colony 3 Cocktail B

Lane 11: Colony 4 Cocktail B

Lane 12: Colony 5 Cocktail B

Lane 13: Colony 6 Cocktail B

Conclusion

HCG was amplified from the colony PCR from the second set of primers, cocktail B. The bands produced are in the correct spot and show bright bands. Next, we will start overnight cultures and do mini preps on them to see if the plasmid DNA is present.

Name: Rehmat Babar

Date: 8/9/18

Goal: Protein expression of recombinant HCG beta subunit

Materials

LB + Amp

1M IPTG

HCG in pGEX Rosetta Cells Colony 3 10 mL overnight culture

HCG in pGEX Rosetta Cells Colony 5 10 mL overnight culture

Protocol

500 mL Mass Culture

1. The grown up HCG in pGEX Rosetta Cells Colony 3 10 mL overnight culture from the day before was dumped into 500 mL of LB + Amp in flask 1 and the grown up HCG in pGEX Rosetta Cells Colony 5 10 mL overnight culture into flask 2.
2. Flask 2 was incubated at 37° and 150 rpm for 4.5 hours and flask 1 was incubated for hours at 37° and 150 rpm.
3. The absorbance at OD 600 was taken for the cultures to ensure they were between 1 and 0.6.
4. Once the absorbance was in that range, 500 µL of 1M IPTG was added to each of the 500 mL cultures and the two flasks were incubated at 30° and 150 rpm for 18 hours.

Results

The IPTG should turn the promoter on and induce protein production.

Sample	Absorbance at OD 600 before addition of IPTG
HCG in pGEX Rosetta Cells Colony 3 500 mL culture	0.734
HCG in pGEX Rosetta Cells Colony 5 500 mL culture	1.015

Conclusion

We will move on with the next steps of protein isolation tomorrow.

Name: Rehmat Babar

Date: 8/9/18

Goal: PCR cleanup on the PCR done on 7/31/18 in order to do another ligation of Beta 3 Loop into pGEX in order to express the protein.

Materials

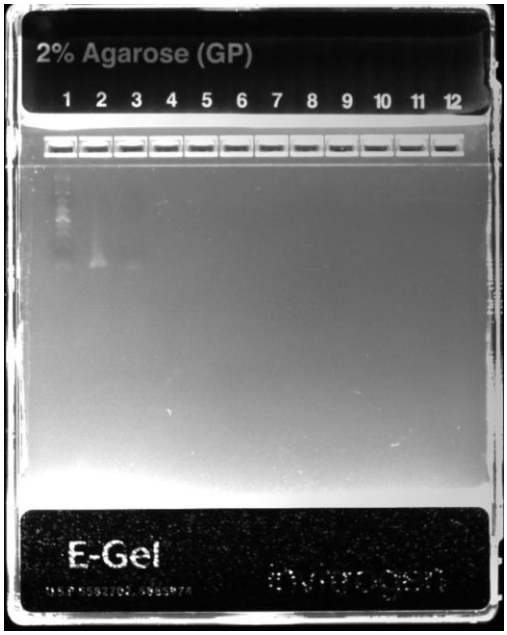
Zymo Research DNA Clean & Concentrator - 5 Lot No: ZRC185825

Beta 3 Loop for pGEX PCR Product 7/31/18

Protocol

1. Transferred 15 µL of Beta 3 Loop for pSB1C3 PCR Product 7/31/18 into a 1.5 Eppendorf tube and 5 volumes of DNA Binding Buffer was added and the tube was vortexed.
2. The sample was transferred to a Zymo-Spin Column in a collection tube
3. Centrifuged for 30 seconds and the flow through was discarded
4. 200 µL of DNA Wash Buffer was added to the column and centrifuged for 30 seconds.
5. Step 4 was repeated again and the flow through was discarded.
6. 30 µL of DNA Elution Buffer was added and the column was incubated at room temperature for 1 minute
7. The column was transferred to a 1.5 mL Eppendorf tube and was centrifuged for 30 seconds to elute the DNA.

Results



Key

Lane 1: GeneRuler 1kb Plus DNA Ladder

Lane 2: Beta 3 Loop PCR 7/31/18 Combined

Lane 3: Beta 3 Loop PCR 7/31/18 Combined PCR Cleanup

Sample	Concentration
Beta 3 Loop for pGEX PCR cleanup product	- ng/µL

Conclusion

The concentration was too low to detect for the PCR product but I went ahead and ran it on an e-gel to see if bright bands or any bands at all are produced. I also ran the combined PCR product from 7/31/18 just to double check that this is indeed Beta 3 Loop for pGEX. A very faint band did show, this may be because the concentration of the original PCR product is low to begin with because its band is faint as well, although not as faint as the PCR cleanup band. I

will do an ethanol precipitate and concentrate the PCR products in a lower volume and do a PCR cleanup again from that.

Name: Rehmat Babar, Saleh Alhassan

Date: 8/9/2018

Goal: Put the Beta 3 Loop into the pGEX plasmid by doing a 10 minute ligation. We will do two different ligations: RD Beta 3 Loop for pGEX (PCR Cleanup) cut with EcoRI and NotI 7/17/18 R.B. and the pGEX restriction digest cleanup 105 ng/µL 7-6-17; PCR Cleanup and gel extraction from today.

Materials:

T4 DNA Ligase

T4 DNA Ligase Buffer

diH2O

Protocol:

1. Six microliters of water was added to the tube, then 1µL of the T4 DNA Ligase Buffer, followed by 1µL of the plasmid and 1µL of the HCG part. The three tubes made were full length HCG in pSB1C3, full length HCG in pGEX, and beta loop HCG in pSB1C3. The pSB1C3 backbone came from the blue 4 tube of the gel extractions. Lastly, 1µL of T4 DNA Ligase was added.
2. The tube was mixed by pipet then incubated at room temperature for 10 minutes.

Name: Saleh Alhassan, Rehmat Babar

Date: 8/6/18

Goal: We have done another ligation of the Beta 3 Loop for pGEX and the pGEX vector. We are going to transform them directly into the Rosetta cells so that they may be used for protein expression and isolation directly from this first transformation instead of using the E.Coli DH5alpha cells which we have found are not optimal for protein expression and isolation.

Materials
LB-Ampicillin plates
Rosetta-gami 2(DE3) competent cells
SOC medium

Protocol

1. Eight LB-Ampicillin plates were pulled out from the fridge and placed in the incubator at 37℃to thaw.

2. Rosetta-gami 2(DE3) competent cells was taken out from the -80 freezer and thawed out on ice.
3. 50 µl of the competent cells was pipetted into a new eppendorf tube and 1 µl of Beta 3 Loop in pGEX construct was added to the eppendorf tube. The tube was gently filcked a few times to mix.
4. The tube sat on ice for 5 minutes then was heated for exactly 30 seconds at 42℃in a water bath
5. It was subsequently placed on ice for 2 minutes then 250 µl of SOC medium was added to the mixture.
6. The LB plates were taken out of the incubator, and under aseptic conditions, 100 µl of the transformed cell mixture was inoculated onto one plate while 75 µl more was inoculated on another plate

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Name: Rehmat Babar

Date: 8/9/18

Goal: To concentrate the PCR products from 7/31/18 so that the PCR cleanup done from the PCR product will have a higher concentration thus making a ligation with this DNA more likely to be successful.

Materials

3M Sodium Acetate

100% ethanol

95% ethanol

Deionized Water

Protocol

1. The volume of the sample was 182 µL. One-tenth volume of 3M sodium acetate was added (18.2 µL) and two and a half volume of 100% ethanol were added (455 µL).
2. The sample was put in the freezer for 15 minutes.
3. The sample was centrifuged at 15,000 rpm at 4°C for 30 minutes.
4. The supernatant was removed.
5. The pellet was washed with 200 µL of 95% ethanol and centrifuged at 15,000 rpm at 4°C for 30 minutes and the supernatant was discarded.
6. 30 µL of diH2O was added and the pellet was resuspended.

Results

Sample Conclusion

Conclusions

Name: Saleh Alhassan

Date: 8/9/18

Goal: To separate the pGEX backbone from mambalgin, do a gel extraction, and use this DNA for a ligation with the Beta 3 Loop for pGEX.

Materials

Qiagen QIAEX II Gel Extraction Kit (Received 6/4/2015)

-Buffer QX1

-QIAEX II

-Buffer PE

Razor Blade, Forceps

Protocol

Gel Extraction

1. After running a restriction digest on the pGEX parts (using EcoRI and NotI), the backbone was cut out using a razor blade, taking care to get as much DNA out while not keeping too much agarose.

2. Empty Eppendorf tubes were weighed before and after the gel extracts were added to them. The weight of the gel was calculated using the difference in tube weight. That weight was multiplied by 3 to get the volume of Buffer QX1 that was added to each tube.

	Empty Tube Weight (g)	Tube with Gel Weight (g)	Weight of Gel (g)	QX1 Buffer (uL)
T1	0.9795	1.0074	0.0279	83.7
T2	0.9922	1.0249	0.0327	98.1
T3	0.9960	1.0199	0.0239	71.7
T4	0.9945	1.0382	0.0437	131.1
T5	0.9977	1.0345	0.0368	110.4
T6	0.9930	1.0193	0.0263	78.9
T7	0.9887	1.0185	0.0298	89.4
T8	0.9975	1.0468	0.0493	147.9

3. Thirty microliters of QIAEX II was added to the samples. Then the tubes were put into the shaking incubator at 50 degrees C for 10 minutes and centrifuged afterwords.

4. The supernatant was removed and 500 ul of Buffer QX1 was added to the tubes and the pellet was resuspended. The tubes were centrifuged for 30 seconds and the supernatant was removed again.

5. The pellet was resuspended using 500 ul of Buffer PE and centrifuged for 30 seconds. The supernatant was removed. This step was done twice.

6. The pellet was left to air dry after the second round of Buffer PE for 30 minutes.

7.The DNA was eluted using 20 ul deionized water and incubated at room temp for 5 minutes. Afterwards, it was centrifuged for 30 seconds and the supernatant was pipetted into a clean tube. Lastly we checked the concentration.

Results

Sample	Concentration ng/ul	Sample	Concentration ng/ul
T1	13.3	T5	14.3
T2	6.8	T6	14.9
T3	10.3	T7	12.8
T4	10.9	T8	15.7

Conclusion

Name:Christina

Date: 8/9/18

Goal: PCR cleanup on the ethanol precipitate 8/9/18

Materials

Zymo Research DNA Clean & Concentrator - 5 Lot No: ZRC185825

Beta 3 Loop for pGEX PCR Product (Ethanol Precipitate)

Protocol

8. Transferred 15 µL of Beta 3 Loop for pSB1C3 PCR Product 7/31/18 into a 1.5 Eppendorf tube and 5 volumes of DNA Binding Buffer was added and the tube was vortexed.
9. The sample was transferred to a Zymo-Spin Column in a collection tube
10. Centrifuged for 30 seconds and the flow through was discarded
11. 200 µL of DNA Wash Buffer was added to the column and centrifuged for 30 seconds.
12. Step 4 was repeated again and the flow through was discarded.
13. 30 µL of DNA Elution Buffer was added and the column was incubated at room temperature for 1 minute
14. The column was transferred to a 1.5 mL Eppendorf tube and was centrifuged for 30 seconds to elute the DNA.

Results

Concentration after cleanup:

45 ng/ul

Conclusion

The ethanol precipitation worked and the PCR product is at a higher concentration and can be used to do a ligation.

Name: Christina

Date: 8/9/2018

Goal: Put the Beta 3 Loop into the pGEX plasmid by doing a 10 minute ligation. We will do two different ligations:.

Materials:

T4 DNA Ligase

T4 DNA Ligase Buffer

diH2O

B3 ethanol precipitate

Protocol:

3. Six microliters of water was added to the tube, then 1µL of the T4 DNA Ligase Buffer, followed by 1µL of the plasmid and 1µL of the HCG part. 1µL of T4 DNA Ligase was added.
4. The tube was mixed by pipet then incubated at room temperature for 10 minutes.

1 is right 2 is added ab extra)

Name: Rehmat Babar

Date: 8/10/18

Goal: Make the ends of the PCR cleanup with the concentration of 45 ng/ul compatible to be put into the pGEX vector. This will be done by doing a restriction digest on the PCR cleanup product and cutting with EcoRI and NotI which is also what the pGEX vector is digested with. A heat kill will also be done so that the sample is ready to ligate.

Materials

Thermo Scientific FastDigest NotI Lot 00537736

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific 10X FastDigest Buffer Lot 00312007

PCR Cleanup Product from 8/9/18

Protocol

20 µL Restriction Digest

a. HCG Beta 3 Loop for pSB1C3
   16 µL of HCG Beta 3 Loop for pGEX PCR Cleanup Product

   1 µL EcoRI

   1 µL NotI

   2 µL of FastDigest Buffer

   Incubate at 37° C for 30 minutes

Heat Kill

a. The restriction digests were placed in the water bath at 75° C for 20 minutes in order to denature the enzymes so that the PCR cleanup product will be ready for ligation.

Results

The sample should be ready to ligate and the heat kill should have inactivated the restriction enzymes.

Conclusion

This sample the products from the pGEX gel extraction from yesterday will be used to ligate the Beta 3 loop for pGEX and the pGEX vector.

Name: Rehmat Babar, Saleh Alhassan

Date: 8/10/18

Goal: HCG recombinant subunit protein expression and isolation

Materials

1X PBS

1M DTT

2 X 500 mL mass culture from 8/9/18

Sigma CellLytic Express Lot SLBN1169V

Roche cOmplete Tablets, Mini EDTA-free, EASYpack Protease Inhibitor Cocktail Tablets Lot 04693159001

Pierce Glutathione Agarose Lot TD262116

Protocol

1. The two 500 mL mass cultures were divided evenly into 10 different falcon tubes each and then centrifuged at 5,000 rpm for 15 minutes to pellet the Rosetta cells.
2. The supernatant was discarded from each of the tubes and the cells were resuspended in 25 mL of 1X PBS with 1 mM DTT, the two different cultures were kept separate.
    a. The PBS was prepared from a 10X stock solution: 5 mL of 10X PBS and 45 mL of diH2O
    b. The 1M DTT was prepared by dissolving 0.15424 grams of DTT in 1 mL of diH2O
    c. Then 25 mL of 1X PBS and 25 µL of 1M DTT were mixed together and used as the resuspension media.
3. Once all of the pelleted cells are fully dissolved in 25 mL of 1X PBS with 1 mM DTT, a Protease Inhibitor Cocktail Tablet was added to the solution and fully dissolved as well. To help with this, it was vortexed repeatedly.
4. Once it is fully dissolved, 2 volumes of a scoopula tip full of Sigma CellLytic Express powder was added to the mixture and vortexed.
5. The solution was allowed to sit benchside for 1 hour.
6. The lysed cell solution was centrifuged at 14,000 rpm for 15 minutes
7. While that ran, 1 mL of Pierce Glutathione Agarose was centrifuged at 13,000 rpm for 1 minute and the ethanol was removed.
    a. The resin was washed with 1X PBS with 1 mM DTT, spun down at 13,000 rpm for 1 minute, and the supernatant was removed. This step was repeated 3 times
8. Then once the 1X PBS with 1 mM DTT was removed, the resin was resuspended in the supernatant that came from the lysed cells being centrifuged which now contains all of the proteins released from the cells
9. This was allowed to shake at 215 rpm in the fridge at 4° for 55 hours.

Results

The chilled solution will be used to continue protein isolation on 8/13/18.

fName: Rehmat Babar

Date: 8/13/18

Goal: Want to continue protein isolation which has been a continuous effort for the past two days. We hope to isolate the recombinant HCG beta subunit by running a Western Blot.

Materials

Glutathione (Reduced) Lot TC263876

Protocol

1. Continuing from yesterday, the protein solution was shaking overnight at 215 rpm in the fridge at 4°. A column was prepared by running some diH2O through it, and the chilled solution was ran through the column and the flow through was collected and labeled.
2. Then about 3 mL 1X PBS with 1 mM DTT was run through the spin column as a wash step and the flow through was collected and labeled. This was done 3 times for 3 different wash stages and the absorbance at 280 was measured using a UVette and recorded.
3. A 10 mM solution of Glutathione (reduced) was prepared by dissolving 0.0307 grams in 10 mL of 1X PBS with 1 mM DTT. This will be used for elution.
4. 3 mL of the solution prepared in step 3 was run through the column and allowed to sit for a couple of minutes and then the flow through was collected and labeled elution 1. This was repeated two more times for a total of 3 separate elutions.
5. The absorbance of the first elution was recorded using a UVette at an absorbance of 280. All of the elutions were combined and concentrated using a molecular weight concentrator and the absorbance of this concentrated combined elution was measured.
6. The samples were prepared to load by taking 25 µL of each of the samples and adding 5 µL of the 6X loading dye to a clean eppendorf tube. These tubes were then boiled for 5 minutes.
7. SDS plates were loaded with 3 µL of Color Protein Standard Broad Range, 10 µL of the cell debris, and 20 µL of all of the other samples . This was run on polyacrylamide gel electrophoresis at 200 volts in the SDS running buffer until the ladder was formed and the loading dye reached the bottom.
8. The two plates ran side by side. The gels were removed and soaked in methanol for a few minutes.
9. The methanol was dumped into a waste container and the gels were soaked in coomassie blue and were left to shake at 130 rpm at room temperature for 30 minutes to dye the gels.
10. The gels were rinsed with diH2O twice and continued to shake overnight at 30 rpm to remove the stain

Results

Sample	Absorbance at 280
Wash 1 colony 3	1.156 A
Wash 2 colony 3	0.104 A
Wash 3 colony 3	0.018 A

Elution 1 colony 3	0.069 A
Elution 2 colony 3	0.042 A
Elution 3 colony 3	0.034 A
Wash 1 colony 5	0.194 A
Wash 2 colony 5	0.032 A
Wash 3 colony 5	0.018 A
Elution 1 colony 5	0.022 A
Elution 2 colony 5	0.010 A
Elution 3 colony 5	-0.012 A

The gel was loaded as follows:

Key for Colony 3

Lane 1: Color Protein Standard Broad Range

Lane 2: Cell Debris from lysis step

Lane 3: left empty

Lane 4: Flow through colony 3

Lane 5: Wash 1 colony 3

Lane 6: Wash 2 colony 3

Lane 7: Wash 3 colony 3

Lane 8: Elution 1 colony 3

Lane 9: Elution 2 colony 3

Lane 10: Elution 3 colony 3

The gel was loaded as follows:

Key for Colony 5

Lane 1: Cell debris from lysis step

Lane 2: left empty

Lane 3: Flow through colony 5

Lane 4: Wash 1 colony 5

Lane 5: Wash 2 colony 5

Lane 6: Wash 3 colony 5

Lane 7: Elution 1 colony 5

Lane 8: Elution 2 colony 5

Lane 9: Elution 3 colony 5

Lane 10: Color Protein Standard Broad Range

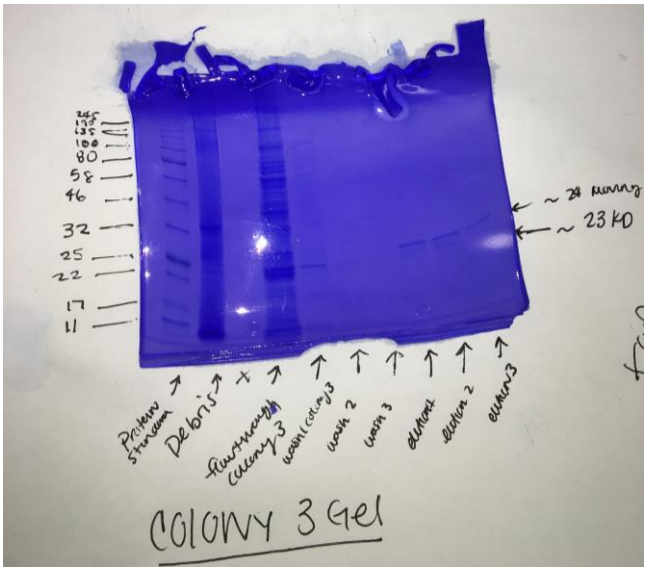
Conclusion

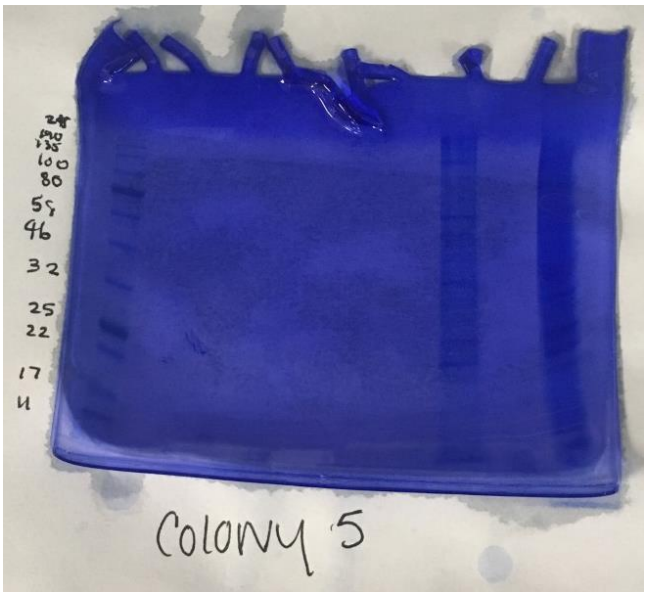
Name: Laura Das Neves

Date**:** 8/14/18

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

Results





From Right

Lane 1: Cell debris from lysis step

Lane 2: left empty

Lane 3: Flow through colony 5

Lane 4: Wash 1 colony 5

Lane 5: Wash 2 colony 5

Lane 6: Wash 3 colony 5

Lane 7: Elution 1 colony 5

Lane 8: Elution 2 colony 5

Lane 9: Elution 3 colony 5

Lane 10: Color Protein Standard Broad Range

Conclusion

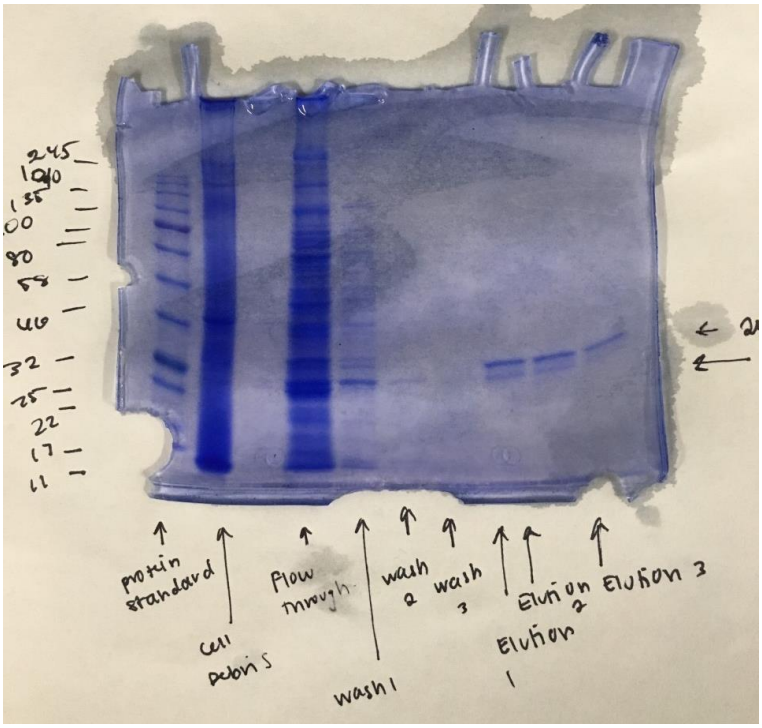
The results from the SDS gel were inconclusive. The image for the first gel, Colony 5, shows a band around 23 kDa, indicating the presence of a small protein. What was expected was a band around 46 kDa. The gels were still blue and not clear as they should have been so the gels were allowed to destain for an additional 48 hours.

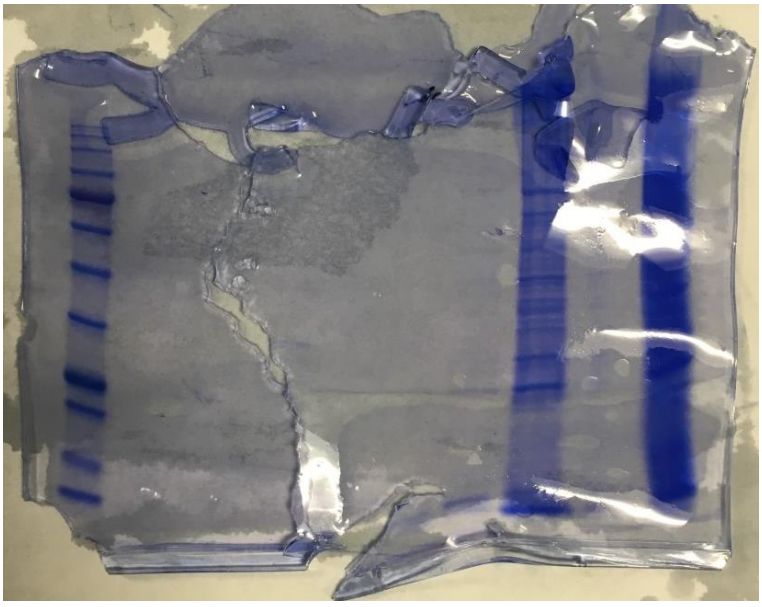
Name: Rehmat Babar

Date**:** 8/16/18

Goal: We hope to have isolated the recombinant HCG beta subunit, this is a coomassie blue which will show all protein present. We are expecting our HCG-GST tagged protein to be about 46 kDa. The gels from 8/14/18 were allowed to detain further for a clearer picture.

Results





From Right

Lane 1: Cell debris from lysis step

Lane 2: left empty

Lane 3: Flow through colony 5

Lane 4: Wash 1 colony 5

Lane 5: Wash 2 colony 5

Lane 6: Wash 3 colony 5

Lane 7: Elution 1 colony 5

Lane 8: Elution 2 colony 5

Lane 9: Elution 3 colony 5

Lane 10: Color Protein Standard Broad Range

Conclusion

We did not see the protein we had hoped to see and instead saw protein about 22-24 kDa which is the size of the GST tag only. We were not able to express HCG yet again. Potential areas of error could be the DNA sequence has an unexpected stop codon, complications in cell lysis, faulty materials/equipment, etc.

Name: Rehmat Babar

Date: 8/16/18

Goal: Do a Western Blot to get a final answer on whether or not the GST tagged HCG protein was expressed and isolated or not.

Materials

Protocol

Results

Conclusion

Name: Rehmat, Julia, Mo, Christina

Date: 8/24/18

Goal: Want to run a Western Blot on the samples from the second and third attempt at protein expression and isolation.

Materials

Color Protein Standard Broad Range Lot 0071601

SuperSignal Molecular Weight Protein Ladder Lot RB231384A

GST TIALA RRmz from another lab as a positive control

Protocol

1. The samples were prepared to load by taking 25 µL of each of the samples and adding 5 µL of the 6X loading dye to a clean eppendorf tube. These tubes were then boiled for 5 minutes.
2. SDS plates were loaded with 3 µL of Color Protein Standard Broad Range, 10 µL of the cell debris, and 20 µL of all of the other samples, 5 µL of the SuperSignal Molecular Weight Protein Ladder. This was run on polyacrylamide gel electrophoresis at 200 volts in the SDS running buffer until the ladder was formed and the loading dye reached the bottom.
3. The two plates (colony 3 and 5) ran side by side.
4. A western blot was set up and allowed to run for 45 minutes.

Results

The gel was loaded as follows:

Key for Colony 5

Lane 1: Color Protein Standard Broad Range

Lane 2: left empty

Lane 3: Cell Debris from lysis step

Lane 4: left empty

Lane 5: Flow through

Lane 6: Wash 1 colony 5

Lane 7: Elution 1 colony 5

Lane 8: Combined elution

Lane 9: GST TIALA RRmz

Lane 10: SuperSignal Molecular Weight Protein Ladder

The gel was loaded as follows:

Key for Colony 3

Lane 1: GST TIALA RRmz

Lane 2: left empty

Lane 3: Cell Debris from lysis step

Lane 4: left empty

Lane 5: Flow through

Lane 6: Wash 1 colony 3

Lane 7: Elution 1 colony 3

Lane 8: Combined elution

Lane 9: Color Protein Standard Broad Range

Lane 10: SuperSignal Molecular Weight Protein Ladder

Names: Rehmat Babar

Date: 8-27-18

Goal: The Western blot was probed with the primary and secondary antibodies to see if GST is present anywhere in the gels that were run on 8-24-18.

Results

Conclusion

Nothing appeared on this imaging so it will be reprobed with new antibodies to see if there is any flouresences.

Names: Mo, Julia, Amirah, Sierra

Date: 8-27-18 12:00pm
(this might go on 8-24)

Goal: After imaging, which did not show results, the paper was reprobed with a new antibody to further analyze the results of the western blot.

Materials:

2 8-24 western blot nitrocellulose paper

6x-His Tag Polyclonal Antibody (#RK240872)

10mL Fast Western Antibody Diluent (#SA245960)

HRP-Goat Anti-Rabbit (#1213156A)

1x Wash Buffer

Protocol:

1.The nitrocellulose paper was washed in 1x wash buffer twice and rinsed with water once while being shaken.

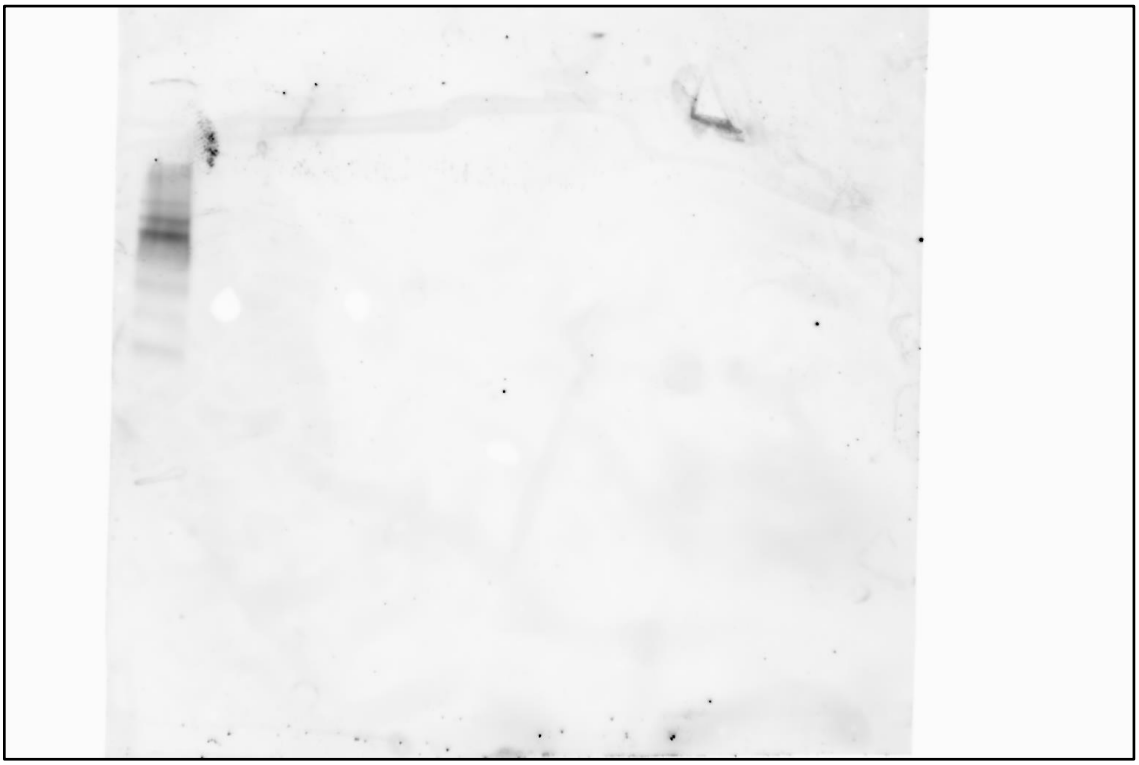
2.Ten microliters of 6x-His Tag Polyclonal Antibody was added to 10mL Fast Western Antibody Diluent and the paper was submerged in the solution for thirty minutes while shaking.

3.After the first thirty minutes, the paper was submerged in another solution containing 10mL Fast Western Antibody Diluent and 10µL of HRP-Goat Anti-Rabbit. The paper was left shaking in this secondary antibody for 30 minutes.

4.The paper was washed twice more using 1x wash buffer while shaking.

5. The paper was taken to the imager and mixed with a solution of 6 mL of and 6 mL of .

Results:



Conclusion: