Name: Julia Kelly

Date: 10-1-18

Goal: Isolate plasmid DNA from the cells that contain the three promoter parts.

Materials:

QIAprep Spin Miniprep Kit (lot#157025733)

Overnight Cultures from 9-30
- PmerT colonies 1-3
- Rcn colonies 1-3
- Scp colonies 1-3

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:

Sample	[DNA] ng/µL	A260/A280
PmerT 1	60.0	1.846
PmerT 2	65.0	1.857
PmerT 3	115	1.840
Scp 1	42.5	1.889
Scp 2	37.5	2.143
Scp 3	75.0	1.785
Rcn 1	80.0	1.778
Rcn 2	75.0	2.143
Rcn 3	82.5	1.941

Conclusion: These promoters parts can be used in the HCG system as a way to detect certain factors like metal presence. The next step in this process would be to do a digest to make sure the part looks okay.

Name: Rehmat Babar

Date: 10/3/18

Goal: Complete a restriction digest on the promoters needed for proof of concept

Materials

Strong Promoter BBa_K541503

RCN Cobalt Sensitive Promoter BBa_K540001

PmerT Promoter Bba_K346002

EcoRI

PstI

Protocol

Restriction Digest

Results

Include pictures of your gel with a key of what is in each lane and a snapshot of what it should look like from SnapGene by simulating a gel.

Conclusion

Did you accomplish your goal? How has your work today helped with the overall project or in lab overall? Discuss gel results here, possible points of error or expected error. How will you proceed?

Name: Rehmat Babar

Date: 10/5/18

Goal: Cleave GST from HCG from Elution 1 and 2 from the most recent protein expression

Materials

Pierce HRV 3C Protease 1,000 Unit Kit Lot# 00669803

Protocol

Combined 200 μL of elution (protein), 30 μL of 10X Reaction buffer, 2 μL of the protease, 68 μL of diH2O. Incubated at 4°C for 1 hour.

Results

To be continued by running western blot using HCG antibodies

Conclusion

The GST should have successfully cleaved from HCG, getting rid of the GST-HCG fusion protein altogether

Name: Rehmat Babar

Date: 10/8/18

Goal: Run a western blot and a coomassie blue stain using the newly cleaved recombinant HCG protein

Materials

Specific brand name and lot number

Protocol

Not too wordy, include bullet points or numbering system for what you did in lab.

Results

Include pictures of your gel with a key of what is in each lane and a snapshot of what it should look like from SnapGene by simulating a gel.

Conclusion

Did you accomplish your goal? How has your work today helped with the overall project or in lab overall? Discuss gel results here, possible points of error or expected error. How will you proceed?

Laura das Neves, Rehmat Babar, Julia Kelly, Yewouemoe Lynda Waku Kouomou
Date: 10/10/2018
Goal:

Pictures Fast W. Blott:

The image is soo clear it’s almost invisible. But it’s there. - GST Western Blot - 2nd Picture.

Same very clear image. - GST Western Blot

hCG Western Blott. Very clear image but there.

Name: Laura das Neves
Date: 10/11/2018
Goal: Generate a more concentrated sample of the elution samples 5 and 6

Materials:
15 mL centrifugal filter unit
Elution sample 5
Elution sample 6

Procedure:
Samples 5 and 6 were placed together into the centrifugal filter unit. The tube was then centrifuged for 15 minutes until the the sample reached 250 microliters.

Goal #2: Cleave GST from HCG from Elutions 5 & 6 from the most recent protein expression

Materials:
Pierce HRV 3C Protease 1,000 Unit Kit Lot# 00669803
50 microliters of the concentrated elution sample (5&6)
6 microliters of 10x Buffer
1 microliter enzyme
3 microliters of H_2O

Procedure:
Combined 50 µL of elution (protein), 6 µL of 10X Reaction buffer, 1 µL of the protease, 3 µL of diH_2O. Incubated at 4°C for overnight.

Name: Julia Kelly, Rehmat Babar

Date: 10/12/18

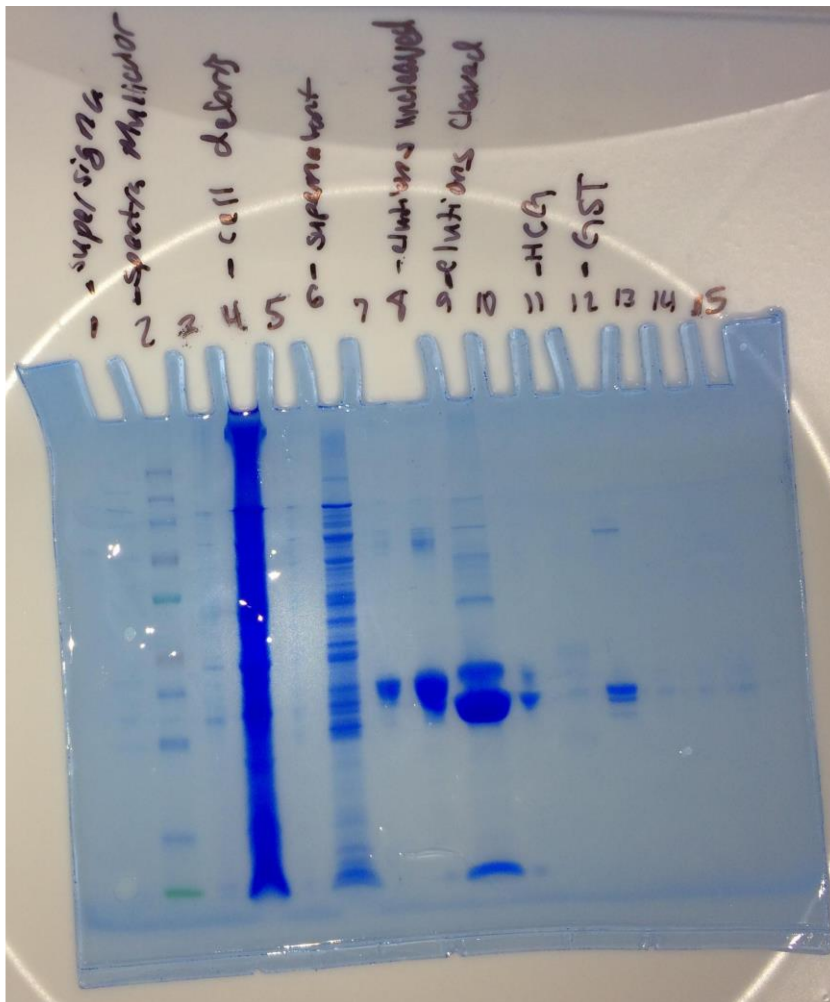
Goal:

Materials:

TruSep 2x SDS Sample Buffer

Protocol:

Results:



Well
1. Supersignal Protein Standard
2. Spectro Multicolor Standard
3.
4. Cell Debris
5.
6. Supernatant
7.
8. Concentrated elutions uncleaved
9. Concentrated elutions cleaved
10.
11. HCG standard
12. GST standard
13.
14.
15.

Conclusion:

Name:Julia Kelly

Date: 10-12-18

Goal: Grow up a 10 mL culture than can be use for a mass culture of B3 Loop in pGEX

Materials:

Luria Broth made on 9-17

B3 Loop in pGEX colony 1a glycerol stock made on 9/27/18

Chloramphenicol

Protocol:
10 mL of LB and 10 μL of Chloramphenicol were added into a falcon tube under a flame. A p10 tip was used to scrape the top of the 1a glycerol stock and it was dropped into the LB+ chloro. The overnight was put into a shaking water bath for 18 hours at 37℃ and 200 rpm.

Results: The overnight showed growth.

Conclusion: The culture will be used for the mass culture of B3 Loop in pGEX for protein expression.

Name: Julia Kelly

Date: 10/13/18

Goal: Protein expression of Beta 3 Loop in pGEX

Materials

LB + Amp

1M IPTG

Beta 3 Loop in pGEX 10 mL overnight culture

Protocol

500 mL Mass Culture

1. The grown up Beta 3 Loop in pGEX 10 mL overnight culture from the day before was dumped into 500 mL of LB + Amp in a flask.
2. The flask was incubated at 37℃ at 150 rpm for several hours.
3. The absorbance at OD 600 was intermittently taken for the culture to ensure it was between 0.6 and 1.0.
4. Once the absorbance was in that range, 500 µL of 1M IPTG was added to the 500 mL culture and the flask was 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	0.625

Conclusion

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

Name: Julia Kelly, Christina Clodomir

Date: 10-14-18

Goal: Express and isolate the Beta 3 Loop in pGEX.

Materials:

1X PBS

1M DTT

500 mL mass culture from 10/13/18

Sigma Cell Lytic Express Lot SLBN1169V

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

Pierce Glutathione Agarose Lot TD262116

Protocol:

1. The 500 mL mass culture were divided evenly into 7 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 sonicated with short pulses while on ice.
6. The lysed cell solution was centrifuged at 13,000xg 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 24 hours.

Results:

Conclusion: The resin and supernatant needs to be put through a column so it can be used in an SDS-PAGE gel and a Western BLot

Name:
Date: 10/15/18

Goal: Want to continue protein isolation which has been a continuous effort for the past two days. We hope to isolate Beta3 Loop 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.
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

Name: Julia Kelly, Rehmat Babar

Date:10/16/18

Goal: Redo the protein transfer from 10/12/18 and run the B3 Loop on a gel to get a coomassie and transfers ready for western blots.

Materials:

Elution samples of B3

Protocol:

Results:

Conclusion: