Name: Laura Das Neves, Rehmat Babar

Date: 7/2/18

Goal: Amplify existing DNA labeled HCG cDNA for pSB1C3, PGEX, and Beta 3 Loop with new HCG primers.

Materials:

HCG for pSB1C3 PCR from 9/22/17
HCG G-Block 5
1:10 Dilution of HCG new prefix primer
1:10 Dilution of HCG new suffix primer
1:10 Dilution of HCG for pGEX F
1:10 Dilution of HCG for pGEX R
1:10 Dilution of Beta 3 loop with biobrick prefix
1:10 Dilution of Beta 3 loop with biobrick suffix
Invitrogen PCR supermix High Fidelity Lot no. 1763959 10 µL
H20

Protocol

PCR of HCG CDNA's

1. HCG for pSB1C3 PCR 9/22/17

A PCR tube was filled with 7 µL of water, 10 µL of HiFi supermix, 1µL of 1:10 Dilution of HCG new prefix primer, 1 µL 1:10 Dilution of HCG new suffix primer , and 1 µL of HCG for pSB1C3 PCR from 9/22/17.

2. HCG for pSB1C3 PCR 9/22/17

A PCR tube was filled with 7 µL of water, 10 µL of HiFi supermix, 1:10 Dilution of HCG for pGEX F,1:10 Dilution of HCG for pGEX R, and 1 µL of HCG for pSB1C3 PCR from 9/22/17.

3. HCG for pSB1C3 PCR 9/22/17

A PCR tube was filled with 7 µL of water, 10 µL of HiFi supermix, 1:10 Dilution of Beta 3 loop with biobrick prefix, 1:10 Dilution of Beta 3 loop with biobrick suffix and 1 µL of HCG for pSB1C3 PCR from 9/22/17.

4. PCR of HCG G-Block 5

A PCR tube was filled with 7 µL of water, 10 µL of HiFi supermix, 1:10 Dilution of HCG for pGEX F,1:10 Dilution of HCG for pGEX R, and 1 µL of HCG G-BLock 5 DNA.

5.PCR of HCG G-Block 5

A PCR tube was filled with 7 μL of water, 10 μL of HiFi supermix, 1μL of 1:10 Dilution of HCG new prefix primer, 1 μL 1:10 Dilution of HCG new suffix primer , and 1 μL of HCG G-BLock 5 DNA

6.PCR of HCG G-Block 5

A PCR tube was filled with 7 μL of water, 10 μL of HiFi supermix, 1:10 Dilution of Beta 3 loop with biobrick prefix, 1:10 Dilution of Beta 3 loop with biobrick suffix and 1 μL of HCG G-BLock 5 DNA.

3. The PCR tubes were placed in the thermocycler at the following settings:

1. 94° C for 3:00 minutes
2. 94° C for 1:00 minute
3. 56° C for 0:45 seconds
4. 72° C for 1:00 minute
5. 35X (Go to Step 2)
6. 72° C for 2:00 minutes

Lid Temperature: 105° C

Results

7/3/18

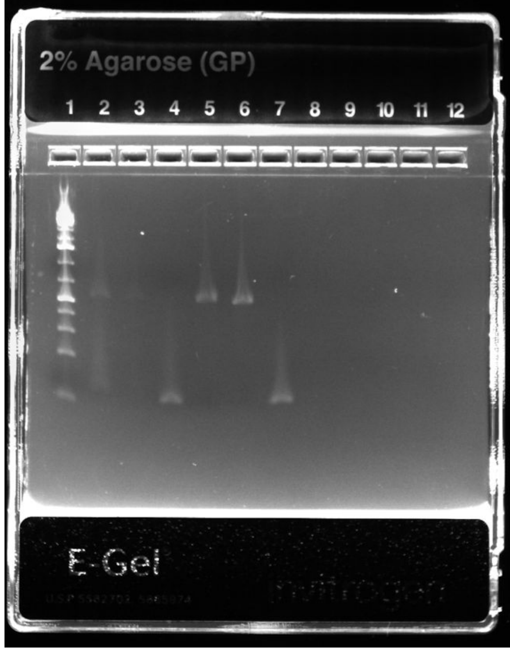
Key

E-Gel #1

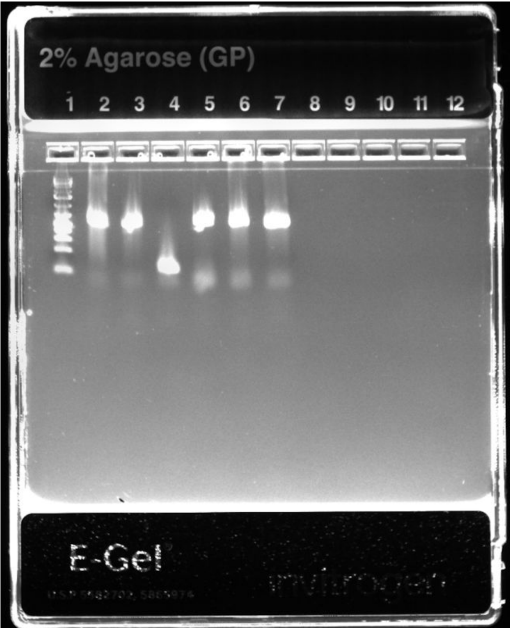
Well 1 GeneRuler 1 kb Plus DNA Ladder ready-to-use Lot 00516263
Well 2 HCG for pSB PCR 9/22/17 Tube 1
Well 3 HCG for pSB PCR 9/22/17 Tube 2
Well 4 HCG for pSB PCR 9/22/17 Tube 3
Well 5 HCG G-block 5 Tube 4
Well 6 HCG G-block 5 Tube 5
Well 7 HCG G-block 5 Tube 6

E-Gel #2

Well 1 GeneRuler 1 kb Plus DNA Ladder ready-to-use Lot 00516263
Well 2 HCG for pSB PCR 9/22/17 Tube HCG
Well 3 HCG for pSB PCR 9/22/17 Tube PLEX
Well 4 HCG for pSB PCR 9/22/17 Tube B3
Well 5 HCG G-block 5 Tube 1 JE
Well 6 HCG G-block 5 Tube 2 JE
Well 7 HCG G-block 5 Tube 3 JE

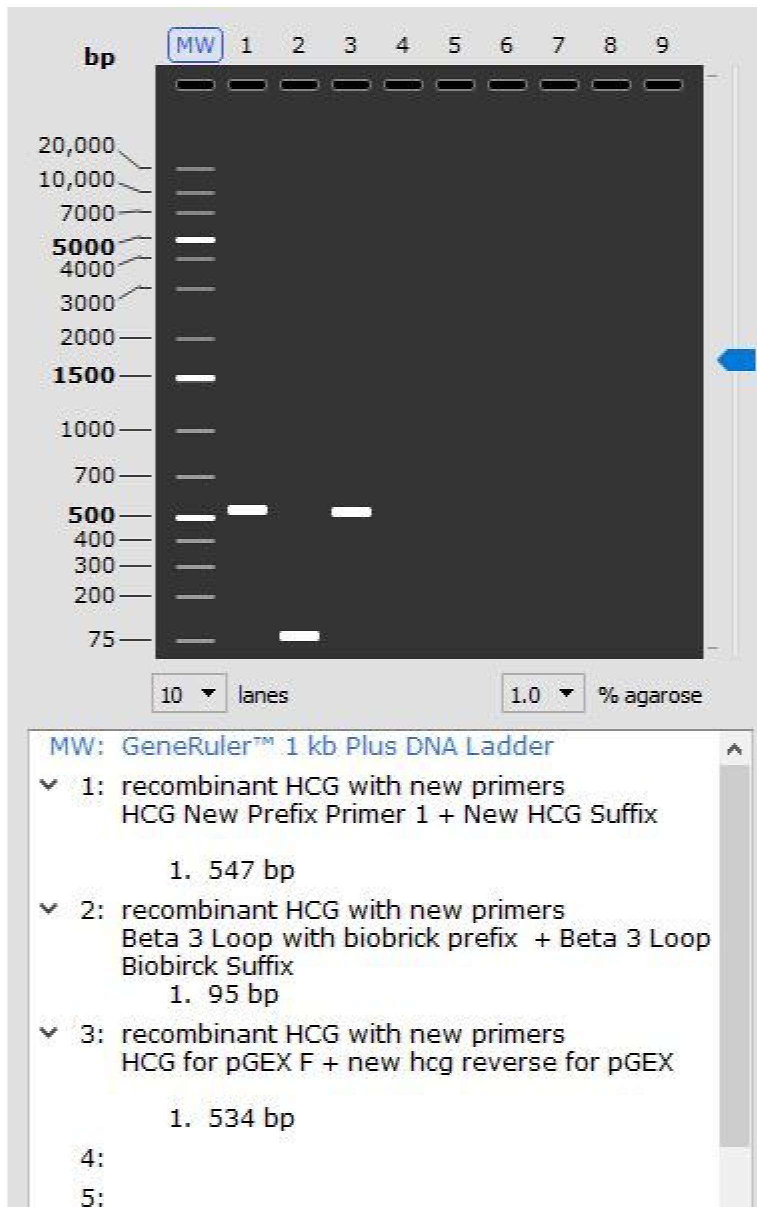


E-Gel #1



E-Gel #2

Expected Results Simulated in SnapGene



Conclusion: The bands were bright and were placed in the correct places. The next step will be to do a PCR cleanup to prepare the samples for ligation.

Name: Yash Y., Julia Kelly

Date: 7/03/18

Goal: To separate the pSB1C3 backbone from the RFP in pSB1C3 and the GFP in pSB1C3 using gel extraction.

Materials:

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

-Buffer QX1

-QIAEX II

-Buffer PE

Razor Blade, Forceps

Specific names including the brand and other important information

Protocol:

1.After running a restriction digest on the pSB1C3 parts (using EcoRI and PstI), 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)
Blue 1	0.9933	1.1067	0.1134	340.2
Blue 2	0.9798	1.1449	0.1651	495.3
Blue 3	0.9904	1.0928	0.1024	307.2
Blue 4	0.9917	1.1455	0.1138	461.4
Purple 1	0.9944	1.1111	0.1167	350.1
Purple 2	0.9952	1.0757	0.0805	241.5
Purple 3	0.9797	1.1137	0.1340	402.0
Purple 4	0.9956	1.0997	0.1041	312.3

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
Blue 1	2.5	Purple 1	5
Blue 2	5	Purple 2	Too Low
Blue 3	Too Low	Purple 3	72.5
Blue 4	128	Purple 4	Too Low

Conclusion: Only two samples showed distinct DNA concentrations so they will be used for the ligation. For the other sample, we will combine them into one tube and do an ethanol precipitation.

Name: Rehmat Babar

Date: 7/03/18

Goal: PCR cleanup on the PCR done with the new primers on 7/02/18. Want to prepare the samples for restriction digests before the samples are ready for ligation.

Materials

Zymo Research DNA Clean & Concentrator - 5

Lot No: ZRC185825

HCG for pSB1C3 PCR Product 7/2/18

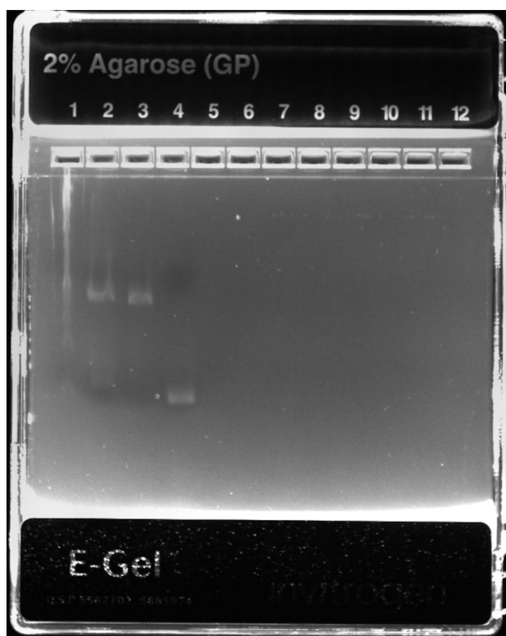
HCG for pSB1C3 PCR Product 7/2/18

HCG Beta 3 Loop for pSB1C3 PCR Product 7/2/18

Protocol

1. Transferred 15 µL of HCG for pSB1C3 PCR Product 7/2/18 into a 1.5 Eppendorf tube and 5 volumes of DNA Binding Buffer was added and the tbe was vortexed. This is repeated for each of the PCR products
2. Each of the samples 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

Well 1 GeneRuler 1 kb Plus DNA Ladder ready-to-use Lot 00516263

Well 2 HCG for pSB1C3 PCR cleanup product

Well 3 HCG for pGEX PCR cleanup product

Well 4 HCG Beta 3 Loop for pSB1C3 PCR cleanup product

Concentrations

Sample	Concentration
HCG for pSB1C3 PCR cleanup product	15 ng/μL
HCG for pGEX PCR cleanup product	17.5 ng/μL
HCG Beta 3 Loop for pSB1C3 PCR cleanup product	20 ng/μL

Conclusion

The PCR Cleanup was executed properly and enough volume of the DNA was recovered however the concentrations appeared to be too low. When the samples were imaged on the E-Gel the bands were present and were present at the appropriate band length but the bands were not that bright.

Name: Rehmat Babar

Date: 7/05/18

Goal: Do a restriction digest on the PCR Cleanup from 7/03/18 to make the ends compatible to be put into the pSB1C3 from the gel extraction. The pSB1C3 vector was cut with EcoRI and PstI. Then a heat kill will be done on the restriction digest products in order to denature the enzymes so that the product is ready for ligation.

Materials

Thermo Scientific FastDigest NotI Lot 00537736

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific FastDigest PstI Lot 00472716

Thermo Scientific 10X FastDigest Buffer Lot 00312007

PCR Cleanup Product from 7/03/18

Protocol

20 µL Restriction Digest on PCR Cleanup Product (HCG for pSB1C3 with new primers, HCG for pGEX with new primers, HCG Beta 3 Loop for pSB1C3)

a. HCG for pSB1C3

    16 µL of HCG for pSB1C3 PCR Cleanup Product

    1 µL EcoRI

    1 µL PstI

    2 µL of FastDigest Buffer

b. HCG for pGEX

    16 µL of HCG for pGEX PCR Cleanup Product

    1 µL EcoRI

    1 µL NotI

    2 µL of FastDigest Buffer

c. HCG Beta 3 Loop for pSB1C3

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

    1 µL EcoRI

    1 µL PstI

    2 µL of FastDigest Buffer

Heat Kill

a. The restriction digests were placed in the water bath at 60° C for 20 minutes in order to denature the enzymes so that the PCR cleanup product will be ready for ligation.

Name: Yash Y., Lynda W, Julia K.

Date: 7/05/2018

Goal: To combine the gel extraction products that were too low in order and do an ethanol precipitation to get a concentration that can be worked with.

Materials:

3M Sodium Acetate

100% ethanol

70% ethanol

Deionized Water

Protocol:

1. The volume of the sample was 95μL. One-tenth volume of 3M sodium acetate was added (9.5 μL) and two and a half volume of 100% ethanol were added (238 μL).
2. The sample was put in the freezer for 15 minutes.
3. The sample was centrifuged at 15000 rpm at 4 degrees Celsius for 30 minutes.
4. The supernatant was taken off and the pellet was air dried for 15 minutes.
5. Thirty microliters of water was added and the pellet was resuspended, then spun down and resuspended again.

Results: The concentration was too low on the sample, 10 ng/μL

Conclusions: Even after the ethanol precipitation, the concentration of DNA was too low. There was not a distinct pellet after the 30 minute centrifuged so a poor concentration was expected. This sample will not be used in the ligation steps.

Name: Yash, Lynda, Mo, Rehmat, Julia

Date: 7/05/2018

Goal: Put the HCG parts in the chosen plasmids by doing a ligation on the full length HCG unit with plasmids pSB1C3 and pGEX and on the beta loop HCG unit with plasmid pSB1C3.

Materials:

T4 DNA Ligase

T4 DNA Ligase Buffer

pSB1C3, pGEX

Full length HCG unit, beta loop subunit

Water

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: Rehmat Babar, Yash Yadav, Lynda W.

Date: 7/05/18

Goal: To do a transformation using electrically competent DH5a E. Coli cells using the DNA that was ligated earlier: Beta HCG in pSB1C3, Beta HCG in pGEX, and Beta 3 Loop HCG in pSB1C3 will be used.

Materials

Ligated Beta HCG in pSB1C3

Ligated Beta HCG in pGEX

Ligated Beta 3 Loop HCG in pSB1C3

SOC Medium

Invitrogen ElectroMAX DH5a Electrocompetent Cells Lot 1932992

Bio-Rad MicroPulser

Protocol

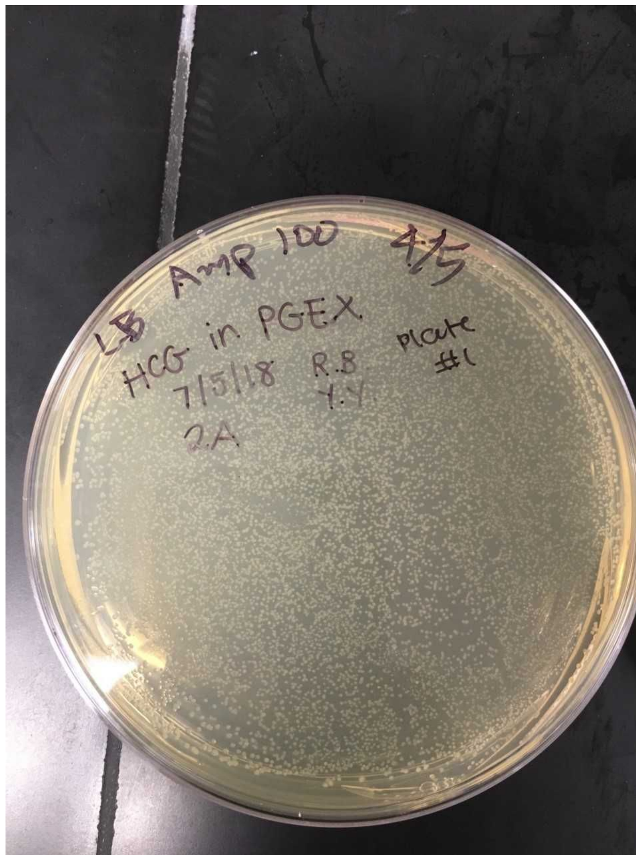
Electroporation Transformation

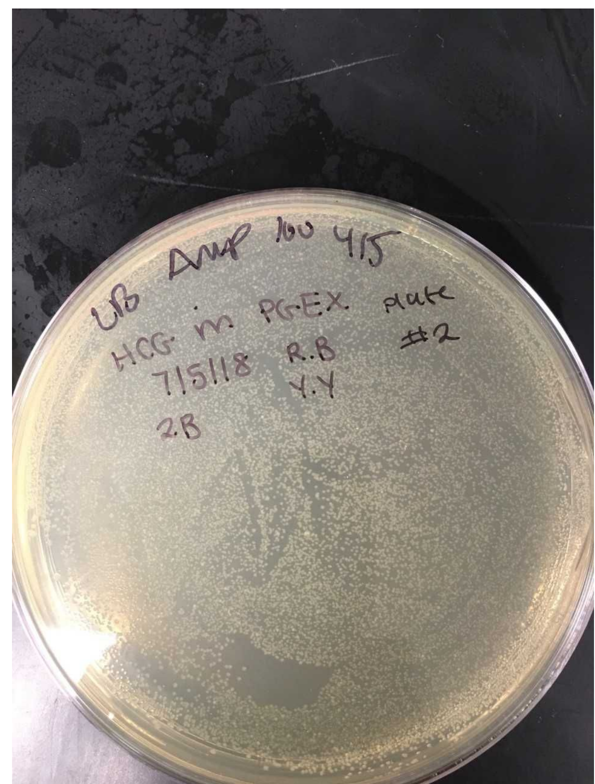
1. 40 µL of the electrically competent cells and 1 µL of ligated DNA was added to an Eppendorf tube.

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.
7. This was repeated for each of the samples of ligated DNA.

There were two trials for HCG in pGEX, two for HCG in pSB1C3, and one trial for Beta 3 Loop HCG in pSB1C3

Results





There was no growth on any of the 3 plates containing the pSB1C3 vector.

Conclusion

We will leave the pSB1C3 plates in the incubator for an additional 6-8 hours and if no growth is observed, then we will restart the transformation and possibly the ligation process for the DNA

containing pSB1C3. We will move on with the pGEX plates and perform a colony PCR to see if various colonies produced what we were expecting.

Name: Rehmat Babar, Olivia, Mo

Date: 7/06/18

Goal: Extract plasmid DNA from the mambalgin in pGEX overnight cultures to eventually do a restriction digest and gel extraction to isolate the pGEX vector to use in a ligation.

Materials

QIAprep Spin Miniprep Kit Lot 157020506

Protocol

1. Mini Preps
    a. 3 mL of bacterial overnight culture was centrifuged at 8,000 rpm for 3 minutes at room temperature
    b. The supernatant was discarded and the pelleted bacterial cells were resuspended in 250 µL Buffer P1 and was transferred to one eppendorf tube
    c. 250 µL of Buffer P2 was added and each tube was inverted 5 times
    d. 350 µL of Buffer N3 was added and immediately mixed by inverting the tubes 5 times.
    e. All the tubes were centrifuged for 10 minutes at 13,000 rpm.
    f. 800 µL of the clear supernatant was micropipetted into a spin column and was centrifuged for 60 seconds and the excess liquid was discarded
    g. 500 µL of PB was added and the spin columns were centrifuged for 60 seconds and the flow through was discarded
    h. 750 µL of PE was added and to the spin columns, they were centrifuged for 60 seconds, and the flow through was discarded
    i. The spin columns were centrifuged again for 60 seconds to remove residual wash buffer and the flow through was discarded
    j. 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
    k. The spin column was allowed to stand for one minute and was centrifuged for one minute
    l. The concentrations for each sample were recorded

Results

Sample	Concentration	A 260/280
Mambalgin in pGEX #1	22.5 ng/µL	4.500
Mambalgin in pGEX #5	30.0 ng/µL	3.000

Mambalgin in pGEX #10	27.5 ng/µL	1.833
Mambalgin in pGEX #6	30.0 ng/µL	2.000
Mambalgin in pGEX #3	100 ng/µL	1.818
Mambalgin in pGEX #8	27.5 ng/µL	1.833
Mambalgin in pGEX #7	40.0 ng/µL	2.000
Mambalgin in pGEX #9	47.5 ng/µL	2.111

Conclusion

The concentrations for most of these mini preps were in a great range and we will continue to do a restriction digest using EcoRI and NotI in order to isolate the pGEX vector and perform a gel extraction.

Name: Rehmat Babar, Olivia, Mo

Date: 7/06/18

Goal: Want to take mambalgin out of the pGEX vector by cutting with EcoRI and NotI and to run it on a gel to do a gel extraction to prep the pGEX vector for ligation.

Materials

Thermo Scientific FastDigest NotI Lot 00340033

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific 10X FastDigest Buffer Lot 0049145

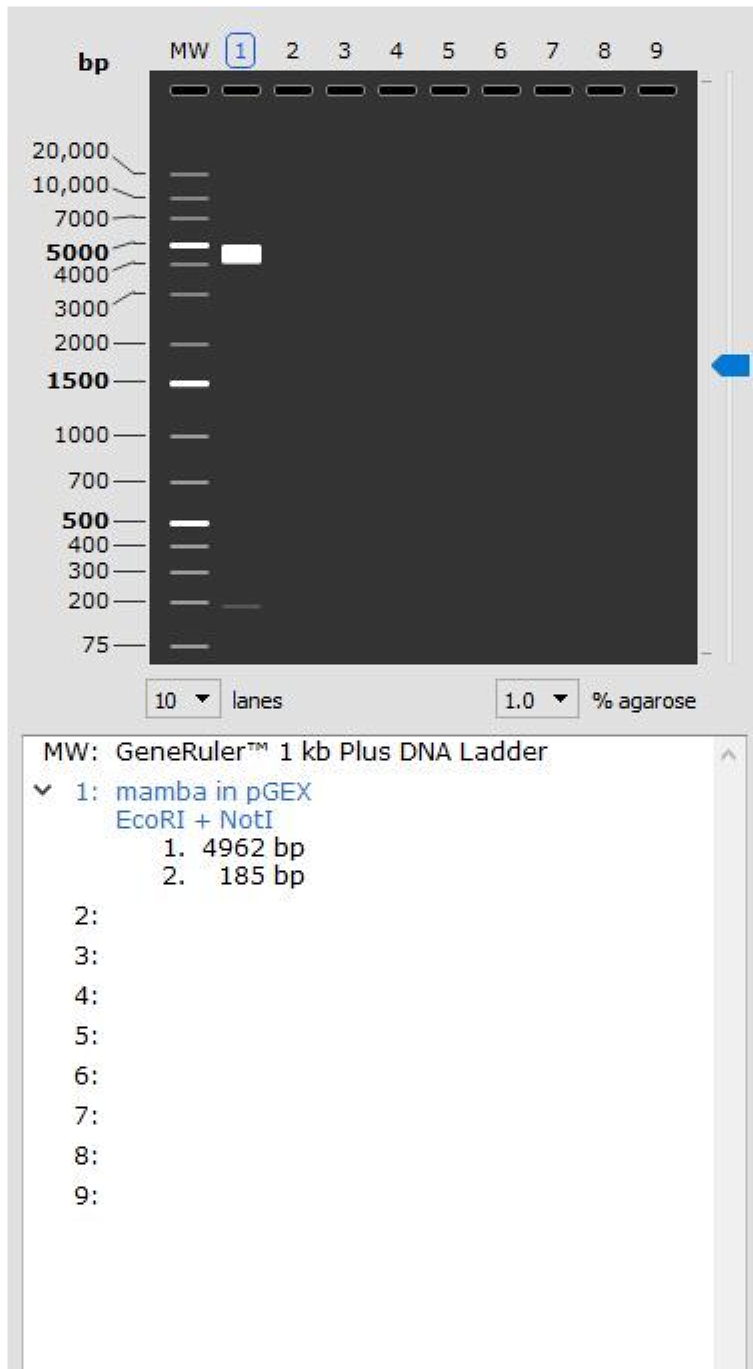
Protocol

30 µL Restriction Digest on pGEX containing mambalgin

1. A 9X cocktail was prepared since there are 8 total samples of DNA of mambalgin in pGEX. The cocktail was prepared as follows: 9 µL EcoRI, 9 µL of NotI, 27µL of Thermo Scientific 10X Fast Digest Buffer, and 135 µL of dI H_2O.
2. Then 20 µL of this cocktail was added to 8 different labeled eppendorf tubes and 10 µL of the corresponding DNA was added to their respective tubes and were incubated at 37° C for 30 minutes.

Results

Expected Results Simulated in SnapGene



Conclusion

The pGEX vector is not immediately needed so the gel extraction will be held off until needed.

Name: Rehmat Babar and Olivia

Date: 7/06/18

Goal: Want to do colony PCR on the colonies that grew on the plates from the transformation done on 7/05/18. There was no growth on the transformations done with the pSB1C3 vector but the transformations done with the pGEX vector had many many colonies.

Materials

PGEX 5’	225043970
Pgex 3’	229398506
New hcg reverse for pGEX	271850405
HCg for pGEX F	226165406

IDT Nuclease-Free Water Lot 285839

PCR SuperMix High Fidelity Lot 1763959

Protocol

Colony PCR

7 colonies each from 2 plates both containing HCG in pGEX

1. Prepare 2 different 15X concentration cocktails which will contain two different sets of primers. The PCR will be 20 µL.
    a. The Cocktail A will include: 150 µL PCR SuperMix High Fidelity, 105 µL IDT Nuclease-Free Water, 15 µL PGEX 5’, and 15 µL Pgex 3’.
    b. The Cocktail B will include: 150 µL PCR SuperMix High Fidelity, 105 µL IDT Nuclease-Free Water, 15 µL New hcg reverse for pGEX, and 15 µL HCg for pGEX F.
2. 19 µL of the first cocktail was added to 7 different PCR tubes and 19 µL of the second cocktail was added to 7 more PCR tubes.
3. 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 14 different tubes containing water and the colonies. Due to the small size of the colonies this was done to ensure the same exact colony will be used for the two different sets of primers and for the overnight cultures.
4. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes.
5. All of the tubes containing cocktail A had the following PCR settings:

    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

6. All of the tubes containing cocktail B 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

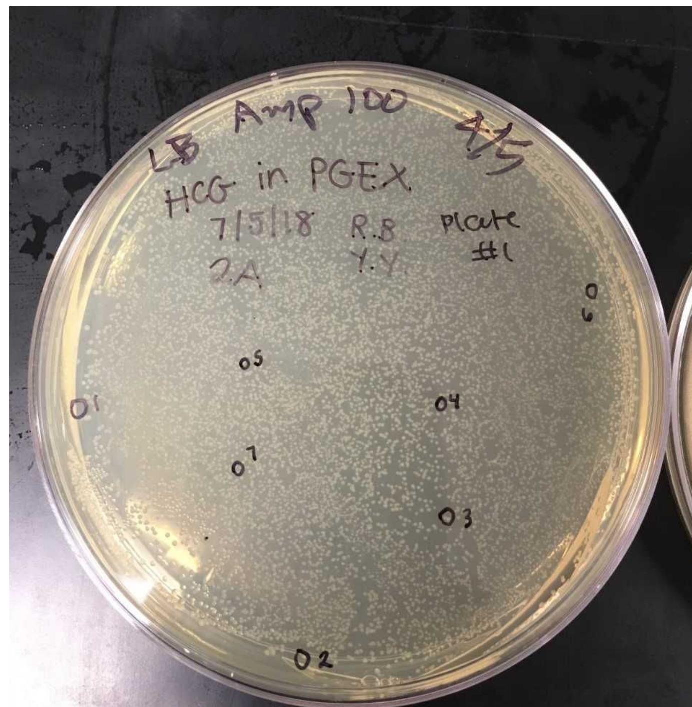
Lid temperature: 105° C

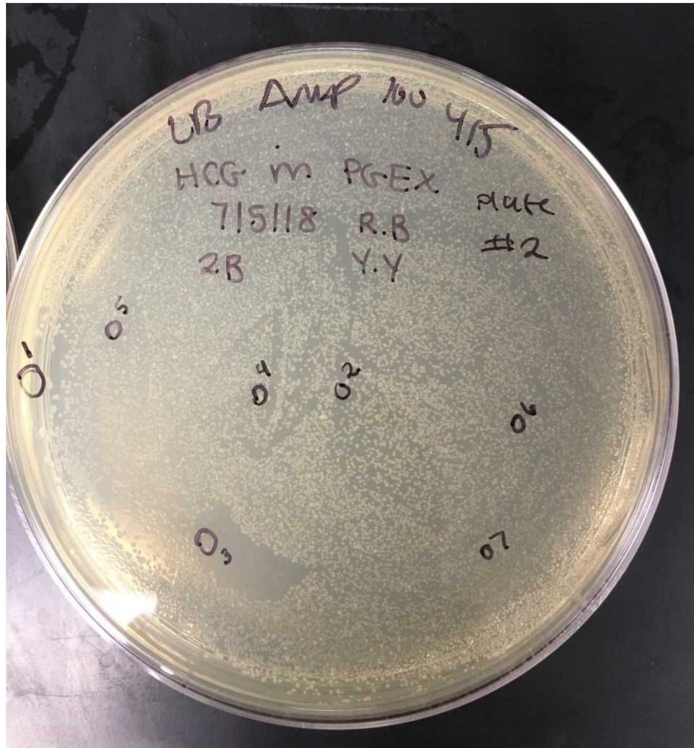
Infinite hold: 4° C

Tubes are labeled for example:

#1A 1	Plate #1, Cocktail A, colony 1
#2B 7	Plate #2, Cocktail B, colony 7

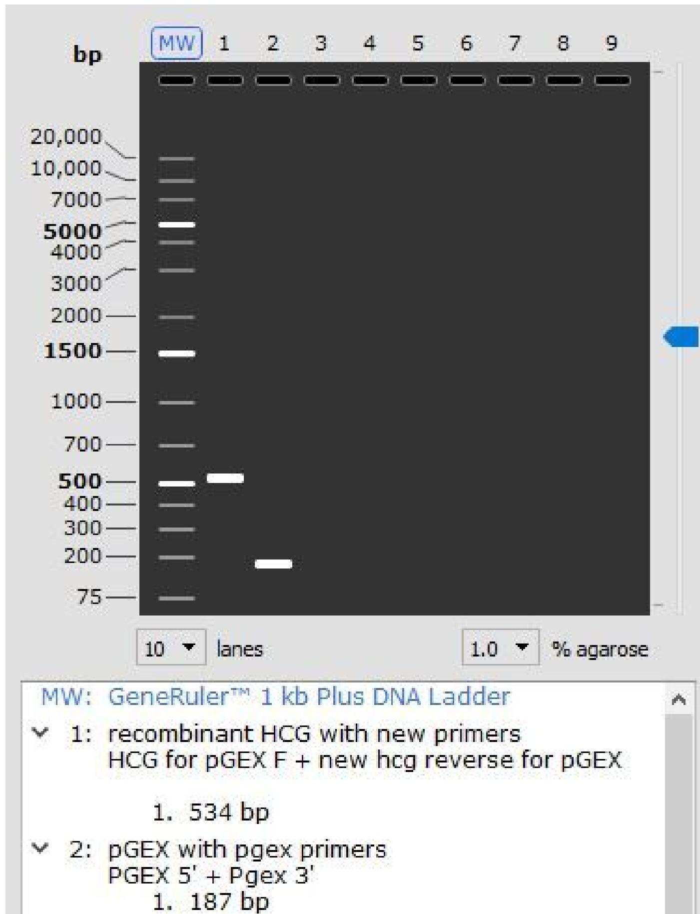
Images of picked colonies:

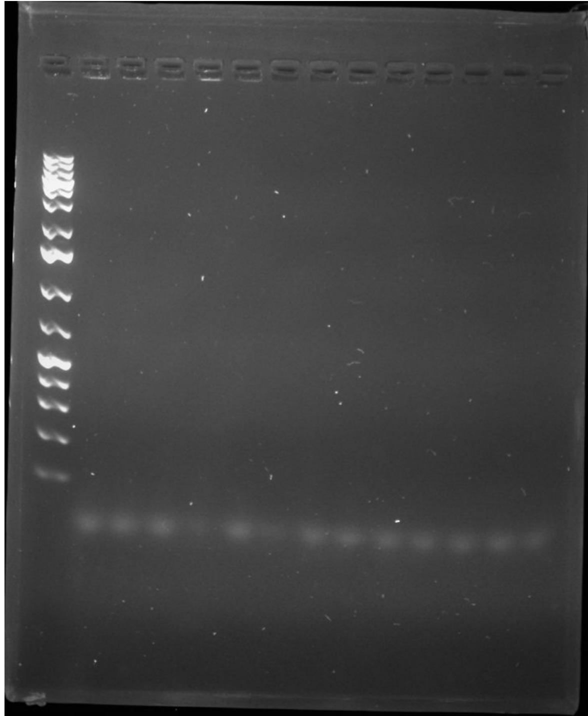




Results

Expected Results Simulated in SnapGene



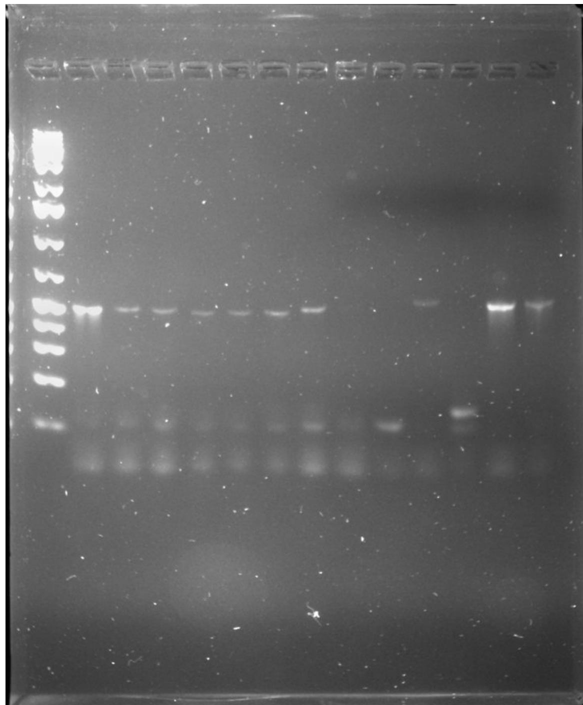


Key

Well 1: GeneRuler 1 kb Plus DNA Ladder

Plate 1A Colony 1-7

Plate 2A Colony 1-7



Key

Well 1: GeneRuler 1 kb Plus DNA Ladder

Plate 1B Colony 1-7

Plate 2B Colony 1-7

Conclusion

Plate 1 and 2 with cocktail A did not result in the correct sized bands and appears to only consist of the primers, but plates 1 and 2 with cocktail B did result in the correct bands and produced the expected results. We will move on with the overnight cultures.

Name: Rehmat Babar

Date: 7/10/18

Goal: Want to extract plasmid DNA from the overnight cultures which contained HCG in pGEX and to hopefully verify the ligation was successful.

Materials

QIAprep Spin Miniprep Kit Lot 157020506

Protocol

1. Mini Preps
   a. 3 mL of bacterial overnight culture was centrifuged at 8,000 rpm for 3 minutes at room temperature
   b. The supernatant was discarded and the pelleted bacterial cells were resuspended in 250 μL Buffer P1 and was transferred to one eppendorf tube
   c. 250 μL of Buffer P2 was added and each tube was inverted 5 times
   d. 350 μL of Buffer N3 was added and immediately mixed by inverting the tubes 5 times.
   e. All the tubes were centrifuged for 10 minutes at 13,000 rpm.
   f. 800 μL of the clear supernatant was micropipetted into a spin column and was centrifuged for 60 seconds and the excess liquid was discarded
   g. 500 μL of PB was added and the spin columns were centrifuged for 60 seconds and the flow through was discarded
   h. 750 μL of PE was added and to the spin columns, they were centrifuged for 60 seconds, and the flow through was discarded
   i. The spin columns were centrifuged again for 60 seconds to remove residual wash buffer and the flow through was discarded
   j. 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
   k. The spin column was allowed to stand for one minute and was centrifuged for one minute
   l. The concentrations for each sample were recorded

Results

Sample	Concentration
HCG in pGEX Plate 2B Colony 1	72.5 ng/μL
HCG in pGEX Plate 2B Colony 2	45 ng/μL

HCG in pGEX Plate 2B Colony 3	75 ng/μL
HCG in pGEX Plate 2B Colony 4	42.5 ng/μL
HCG in pGEX Plate 2B Colony 5	35 ng/μL
HCG in pGEX Plate 2B Colony 6	42.5 ng/μL
HCG in pGEX Plate 2B Colony 7	40 ng/μL

Conclusion

The concentrations of the mini preps were in a good range, they were not too high or too low.

Name: Escolivia Birungi

Date: 7/11/18

Goal: The pSB1C3 plates were left in the incubator for longer, and a few colonies grew. These colonies were picked and colony PCR was performed to give more information on what actually grew.

Materials:

IDT Nuclease-Free Water Lot 285839

PCR SuperMix High Fidelity Lot 1763959

B 3 Loop Biobrick Suffix

B 3 Loop Biobrick Prefix

New HCG Suffix

New HCG Prefix

Protocol:
Colony PCR

2 colonies each from 2 plates both containing HCG in pSB1C3 and HCG Beta 3 loop in pSB1C3

1. The PCR tubes will be 20 µL.
    a. HCG in pSB1C3 (2 TUBES): 10 µL PCR SuperMix High Fidelity, 7 µL IDT Nuclease-Free Water, 1µL New HCG Prefix , and 1 µL New HCG Suffix.
    b. HCG Beta 3 Loop in pSB1C3 (2 TUBES): 10 µL PCR SuperMix High Fidelity, 7 µL IDT Nuclease-Free Water, 1 µL B 3 Loop Biobrick Prefix, and 1 µL B 3 Loop Biobrick Suffix.
2. 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. Due to the small size of the colonies this was done to ensure the same exact colony will be used for the two different sets of primers and for the overnight cultures.
3. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes.
4. All of the tubes containing HCG in pSB1C3 had the following PCR settings:

    1. 95° C for 3:00 minutes

    2. 95° C for 1:00 minutes

    3. 57° 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

5. 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

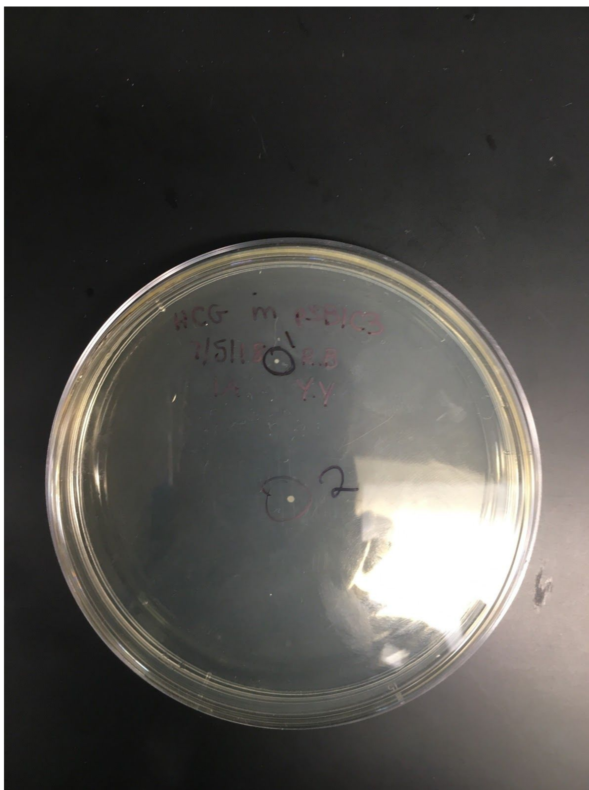
    5. 30X Cycles

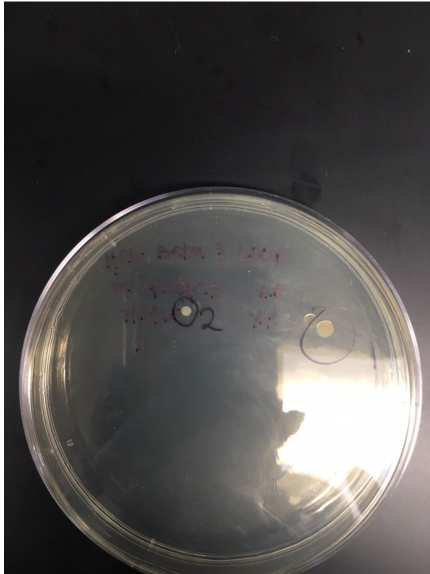
    72° C for 5:00 minutes

    Lid temperature: 105° C

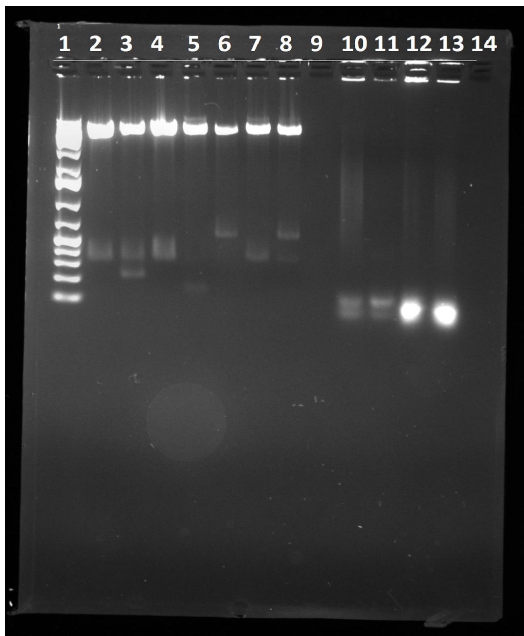
    Infinite hold: 4° C

Images of picked colonies





Results



1. GeneRuler 1kb plus ladder
2. HCG in pGEX Colony 1
3. HCG in pGEX Colony 2
4. HCG in pGEX Colony 3
5. HCG in pGEX Colony 4
6. HCG in pGEX Colony 5
7. HCG in pGEX Colony 6
8. HCG in pGEX Colony 7
9.
10. HCG Beta 3 in pSB1C3 Colony 1
11. HCG Beta 3 in pSB1C3 Colony 2
12. HCG in pSB1C3 Colony 1
13. HCG in pSB1C3 Colony 2
14.

Conclusion:
The gel shows only the presence of the primers, and no HCG DNA. Minipreps will be performed to determine if any of the transformations were successful.

Name: Julia Kelly

Date: 7/12/18

Goal: Extract plasmid DNA from the HCG in pGEX and HCG Beta Loop 3 in pGEX overnight cultures to eventually send them off for sequencing to see whether the ligation worked.

Materials: QIAprep Spin Miniprep Kit Lot 157025733

Protocol:

Mini Preps

a. 3 mL of bacterial overnight culture was centrifuged at 8,000 rpm for 3 minutes at room temperature
b. The supernatant was discarded and the pelleted bacterial cells were resuspended in 250 µL Buffer P1 and was transferred to one eppendorf tube
c. 250 µL of Buffer P2 was added and each tube was inverted 5 times
d. 350 µL of Buffer N3 was added and immediately mixed by inverting the tubes 5 times.
e. All the tubes were centrifuged for 10 minutes at 13,000 rpm.
f. 800 µL of the clear supernatant was micropipetted into a spin column and was centrifuged for 60 seconds and the excess liquid was discarded
g. 500 µL of PB was added and the spin columns were centrifuged for 60 seconds and the flow through was discarded
h. 750 µL of PE was added and to the spin columns, they were centrifuged for 60 seconds, and the flow through was discarded
i. The spin columns were centrifuged again for 60 seconds to remove residual wash buffer and the flow through was discarded
j. 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
k. The spin column was allowed to stand for one minute and was centrifuged for one minute
l. The concentrations for each sample were recorded

Results: HCG in pSB1C3 colony 1- too low
HCG in pSB1C3 colony 2- too low
Beta 3 Loop in pSB1C3 1- 20.0
Beta 3 Loop in pSB1C3 2- 22.5

Conclusion: Concentrations were too low, This combined with the poor result of colony PCR shows that these plate samples cannot be worked with. Transformations will be done again.

Name: Julia Kelly

Date:7-12-18

Goal: Cut the vector and plasmid with EcoRI and NotI to further test to see if the ligation of the two together was successful.

Materials:

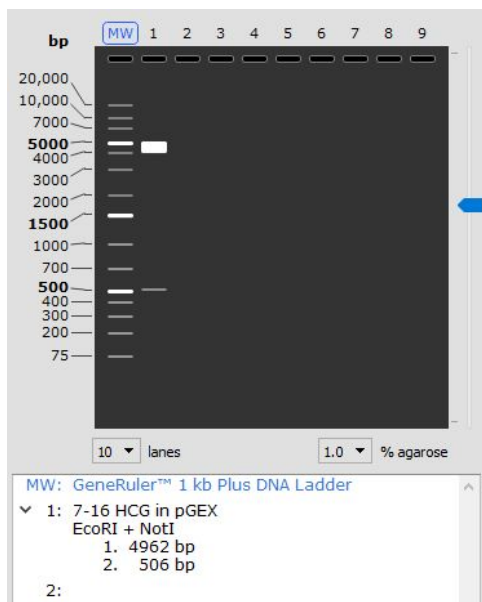
Thermo Scientific FastDigest NotI Lot 00537736

Thermo Scientific FastDigest EcoRI Lot 00246951

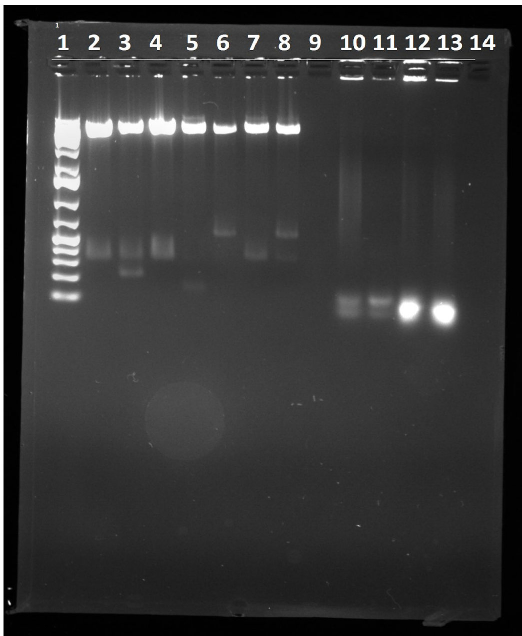
Thermo Scientific 10X FastDigest Buffer Lot 00440534

Protocol:

1. A 20 μL reaction was prepared using a 9x cocktail. Nine microliters of NotI, 9 μL of EcoRI, 18 μL of Fastdigest Buffer and 54 μL of water were mixed together.
2. Ten microliters of the cocktail was put into each of the seven eppendorf tubes.
3. Ten microliters of each miniprep DNA (HCG in pGEX) from 7/10/18 was added to one of the tubes.
4. The samples were incubated at 37 degrees for 15 minutes.
5. After incubation, the samples were run on a gel.



Results:



1. GeneRuler 1kb plus ladder
2. HCG in pGEX Colony 1
3. HCG in pGEX Colony 2
4. HCG in pGEX Colony 3
5. HCG in pGEX Colony 4
6. HCG in pGEX Colony 5
7. HCG in pGEX Colony 6
8. HCG in pGEX Colony 7
9.
10. Colony PCR tube 1
11. Colony PCR tube 2
12. Colony PCR tube 3
13. Colony PCR tube 4
14.

Conclusion: Colonies five and seven look most like the snapgene simulation, so three PCR reactions will be performed on each of those samples to see if they are good enough to go to out for sequencing.

Name: Julia Kelly

Date: 7/16/18

Goal: Do a PCR on the HCG in pGEX results from 7/10/18 before they are sent off for sequencing.

Materials:

HCG in pGEX miniprep samples colonies 1-7 from 7/10/18
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
1:10 Dilution of Beta 3 loop with biobrick prefix
1:10 Dilution of Beta 3 loop with biobrick suffix
Invitrogen PCR supermix High Fidelity Lot no. 1763959 10 µL
H20

Protocol:

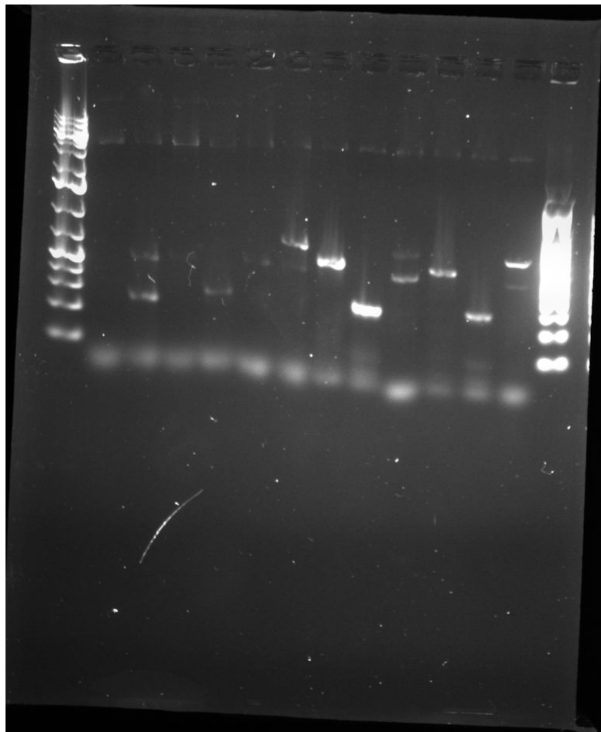
1. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 1 was added to tube 1.
2. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 2 was added to tube 2.
3. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 3 was added to tube 3.
4. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 4 was added to tube 4.
5. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 5 was added to tube 5A.
6. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 6 was added to tube 6.
7. Ten microliters of PCR supermix, 7 µL water, 1 µL of pGEX 5' primer, 1 µL of pGEX 3' primer and 1 µL of HCG in pGEX colony 7 was added to tube 7A.
8. Ten microliters of PCR supermix, 7 µL water, 1 µL of HCG for pGEX reverse' primer, 1 µL of HCG for pGEX forward primer and 1 µL of HCG in pGEX colony 5 was added to tube 5B.
9. Ten microliters of PCR supermix, 7 µL water, 1 µL of HCG for pGEX reverse' primer, 1 µL of HCG for pGEX forward primer and 1 µL of HCG in pGEX colony 7 was added to tube 7B.
10. Ten microliters of PCR supermix, 7 µL water, 1 µL of Beta 3 loop with biobrick prefix primer, 1 µL of Beta 3 loop with biobrick suffix primer and 1 µL of HCG in pGEX colony 5 was added to tube 5C.

11. Ten microliters of PCR supermix, 7 µL water, 1 µL of Beta 3 loop with biobrick prefix primer, 1 µL of Beta 3 loop with biobrick suffix primer and 1 µL of HCG in pGEX colony 7 was added to tube 7C.
12. Tubes 5B, 5C, 7B and 7C were placed in the thermocycler (Block B) with the protocol
    1. 94° C for 3:00 minutes
    2. 94° C for 1:00 minute
    3. 56° C for 0:45 seconds
    4. 72° C for 1:00 minute
    5. 35X (Go to Step 2)
    6. 72° C for 2:00 minutes

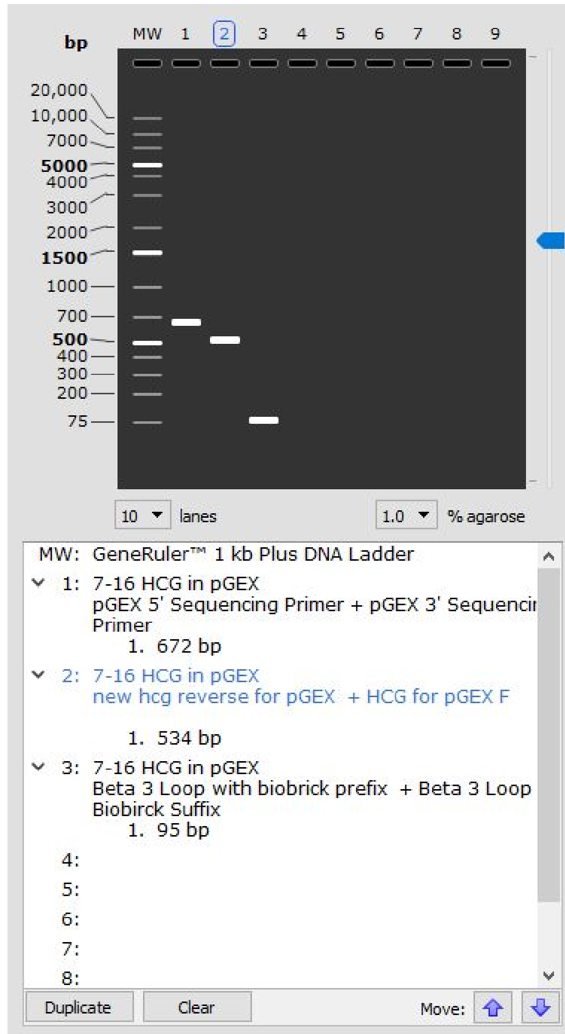
    Lid Temperature: 105° C

13. Tubes 1, 2, 3, 4, 5A, 6, and 7A were placed in the other thermocycler with the protocol

Results



1. GeneRuler 1kb plus ladder
2. PCR tube 1
3. PCR tube 2
4. PCR tube 3
5. PCR tube 4
6. PCR tube 6
7. PCR tube 5A
8. PCR tube 5B
9. PCR tube 5C
10. PCR tube 7A
11. PCR tube 7B
12. PCR tube 7C
13. PCR tube 5A
14. Bullseye 100bp ladder



Conclusion

Colony 5 matches up perfectly with the simulated gel in SnapGene and we are confident that it contains HCG in pGEX and the ligation was successful. The next step will be to do a mass overnight culture of about 100 mL and perform a midi prep to extract as much plasmid DNA as we can.

Name: Christina Clodomir, Mary Gonzalez

Date: 7/16/18

Goal: To re-do transformations for HCG in PSB1C3 and Beta Loop in PSB1C3 because we didn't have any growth on plates "1a, 1b plates as well as plate 3".

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.
7. This was repeated for each of the samples of ligated DNA.

There were two trials for HCG in pGEX, two for HCG in pSB1C3, and one trial for Beta 3 Loop HCG in pSB1C3

Streaking Plates

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 minutes
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

The plates showed no growth

Conclusion

Something is wrong with the pSB1C3 plasmid so we will redo the ligations and transformations with pSB1C3 from a new source.

Name: Rehmat Babar

Date: 7/17/18

Goal: Amplify Beta 3 Loop for pGEX

Materials

HCG for pGEX PCR from 9/22/17

Thermo Scientific PCR Master Mix (2X) Lot 00391316

1:10 Dilution of Beta 3 Loop primer for pGEX F

1:10 Dilution of Beta 3 Loop Biobrick pGEX R

Protocol

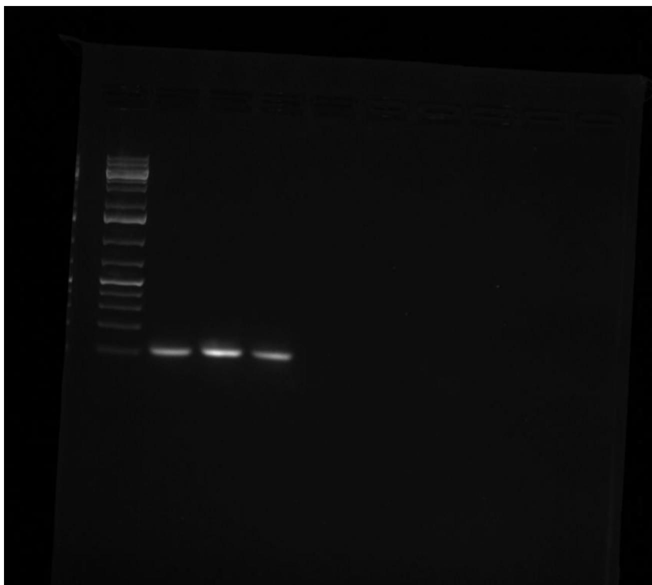
Beta 3 Loop PCR

1. A PCR tube was filled with 7 µL of water, 10 µL of PCR Master Mix (2X), 1µL of 1:10 Dilution of Beta 3 Loop primer for pGEX F, 1 µL 1:10 Dilution of Beta 3 Loop Biobrick pGEX R, and 1 µL of HCG for pGEX PCR from 9/22/17.
2. The PCR tubes were placed in the thermocycler at the following settings:

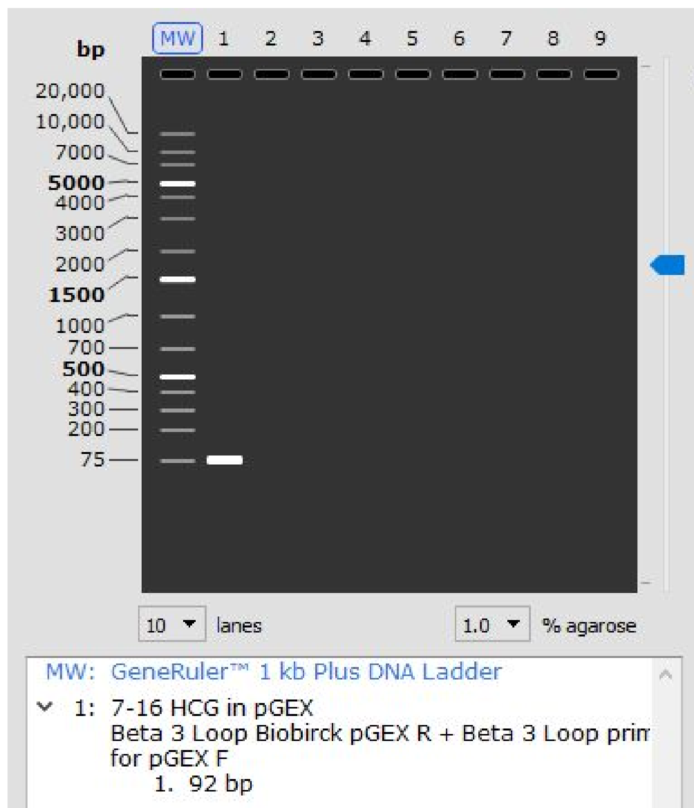
    1. 94° C for 3:00 minutes
    2. 94° C for 1:00 minute
    3. 56° C for 0:45 seconds
    4. 72° C for 1:00 minute
    5. 35X (Go to Step 2)
    6. 72° C for 2:00 minutes

    Lid Temperature: 105° C

Results



1. GeneRuler 1kb plus ladder
2. Beta 3 Loop in pGEX Trial 1
3. Beta 3 Loop in pGEX Trial 2
4. Beta 3 Loop in pGEX Trial 3



Conclusion: The bands are located at the same spot as they were shown on the Snapgene Simulation.

Name: Christina Clodomir, Mary Gonzalez

Goal: To create the appropriate sticky ends to pSB1C3 to get ready for ligation.

Materials:

Thermo Scientific 10X Fast Digest Buffer

Thermo Scientific Fast Digest EcoRI Lot#00246951

Thermo Scientific Fast Digest PSTI Lot#00472716

PSB1C3 Linearized Plasmid Backbone (Green Cap Batch 003)

Protocol

1. Made a 10microllitrer reaction by doing the following in this specific order:

a. 3microL of water
b. 5microL of PSB1C3 backbone
c. 1microL of Fast Digest Buffer
d. .5 of EcoRI
e. .5 of PSTI

2. Incubated at 37degrees Celsius for 30 minutes
3. Heat Kill for 20 minutes at 75degrees celsius

Name: Rehmat Babar

Date: 7/18/18

Goal: PCR cleanup on the Beta 3 Loop for pGEX PCR done with the new primers on 7/17/18. Want to prepare the samples for restriction digests before the samples are ready for ligation.

Materials

Zymo Research DNA Clean & Concentrator - 5

Lot No: ZRC185825

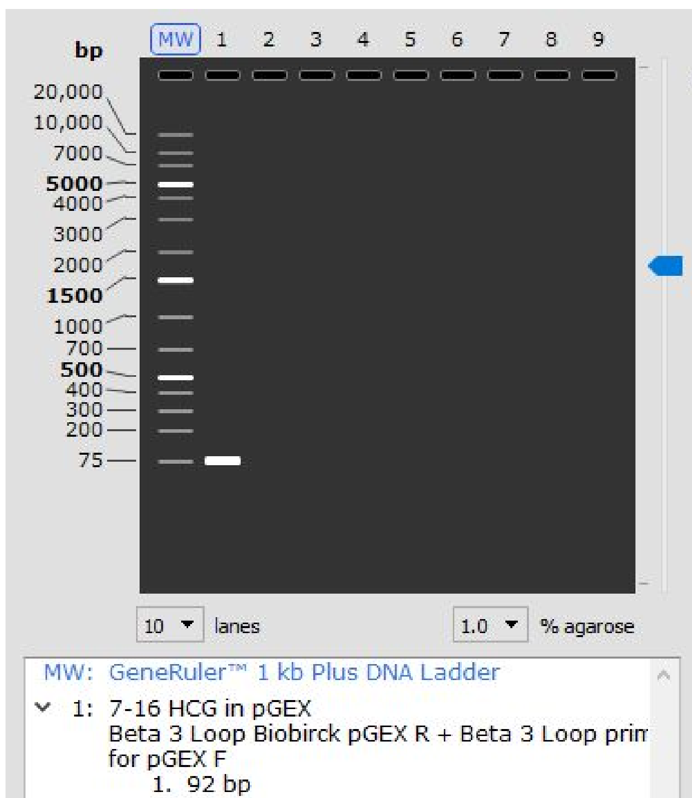
Trials 1,2,3 HCG Beta 3 Loop for pGEX PCR Product 7/17/18

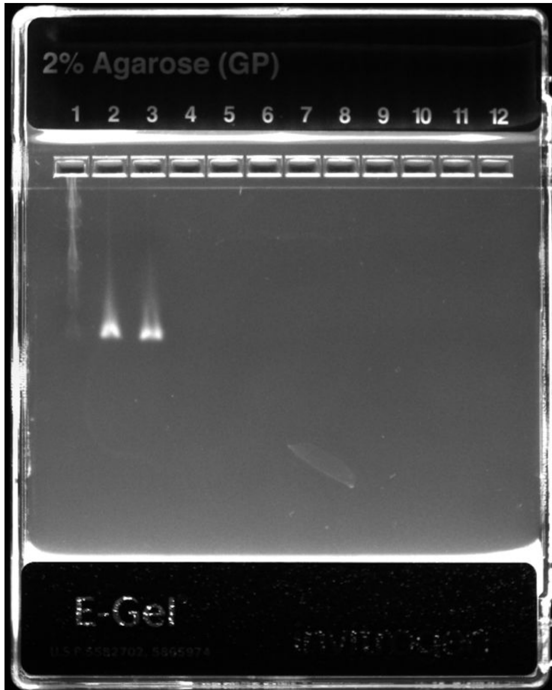
Protocol

1. The 3 tubes of HCG Beta 3 Loop for pGEX PCR Product 7/17/18 were combined in a 1.5 mL Eppendorf tube
2. 225 µL of DNA Binding Buffer was added and the tube was vortexed.
3. The sample was transferred to a Zymo-Spin Column in a collection tube
4. Centrifuged for 30 seconds and the flow through was discarded
5. 200 µL of DNA Wash Buffer was added to the column and centrifuged for 30 seconds.
6. Step 5 was repeated again and the flow through was discarded.
7. 30 µL of DNA Elution Buffer was added and the column was incubated at room temperature for 1 minute
8. The column was transferred to a 1.5 mL Eppendorf tube and was centrifuged for 30 seconds to elute the DNA.

Results

22.5 ng/µL of HCG Beta 3 Loop for PGEX





Key: For E-GEL

Lane 1: GeneRuler 1kb Plus DNA Ladder Lot# 00516263

Lane 2: Beta 3 loop for pGEX PCR cleanup

Lane 3: Beta 3 Loop for pGEX PCR Cleanup

*Dye used: Purple Gel loading Dye (6x) New England Labs

Conclusion

Although the concentration was relatively low, I will continue to use this PCR cleanup product to do a restriction digest and ligation, since bright bands are still being produced and there is enough DNA present to work with.

Name: Rehmat Babar

Date: 7/18/18

Goal: Do a restriction digest on the PCR Cleanup from earlier to make the ends compatible to be put into pGEX. The pGEX vector was cut with EcoRI and NotI. Then a heat kill will be done on the restriction digest products in order to denature the enzymes so that the product is ready for ligation.

Materials

Thermo Scientific FastDigest NotI Lot 00537736

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific 10X FastDigest Buffer Lot 00350512

Beta 3 Loop for pGEX PCR Cleanup Product from the previous entry

Protocol

20 µL Restriction Digest on PCR Cleanup Product (Beta 3 Loop for pGEX)

a. Beta 3 Loop for pGEX

16 µL of Beta 3 Loop for pGEX PCR Cleanup Product

1 µL EcoRI

1 µL NotI

2 µL of FastDigest Buffer

Heat Kill

a. The restriction digests were placed in the water bath at 60° C for 20 minutes in order to denature the enzymes so that the PCR cleanup product will be ready for ligation.

Name: Rehmat, Julia, Christina, Yash

Date: 7/18/2018

Goal: Put the HCG parts in the chosen plasmids by doing a ligation on the recombinant HCG Beta unit with the pSB1C3 plasmid and on the Beta 3 Loop with plasmid pSB1C3 and pGEX.

Materials:

Thermo Scientific T4 DNA Ligase Lot 00398345

Thermo Scientific T4 DNA Ligase Buffer

pSB1C3 digested with EcoRI and PstI

pGEX digested with EcoRI and NotI

Recombinant HCG beta subunit PCR Cleanup

Beta 3 Loop for pGEX PCR Cleanup/Restriction Digest with EcoRI and NotI

Beta 3 Loop for pSB1C3 PCR Cleanup/Restriction Digest with EcoRI and PstI

Water

Protocol:

1. Beta 3 Loop in pGEX
    a. 6 µLof water was added to the tube, then 1µL of the T4 DNA Ligase Buffer, followed by 1µL of the plasmid and 1µL of the Beta 3 Loop for pGEX
2. Beta 3 Loop in pSB1C3
    a. 6 µLof water was added to the tube, then 1µL of the T4 DNA Ligase Buffer, followed by 1µL of the plasmid and 1µL of the Beta 3 Loop for pSB1C3
3. HCG Beta subunit in pSB1C3

    a. 6 µLof 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 Beta subunit for pSB1C3
4. The tube was mixed by pipet then incubated at room temperature for 10 minutes.

Name: Rehmat, Julia, Christina, Yash

Date: 7/18/18

Goal: To do a transformation using electrically competent DH5a E. Coli cells using the DNA that was ligated earlier: Beta 3 Loop in pSB1C3, Beta 3 Loop in pGEX, and Recombinant HCG Beta subunit in pSB1C3 will be used.

Materials

Ligated Beta 3 Loop in pSB1C3

Ligated Beta 3 Loop in pGEX

Ligated Recombinant HCG beta subunit in pSB1C3

SOC Medium

Invitrogen ElectroMAX DH5a Electrocompetent Cells Lot 1932992

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 (chloramphenicol for pSB1C3 and ampicillin for pGEX).
7. This was repeated for each of the samples of ligated DNA.

Tubes are labeled as follows:

B3 pGEX 1: B3 loop in pGEX ligation 7/18 YY

- 1A - trial 1
- 1B - trial 2

B3 pSB 2: B3 loop in pSB1C3 ligation 7/18 YY

- 2A - trial 1
- 2B - trial 2

HCG pSB 3: HCG in pSB1C3 ligation 7/18 YY

- 3A - trial 1
- 3B - trial 2

Results

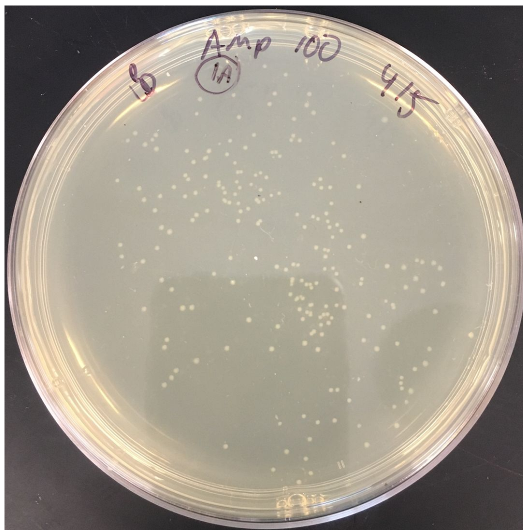


Plate 1A: Beta 3 Loop in pGEX

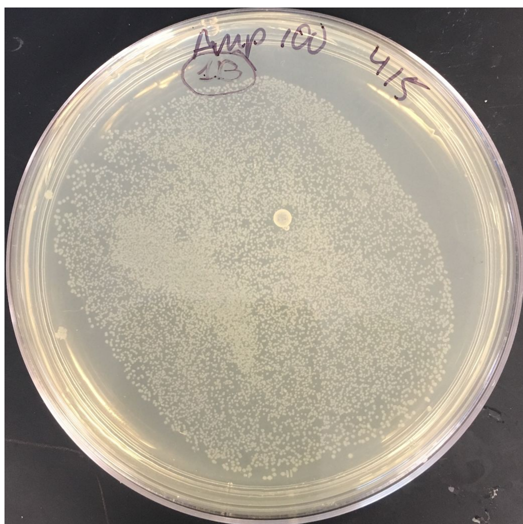


Plate 1B: Beta 3 Loop in pGEX

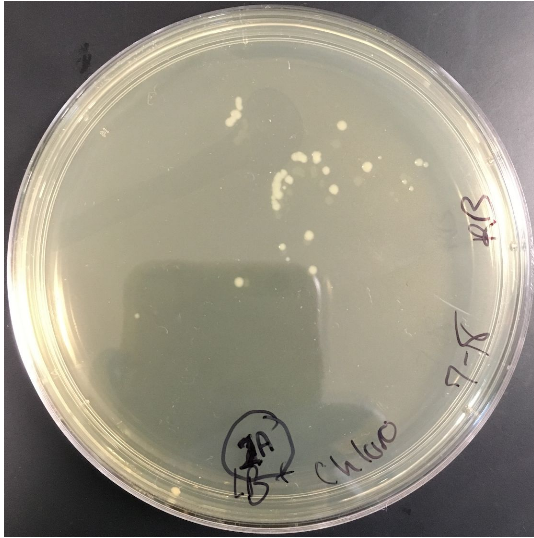


Plate 2A: Beta 3 Loop in pSB1C3

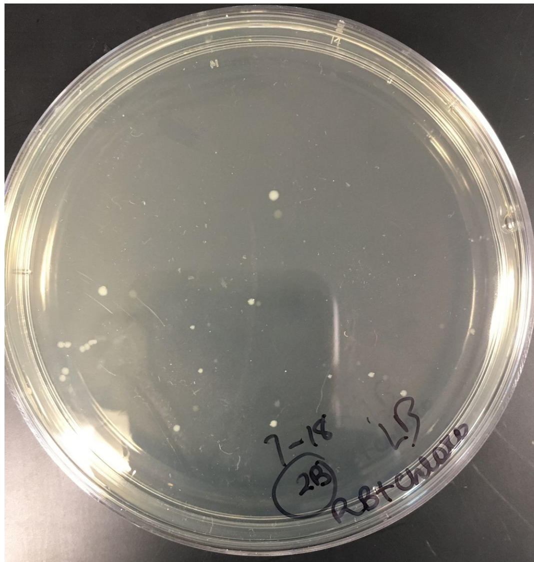


Plate 2B: Beta 3 Loop in pSB1C3

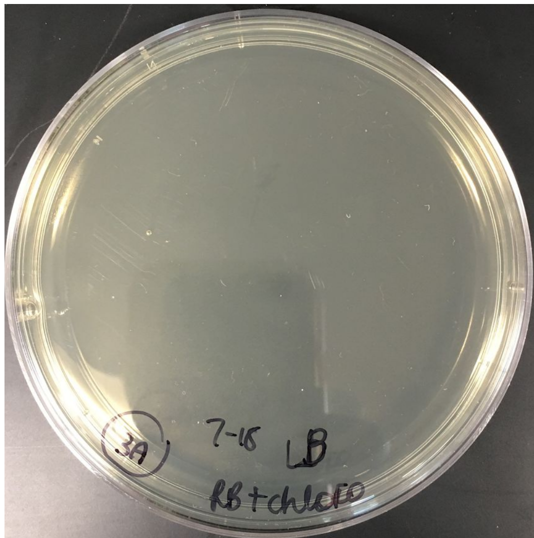


Plate 3A: HCG in pSB1C3

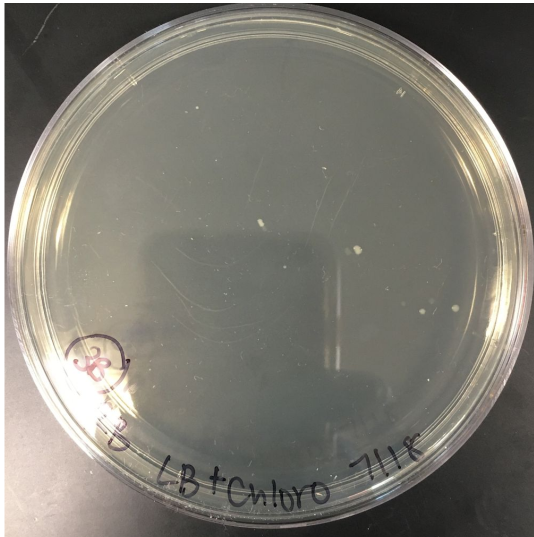


Plate 3B: HCG in pSB1C3

Conclusion

The plates have growth for all of the different ligations (Beta 3 Loop in pGEX, Beta 3 Loop in pSB1C3, and HCG in pSB1C3) which is a great sign. We will continue with these plates by picking at least 10 colonies, if possible, and perform colony PCR to see what each colony is.

Name: Julia, Yash, Christina, Rehmat

Date:7/18/18

Goal: Grow up more of the colony 5 HCG in pGEX to get it into cells for protein expression. A larger culture will be made for midipreps.

Materials:

Liquid LB

Ampicillin

HCG in pGEX Colony 5 glycerol stock

Protocol:

1. Twenty milliliters of LB was added to each of the five falcon tubes (100 mL used total) under a flame.
2. Twenty microliters of ampicillin was added to each tube.
3. A p10 tip was used to scrap the top of the colony 5 glycerol stock and placed into the tube with ampicillin and LB. This was done for each falcon tube.
4. The falcon tubes were incubated at 37 degrees for 18 hours.

Results: All the tubes were cloudy after 18 hours.

Conclusion: A midiprep will be performed to isolate the plasmid DNA.

Name: Christina Clodomir...

Goal: PCR HCG for PSB1C3 (AMPLIFY HCG so that we can cut with XbaI and SpeI)

Protocol:

A PCR tube was filled with 7 μL of water, 10 μL of PCR Master Mix (2X), 1μL of 1:10 Dilution of Beta 3 Loop w/ biobrick prefix, 1 μL 1:10 Dilution of Beta 3 Loop w/ biobrick suffix, and 1 μL of b3 loop for PSB1C3 PCR cleanup.

A PCR tube was filled with 7microliters water, 10 microliters of PCR Master Mix (2x), 1microliter of 1:10 Dilution of HCG suffix, 1 microliter of 1:10 dilution of hcg prefix, and 1 microliter of the HCG for PSB1C3 PCR cleanup.

Tubes 1-2 are Full length HCG for PSB
Tubes 4-6 are b3 loop for PSB

ANNEALING TEMP FOR B3 PREFIX PRIMER IS 59 AND SUFFIX PRIMER IS 58-59

ANNEALING TEMP FOR HCG PREFIX IS 58 AND SUFFIX PRIMER IS 57

For Thermocycler for B3 loop in block A:

1. 94° C for 3:00 minutes
2. 94° C for 1:00 minute
3. 57° C for 0:45 seconds
4. 72° C for 1:00 minute
5. 35X (Go to Step 2)
6. 72° C for 2:00 minutes

Lid Temperature: 105° C

For Thermocycler for HCG in block B:

1. 94° C for 3:00 minutes
2. 94° C for 1:00 minute
3. 56° C for 0:45 seconds
4. 72° C for 1:00 minute
5. 35X (Go to Step 2)
6. 72° C for 2:00 minutes

Lid Temperature: 105° C

Results

In the gels below with the colony PCR

Conclusion

We got primer dimers for all of the PCR and did not amplify HCG for pSB1C3 or Beta 3 Loop for pSB1C3, this could be due to incorrect annealing temperatures or too much primer. The PCR will be run again with different settings and amounts to amplify the desired sequence correctly.

Name: Rehmat, Julia, Mary

Date: 7/19/18

Goal: Colony PCR from the plates containing the Beta 3 Loop in pGEX, Beta 3 Loop in pSB1C3, and Recombinant HCG in pSB1C3 and want to amplify the DNA to see if the transformation was done correctly. This will help in deciding which colonies will have overnight cultures started for them.

Materials

Thermo Scientific PCR Master Mix (2X) Lot 00391316

Beta 3 Loop Biobrick pGEX R 10 µM

Beta 3 Loop primer for pGEX F 10 µM

Beta 3 loop Biobrick Suffix

Beta 3 loop with Biobrick

New HCG Suffix

HCG new Prefix Primer 1

Thermo Scientific Water, nuclease-free Lot 00598298

Protocol

1. Colony PCR for Beta 3 Loop in pGEX (Plates 1A and 1B)

    10 colonies were picked from each plate

    1. Prepare a 21X concentration cocktail, the PCR will be 20 µL.
        a. The cocktail will include: 210 µL Thermo Scientific PCR Master Mix (2X), 147 µL Thermo Scientific Water, nuclease-free, 21 µL Beta 3 Loop primer for pGEX F, and 21 µL Beta 3 Loop Biobrick pGEX R.
    2. 19 µL of the cocktail was added to the 20 different PCR tubes.
    3. Then a p10 tip was dipped into the picked colonies and was swirled around in 10 µL of di water and vortexed so that there are 14 different tubes containing water and the colonies. Due to the small size of the colonies this was done to ensure the same exact colony will be used for the colony PCR and the overnight cultures

4. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes.
5. All of the tubes containing cocktail A had the following PCR settings:

   1. 95° C for 3:00 minutes

   2. 95° C for 1:00 minutes

   3. 55° 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

2. Colony PCR for Beta 3 Loop in pSB1C3 (Plates 2A and 2B)

   10 colonies were picked from each plate

6. Prepare a 22X concentration cocktail, the PCR will be 20 µL.
   a. The cocktail will include: 220 µL Thermo Scientific PCR Master Mix (2X), 154 µL Thermo Scientific Water, nuclease-free, 22 µL Beta 3 loop with Biobrick, and 22 µL Beta 3 loop Biobrick Suffix.
7. 19 µL of the cocktail was added to the 20 different PCR tubes.
8. Then a p10 tip was dipped into the picked colonies and was swirled around in 10 µL of di water so that there are 14 different tubes containing water and the colonies. Due to the small size of the colonies this was done to ensure the same exact colony will be used for the colony PCR and the overnight cultures
9. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes.
10. All of the tubes containing cocktail A had the following PCR settings:

   1. 95° C for 3:00 minutes

   2. 95° C for 1:00 minutes

   3. 55°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

3. Colony PCR for Recombinant HCG Beta Subunit in pSB1C3 (Plate 3B)

4 colonies were picked from plate 3B, there was no growth on plate 3A

11. Prepare a 5X concentration cocktail, the PCR will be 20 µL.
    a. The cocktail will include: 50 µL Thermo Scientific PCR Master Mix (2X), 35 µL Thermo Scientific Water, nuclease-free, 5 µL New HCG Suffix, and 5 µL HCG new Prefix Primer 1.
12. 19 µL of the cocktail was added to the 20 different PCR tubes.
13. Then a p10 tip was dipped into the picked colonies and was swirled around in 10 µL of di water so that there are 14 different tubes containing water and the colonies. Due to the small size of the colonies this was done to ensure the same exact colony will be used for the colony PCR and the overnight cultures
14. 1 µL of each of the colony solution was added to the respectively labeled PCR tubes.
15. All of the tubes containing cocktail A had the following PCR settings:

    1. 95° C for 3:00 minutes

    2. 95° C for 1:00 minutes

    3. ° 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

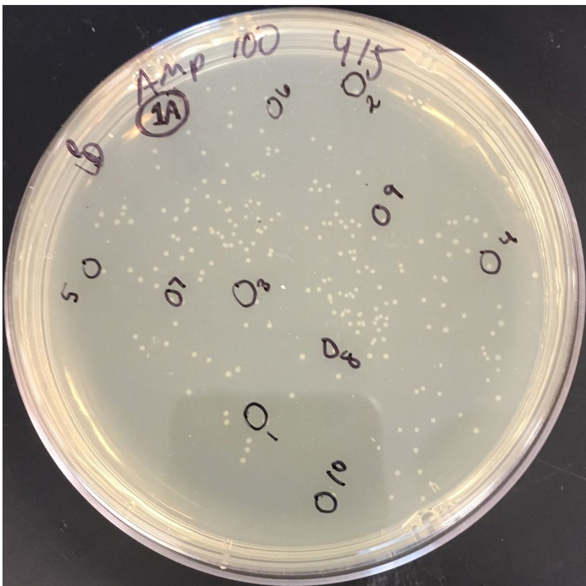


Plate 1A: Beta 3 Loop in pGEX 10 Colonies

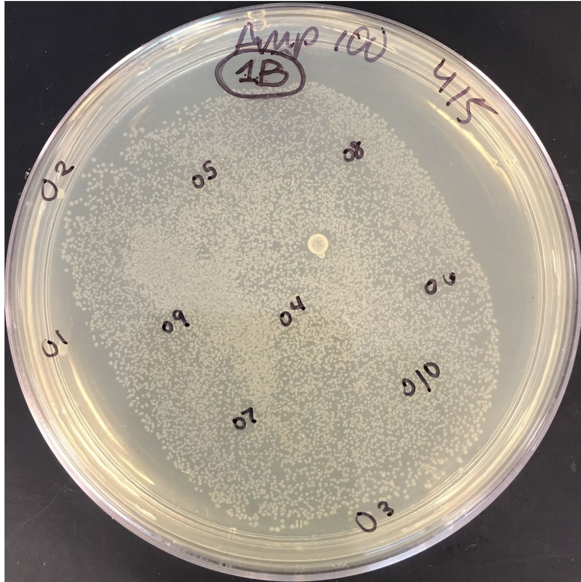


Plate 1B: Beta 3 Loop in pGEX 10 Colonies

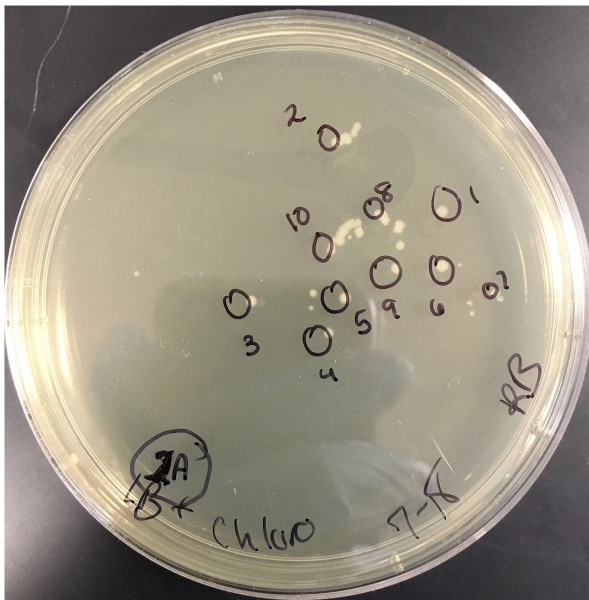


Plate 2A: Beta 3 Loop in pSB1C3 10 Colonies

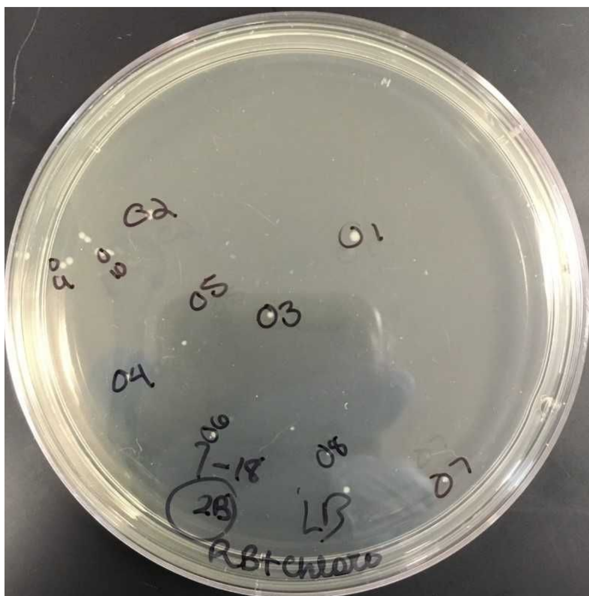
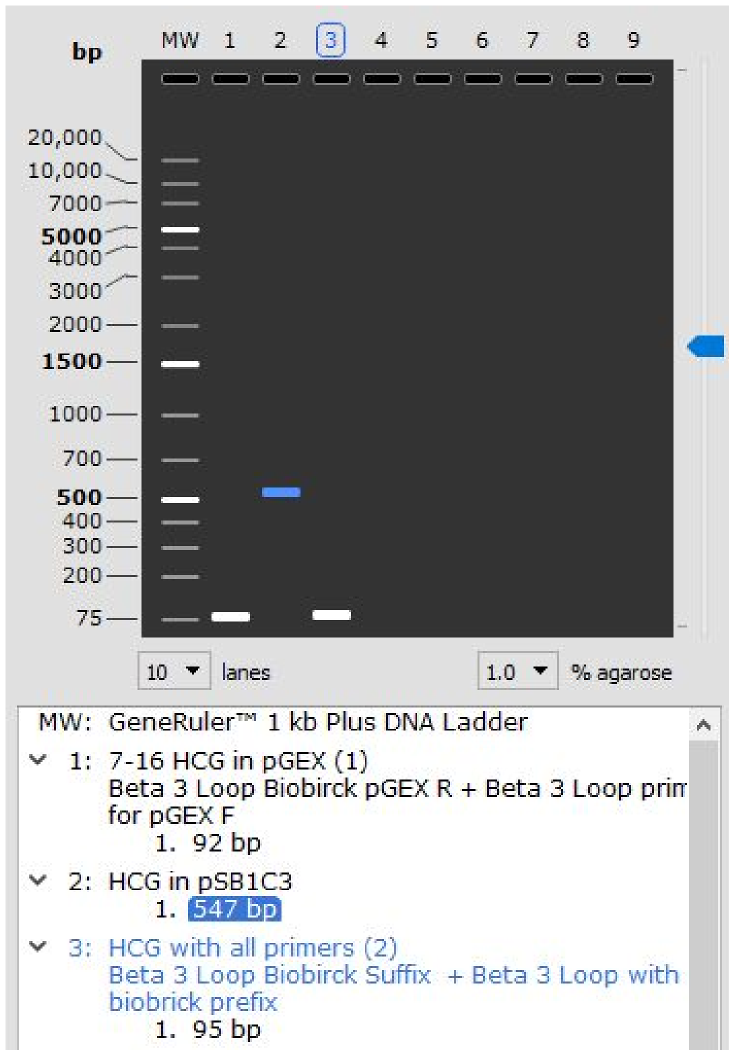


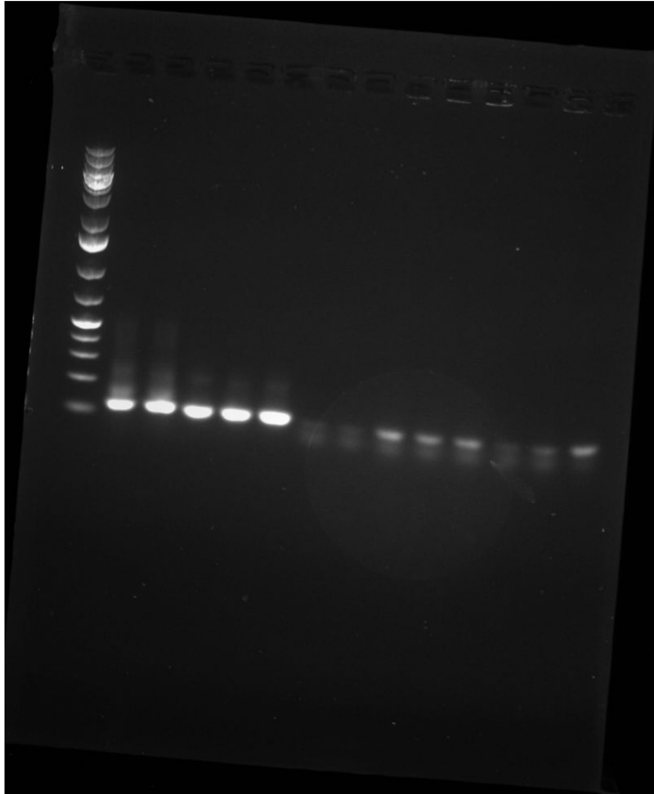
Plate 2B: Beta 3 Loop in pSB1C3 10 Colonies

There was no growth on plate 3A so no colonies were picked for that transformation.

Results

Expected Results Simulated in SnapGene





Lane 1: GeneRuler 1 kb Plus DNA Ladder

Lane 2: 1 HCG for pSB1C3

Lane 3: 2 HCG for pSB1C3

Lane 4: 4 Beta 3 Loop for pSB1C3

Lane 5: 5 Beta 3 Loop for pSB1C3

Lane 6: 6 Beta 3 Loop for pSB1C3

Lane 7: Plate 1A Colony 1

Lane 8: Plate 1A Colony 2

Lane 9: Plate 1A Colony 3

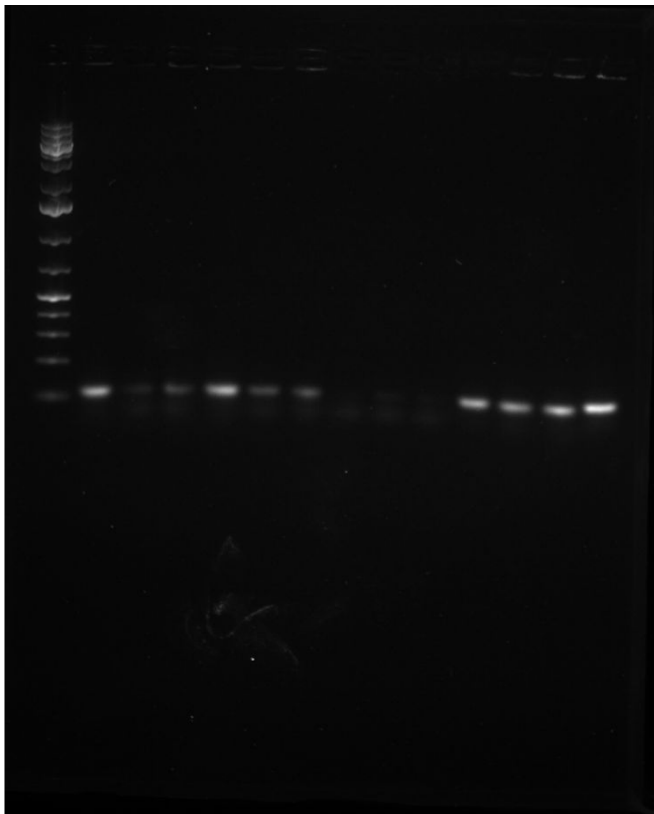
Lane 10: Plate 1A Colony 4

Lane 11: Plate 1A Colony 5

Lane 12: Plate 1A Colony 6

Lane 13: Plate 1A Colony 7

Lane 14: Plate 1A Colony 8



Lane 1: GeneRuler 1 kb Plus DNA Ladder

Lane 2: Plate 1A Colony 9

Lane 3: Plate 1A Colony 10

Lane 4: Plate 1B Colony 1

Lane 5: Plate 1B Colony 2

Lane 6: Plate 1B Colony 3

Lane 7: Plate 1B Colony 4

Lane 8: Plate 1B Colony 5

Lane 9: Plate 1B Colony 6

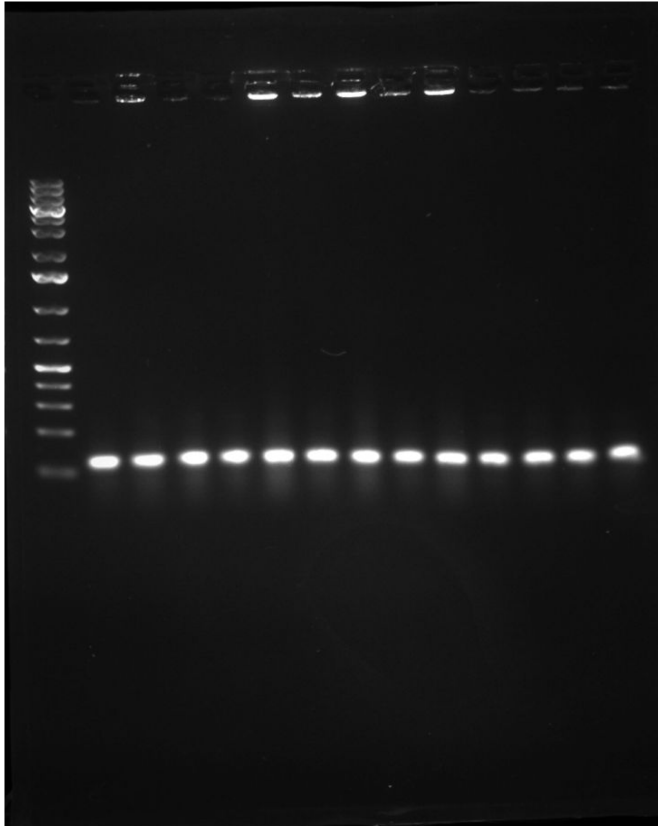
Lane 10: Plate 1B Colony 7

Lane 11: Plate 1B Colony 8

Lane 12: Plate 1B Colony 9

Lane 13: Plate 1B Colony 10

Lane 14: Plate 2A Colony 1



Lane 1: GeneRuler 1 kb Plus DNA Ladder

Lane 2: Plate 2A Colony 2

Lane 3: Plate 2A Colony 3

Lane 4: Plate 2A Colony 4

Lane 5: Plate 2A Colony 5

Lane 6: Plate 2A Colony 6

Lane 7: Plate 2A Colony 7

Lane 8: Plate 2A Colony 8

Lane 9: Plate 2A Colony 9

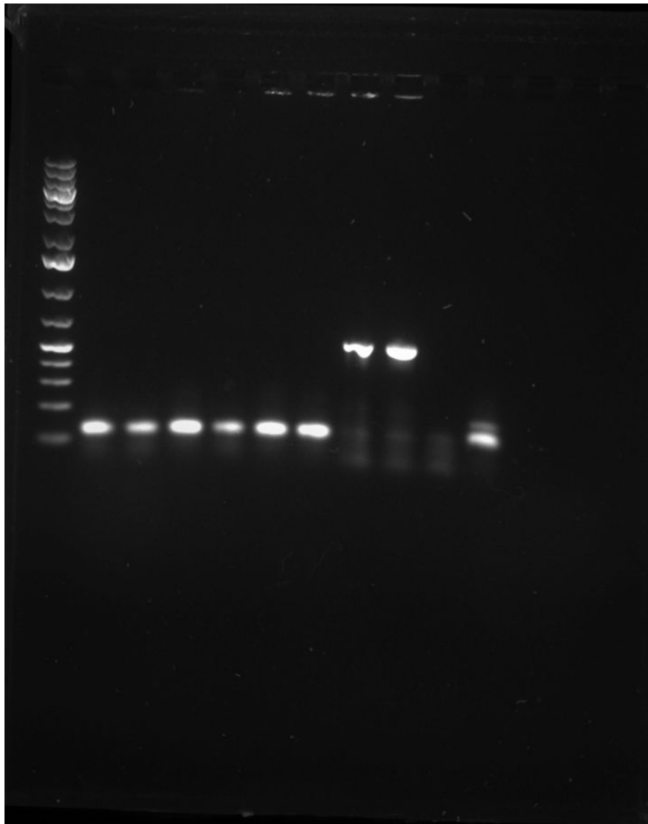
Lane 10: Plate 2A Colony 10

Lane 11: Plate 2B Colony 1

Lane 12: Plate 2B Colony 2

Lane 13: Plate 2B Colony 3

Lane 14: Plate 2B Colony 4



Lane 1: GeneRuler 1 kb Plus DNA Ladder

Lane 2: Plate 2B Colony 5

Lane 3: Plate 2B Colony 6

Lane 4: Plate 2B Colony 7

Lane 5: Plate 2B Colony 8

Lane 6: Plate 2B Colony 9

Lane 7: Plate 2B Colony 10

Lane 8: Plate 3B Colony 1

Lane 9: Plate 3B Colony 2

Lane 10: Plate 3B Colony 3

Lane 11: Plate 3B Colony 4

Conclusion

As far as the first gel goes, colonies 3, 4, 5, and 8 look good for plate 1A and everything else besides those colonies and wells 2-6 seem to just not have enough DNA present. Wells 2-6 appear to be primer dimers where maybe there was too much primer present or the annealing temperatures were not run at the optimal settings. For the second gel, colony 9 for plate 1A looks good and colonies 2, 8, 9, and 10 for plate 1B look good and colony 1 for plate 2A looks good as well. They all appear to have bright bands at the correct spots. One concern is that the size of the primer dimers and the Beta 3 Loop seem to be very close so it is possible that one be taken for another. As of right now we are are confident that the bands present at the wells mentioned above are the Beta 3 Loop but further diagnostics and experiments are necessary to verify. The third gel looks perfect, there is a single bright band exactly where Beta 3 Loop for pSB1C3 should be and the fourth gel looks good for the Beta 3 Loop as well. The last four wells are HCG in pSB1C3 and only the first two of the four are bright and are located at the correct band size. We will move forward and start overnight cultures of each of the colonies that were correctly placed and we will do mini preps on all of the overnight cultures to extract the plasmid DNA.

Name: Rehmat Babar

Date: 7/19/18

Goal: Midi preps on the mass culture of colony 5 HCG in pGEX to extract plasmid DNA

Materials

QIAGEN Plasmid Midi Kit Lot 148044575

Buffer EB Elution Buffer Lot 160012298

Colony 5 of HCG in pGEX overnight culture

Protocol

Midi Prep on Colony 5 of HCG in pGEX

1. 100 mL of bacterial overnight culture distributed in 5 separate 50 mL falcon tubes was centrifuged at 6,000 rpm for 15 minutes at 4°C.
2. The supernatant was discarded from each of the tubes.
3. 4 mL of Buffer P1 was added to one tube, pipet mixed, and transferred to another tube. The contents of that tube was mixed and transferred to the next tube with pelleted cells. This was repeated until all of the cells from the tubes were combined.
4. 4 mL of Buffer P2 was added to the one tube containing 4 mL of Buffer P1 and the resuspended pelleted cells and the tube was vigorously inverted 6 times.
5. The tube was incubated at room temperature for 3 minutes

6. 4 mL of Buffer P3 was added to the tube and the tube was vigorously inverted 10 times.
7. The tube was allowed to incubate on ice for 15 minutes.
8. The tube was centrifuged at 20,000 x g at 4°C for 30 minutes.
9. Once it was centrifuged, the clear supernatant was transferred to another centrifuge tube while avoiding all of the flakes on the sides and in the solution.
10. The tube was centrifuged again at 20,000 x g at 4°C for 15 minutes and while this ran the QIAGEN-tip was equilibrated by adding 4 mL of QBT to the QIAGEN-tip.
11. All of the clear solution was added to the QIAGEN-tip and allowed to enter the resin by gravity flow
12. Next, 10 mL of Buffer QC was added to the QIAGEN-tip and allowed to gravity drip down.
13. Once that had passed through, 10 mL more of Buffer QC was added and allowed to flow through.
14. Then, 5 mL of Buffer QF was added and allowed to flow through.
15. 3.5 mL of room temperature of isopropanol was added to elute the DNA and mixed and then centrifuged for 15,000 x g for 30 minutes at 4°C.
16. The supernatant was carefully taken out, making sure not to disrupt the clear pellet.
17. 2 mL of room-temperature 70% ethanol was added and was centrifuged for 10 minutes at 15,000 x g at 4°C and the supernatant was discarded leaving as little liquid behind as possible, careful not to disrupt the clear pellet.
18. The pellet was air-dried for 20 minutes and redissolved in 100 μL of Buffer EB.

<u>Note: Anytime the centrifuge was loaded, the samples were balanced with water with an equal mass.</u>

Results

Sample	Concentration
Colony 5 of HCG in pGEX	215 ng/μL

Conclusion

The final concentration of the MidiPrep of colony 5 of HCG in pGEX was in a great range and we will use it in further experiments moving forward.

Name: Rehmat Babar and Christina Clodomir

Date: 7/24/18

Goal: Start overnight cultures for each of the colonies from each of the plates that showed bright and correctly placed bands on the colony PCR. Then mini preps will be done tomorrow and eventually a restriction digest to verify the ligation was successful.

Materials

LB

Chloramphenicol

Ampicillin

Colony solutions prepared on 7/19/18

Protocol

1. Overnight Cultures of Beta 3 in pGEX
    a. 5 mL of LB and 5 μL of Ampicillin was added into 12 different falcon tubes and 2 μL of the respective colony solution prepared on 7/19/18 was added along with the p10 tip used to pipette into the tube.
2. Overnight Cultures of Beta 3 in pSB1C3
    a. 5 mL of LB and 5 μL of Chloramphenicol was added into 12 different falcon tubes and 2 μL of the respective colony solution prepared on 7/19/18 was added along with the p10 tip used to pipette into the tube.
3. Overnight Cultures of HCG in pSB1C3
    a. 5 mL of LB and 5 μL of Chloramphenicol was added into 12 different falcon tubes and 2 μL of the respective colony solution prepared on 7/19/18 was added along with the p10 tip used to pipette into the tube.
4. All of the tubes were incubated for 18 hours at 37°C at 220 rpm.

Results

All overnights were cloudy.

Conclusion

Mini preps will be done tomorrow.

Name: Rehmat Babar, Christina Clodomir

Date: 7/25/18

Goal: Want to extract plasmid DNA from each of the picked colonies that showed the correct band size from the colony PCR. After the plasmid DNA is recovered we will perform a restriction digest and run a diagnostic gel which will hopefully show two bands which will be the plasmid and the insert separated out.

Materials

QIAprep Spin Miniprep Kit Lot 157020506

Overnight cultures started on 7/24/18

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	Concentration
Plate 1A Beta 3 Loop in pGEX Colony 3	too low
Plate 1A Beta 3 Loop in pGEX Colony 4	too low
Plate 1A Beta 3 Loop in pGEX Colony 5	too low
Plate 1A Beta 3 Loop in pGEX Colony 8	too low
Plate 1A Beta 3 Loop in pGEX Colony 9	too low
Plate 1B Beta 3 Loop in pGEX Colony 1	too low
Plate 1B Beta 3 Loop in pGEX Colony 2	too low
Plate 1B Beta 3 Loop in pGEX Colony 3	too low
Plate 1B Beta 3 Loop in pGEX Colony 4	too low
Plate 1B Beta 3 Loop in pGEX Colony 8	too low
Plate 1B Beta 3 Loop in pGEX Colony 9	too low
Plate 1B Beta 3 Loop in pGEX Colony 10	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 1	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 2	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 3	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 4	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 5	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 6	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 7	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 8	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 9	too low
Plate 2A Beta 3 Loop in pSB1C3 Colony 10	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 1	too low

Plate 2B Beta 3 Loop in pSB1C3 Colony 2	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 3	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 4	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 5	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 6	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 7	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 8	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 9	too low
Plate 2B Beta 3 Loop in pSB1C3 Colony 10	too low
Plate 3B Recombinant HCG Beta subunit in pSB1C3 Colony 1	too low
Plate 3B Recombinant HCG Beta subunit in pSB1C3 Colony 2	too low

Conclusion

Concentrations were way too low. We had to redo overnight cultures with the colonies with the highest concentration.

Name: Escolivia Birungi, Christina Clodomir

Goal: Want to do another miniprep from the picked colonies that had the highest concentration from yesterday's miniprep (We re-did overnight cultures with the colonies that had the highest concentration). After the plasmid DNA is recovered we will perform a restriction digest and run a diagnostic gel which will hopefully show two bands which will be the plasmid and the insert separated out.

Materials

QIAprep Spin Miniprep Kit Lot 157020506

Overnight cultures started on 7/24/18

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	Concentration
Plate 1A Beta 3 Loop in pGEX Colony 3	175 ng/μL
Plate 1A Beta 3 Loop in pGEX Colony 4	153 ng/μL
Plate 1A Beta 3 Loop in pGEX Colony 5	188 ng/μL
Plate 1A Beta 3 Loop in pGEX Colony 8	148 ng/μL
Plate 1A Beta 3 Loop in pGEX Colony 9	123 ng/μL
Plate 1B Beta 3 Loop in pGEX Colony 1	175 ng/μL
Plate 1B Beta 3 Loop in pGEX Colony 2	208 ng/μL
Plate 1B Beta 3 Loop in pGEX Colony 9	158 ng/μL
Plate 2A Beta 3 Loop in pSB1C3 Colony 5	115 ng/μL
Plate 2A Beta 3 Loop in pSB1C3 Colony 7	50 ng/μL
Plate 2A Beta 3 Loop in pSB1C3 Colony 8	2.5 ng/μL
Plate 2A Beta 3 Loop in pSB1C3 Colony 10	5.0 ng/μL
Plate 2B Beta 3 Loop in pSB1C3 Colony 1	7.5 ng/μL
Plate 2B Beta 3 Loop in pSB1C3 Colony 2	10.0 ng/μL
Plate 2B Beta 3 Loop in pSB1C3 Colony 3	110 ng/μL
Plate 2B Beta 3 Loop in pSB1C3 Colony 4	82.5 ng/μL
Plate 3B Recombinant HCG Beta subunit in pSB1C3 Colony 1	10.0 ng/μL
Plate 3B Recombinant HCG Beta subunit in pSB1C3 Colony 2	7.5 ng/μL

Conclusion: Some colonies had really great concentrations. We must redo overnights for the HCG Beta subunit in pSB1C3 with higher volume. Total volume for each colony is 100mL. Midiprep will be done following day.

Name: Christina Clodomir
Date: 7/26/18

Goal: Cut the insert and vector (for B3 in PGEX) with EcoRI and NotI to further test to see if the ligation of the two together was successful. And to also cut the insert and vector (for B3 in PSB) with EcoRI and PstI to see if ligation was successful.

Materials:

Thermo Scientific FastDigest NotI Lot 00537736

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific FastDigest PstI Lot 00472716

Thermo Scientific 10X FastDigest Buffer Lot 00312007

Miniprep tubes from (7/26/18) 1A: 3,4,5,8,9. 1B:1,2,9. 2A: 5,7 2B: 3,4.

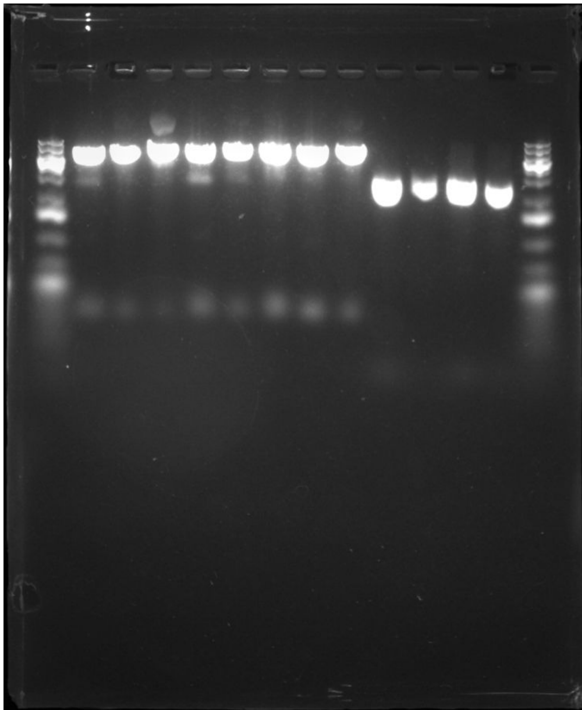
Protocol for Plates 1A/1B (B3 in PGEX):

1. A 20 µL reaction was prepared using a 9x cocktail. 9 microliters of NotI, 9 µL of EcoRI, 18µL of Fastdigest Buffer and 54 µL of water were mixed together.
2. Ten microliters of the cocktail was put into each of the 8 eppendorf tubes.
3. Ten microliters of each miniprep DNA (B3 in pGEX) from 7/26/18 was added to one of the tubes.
4. The samples were incubated at 37 degrees for 15 minutes.
5. After incubation, the samples were run on a gel.

Protocol for Plates 2A/2B (B3 in PSB):

6. A 20 µL reaction was prepared using a 5x cocktail. 5 microliters of PstI, 5 µL of EcoRI, 10µL of Fastdigest Buffer and 30 µL of water were mixed together.
7. Ten microliters of the cocktail was put into each of the 4 eppendorf tubes.
8. Ten microliters of each miniprep DNA (B3 in PSB) from 7/26/18 was added to one of the tubes.
9. The samples were incubated at 37 degrees for 15 minutes.
10. After incubation, the samples were run on a gel

Results:



Lane1:GeneRuler 1kb Plus DNA Ladder Lot# 00516263

Lane 2: 1A Colony 3,

Lane 3: 1A Colony 4,

Lane 4: 1A Colony 5,

Lane 5: 1A Colony 8

Lane 6: 1A Colony 9

Lane 7: 1B Colony 1,

Lane 8 1B Colony 2,

Lane 9: 1B Colony 9

Lane 10: 2A: Colony 5

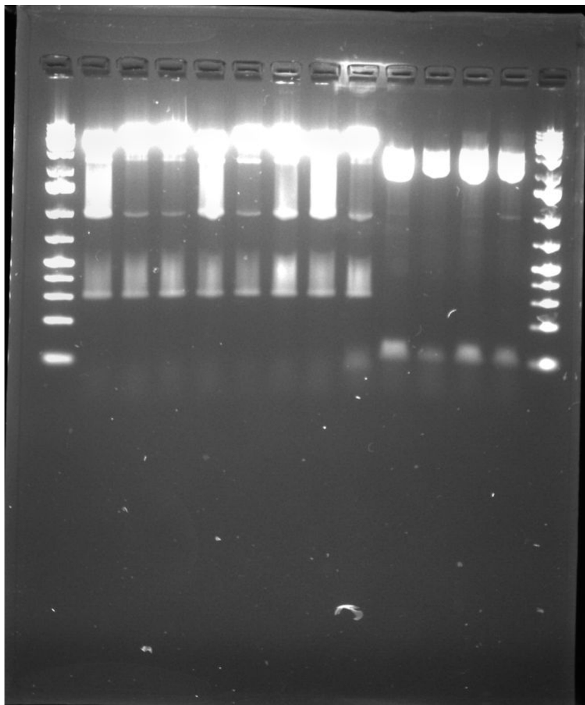
Lane 11: 2A Colony 7

Lane 12: 2B Colony 3

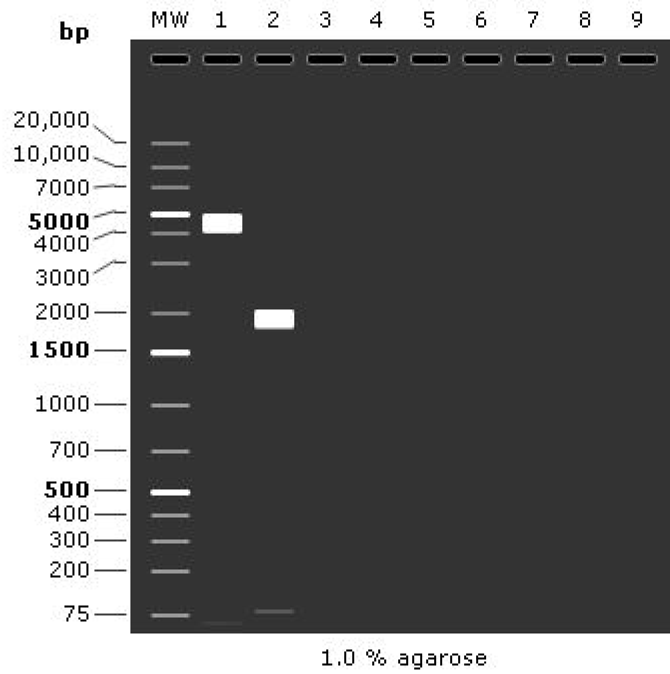
Lane 13: 2B Colony 4

Lane 14: GeneRuler 1kb Plus DNA Ladder

This gel was about 4 days old, so we made a new gel and ran the restriction digest product again with the same gel key as above.



Expected Results:



Conclusion: B3 in PSB have correct size bands. Looks like the ligation was successful so we will prepare the minipreps we did on 7/26/18 for sequencing to further verify.

Name: Rehmat Babar
Date: 7/27/18

Goal: Midi preps on the mass culture of colony 5 HCG in pSB1C3 to extract plasmid DNA at a high enough concentration.

Materials

QIAGEN Plasmid Midi Kit Lot 148044575

Buffer EB Elution Buffer Lot 160012298

Colonies 1 and 2 of HCG in pSB1C3 overnight culture from plate 3B

Protocol

Midi Prep on Colony 5 of HCG in pGEX

1. 100 mL of bacterial overnight culture distributed in 5 separate 50 mL falcon tubes was centrifuged at 6,000 rpm for 15 minutes at 4°C.
2. The supernatant was discarded from each of the tubes.
3. 4 mL of Buffer P1 was added to one tube, pipet mixed, and transferred to another tube. The contents of that tube was mixed and transferred to the next tube with pelleted cells. This was repeated until all of the cells from the tubes were combined.
4. 4 mL of Buffer P2 was added to the one tube containing 4 mL of Buffer P1 and the resuspended pelleted cells and the tube was vigorously inverted 6 times.
5. The tube was incubated at room temperature for 3 minutes
6. 4 mL of Buffer P3 was added to the tube and the tube was vigorously inverted 10 times.
7. The tube was allowed to incubate on ice for 15 minutes.
8. The tube was centrifuged at 20,000 x g at 4°C for 30 minutes.
9. Once it was centrifuged, the clear supernatant was transferred to another centrifuge tube while avoiding all of the flakes on the sides and in the solution.
10. The tube was centrifuged again at 20,000 x g at 4°C for 15 minutes and while this ran the QIAGEN-tip was equilibrated by adding 4 mL of QBT to the QIAGEN-tip.
11. All of the clear solution was added to the QIAGEN-tip and allowed to enter the resin by gravity flow
12. Next, 10 mL of Buffer QC was added to the QIAGEN-tip and allowed to gravity drip down.
13. Once that had passed through, 10 mL more of Buffer QC was added and allowed to flow through.
14. Then, 5 mL of Buffer QF was added and allowed to flow through.
15. 3.5 mL of room temperature of isopropanol was added to elute the DNA and mixed and then centrifuged for 15,000 x g for 30 minutes at 4°C.

16. The supernatant was carefully taken out, making sure not to disrupt the clear pellet.
17. 2 mL of room-temperature 70% ethanol was added and was centrifuged for 10 minutes at 15,000 x g at 4°C and the supernatant was discarded leaving as little liquid behind as possible, careful not to disrupt the clear pellet.
18. The pellet was air-dried for 20 minutes in the vent hood and redissolved in 100 μL of Buffer EB.

Note: Anytime the centrifuge was loaded, the samples were balanced with water with an equal mass.

Results

Sample	Concentration
Colony 1 of Recombinant HCG Beta subunit in pSB1C3	145 ng/μL
Colony 2 of Recombinant HCG Beta subunit in pSB1C3	343 ng/μL

Conclusion

The concentrations for the midipreps were in a great range, the concentration wasn't too high or too low. Next, we will perform a restriction digest to see if this plasmid DNA includes the HCG insert we inserted during ligation.

Name: Rehmat Babar, Yash Yadav

Date: 7/30/18

Goal: Restriction digest on the midi preps from 7/27/18 in order to separate out the vector and the insert and see if the ligation was successful.

Materials

Thermo Scientific 10X FastDigest Buffer Lot 00312007

Thermo Scientific FastDigest PstI Lot 00472716

Thermo Scientific FastDigest EcoRI Lot 00246951

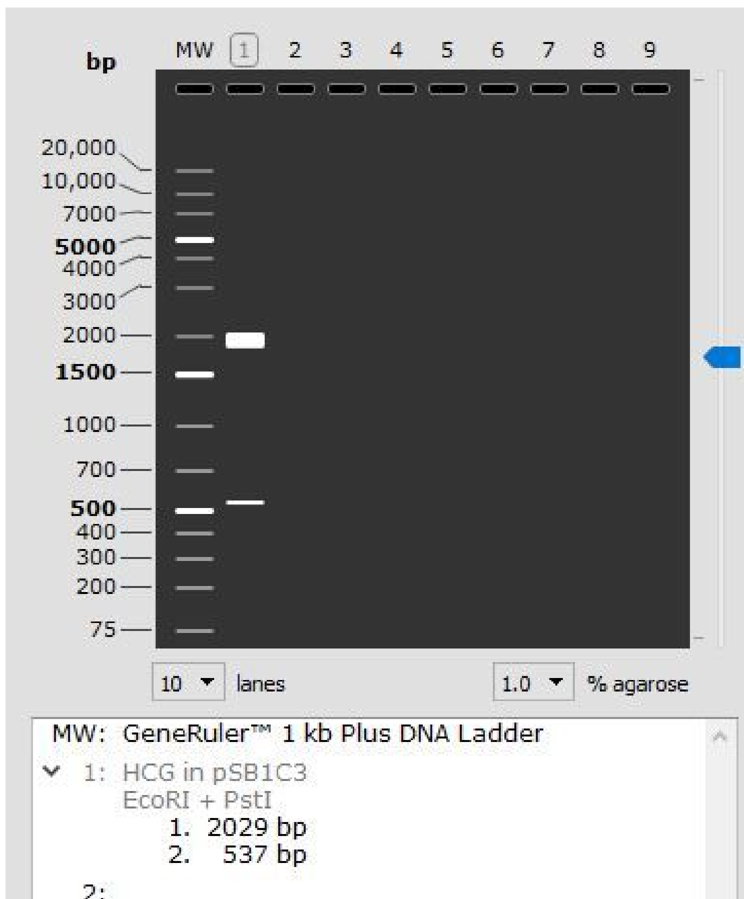
Midi preps from 7/27/18 Recombinant HCG Beta subunit Plate 3B Colony 1 and 2

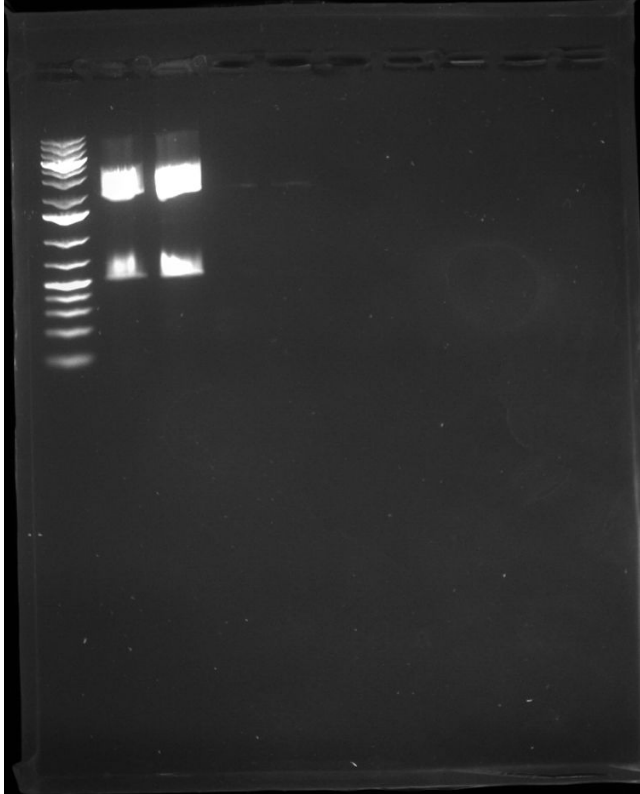
Protocol

Restriction Digest for Plates 3B (Recombinant HCG Beta subunit in pSB1C3)

1. A 30 µL reaction was prepared using a 3x cocktail. 3 µL of PstI, 3 µL of EcoRI, 9 µL of Fastdigest Buffer and 45 µL of water were mixed together.
2. 20 µLof the cocktail was put into the 2 eppendorf tubes.
3. 10 µL of each midiprep DNA (Recombinant HCG Beta subunit in pSB1C3) from 7/27/18 was added to the tubes.
4. The samples were incubated at 37 degrees for 30 minutes.
5. After incubation, the samples were run on a gel.

Results





Key

Lane 1: GeneRule 1 kb Plus DNA Ladder

Lane 2: RD Midi Prep Recombinant HCG Beta subunit Plate 3B colony 1

Lane 3: RD Midi Prep Recombinant HCG Beta subunit Plate 3B colony 2

Conclusion

The bands appear to be quite thick and spread out and bright but this may be due to the fact that we loaded the gel with 10 µL of the restriction digest reaction and since the concentrations of the midi preps were so high this caused the bands to be too thick and plus we loaded a relatively volume to begin with. However, the bands are placed correctly and the restriction digest successfully separated out the vector and the HCG insert. We believe the ligation was successful and this midiprep sample will be sent for sequencing to verify that we have successfully ligated HCG and pSB1C3.

Name: Christina Clodomir, Yash Yadav, Rehmat Babar
Date: 7/30/18

Goal: Cut the insert and vector (for B3 in PGEX) with BamHI/PstI and EcoRI/PstI to further test to see if the ligation of the two together was successful.

Materials:

Thermo Scientific FastDigest BamHI Lot

Thermo Scientific FastDigest EcoRI Lot 00246951

Thermo Scientific FastDigest PstI Lot 00472716

Thermo Scientific 10X FastDigest Buffer Lot 00312007

Miniprep tubes from (7/26/18) 1A: 3,4,5,8,9.   1B:1,2,9.

Protocol

Protocol for Plates 1A/1B (B3 in PGEX) CUTw/BAMHI+PSTI:

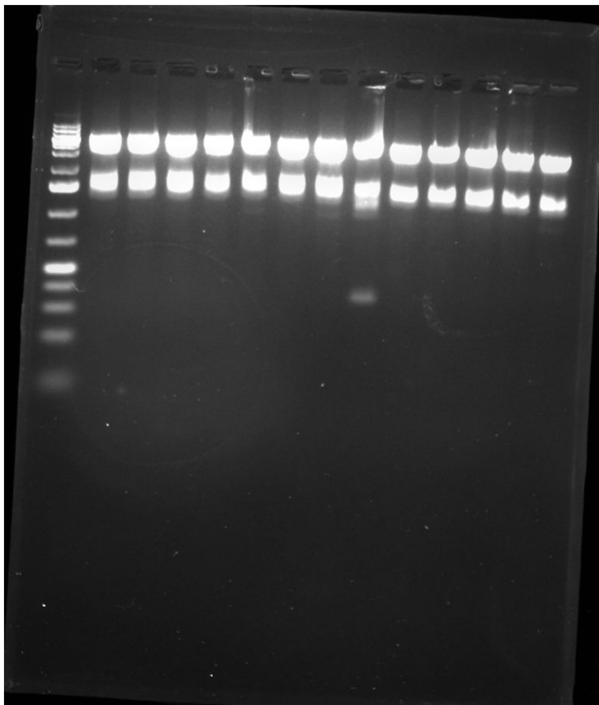
1. A 10 µL reaction was prepared using a 9x cocktail. 4.5 microliters of BamHI, 4.5 µL of PstI, 13.5µL of Fastdigest Buffer and 22.5µL of water were mixed together.
2. Five microliters of the cocktail was put into each of the 8 eppendorf tubes.
3. Five microliters of each miniprep DNA (B3 in pGEX) from 7/26/18 was added to one of the tubes.
4. The samples were incubated at 37 degrees for 15 minutes.
5. After incubation, the samples were run on a gel.

Protocol for Plates 1A/1B (B3 in PGEX) Cutw/EcoRI +PstI

6. A 10 µL reaction was prepared using a 9x cocktail. 4.5 microliters of EcoRI, 4.5 µL of PstI, 13.5µL of Fastdigest Buffer and 22.5µL of water were mixed together.
7. Five microliters of the cocktail was put into each of the 8 eppendorf tubes.
8. Five microliters of each miniprep DNA (B3 in pGEX) from 7/26/18 was added to one of the tubes.
9. The samples were incubated at 37 degrees for 30 minutes.
10. After incubation, the samples were run on a gel.

Results:

The Key for the gel is as follows:



Lane1:GeneRuler 1kb Plus DNA Ladder Lot# 00516263

Lane 2: 1A Colony 3,

Lane 3: 1A Colony 4,

Lane 4: 1A Colony 5,

Lane 5: 1A Colony 8

Lane 6: 1A Colony 9

Lane 7: 1B Colony 1,

Lane 8 1B Colony 2,

Lane 9: 1B Colony 9

Name: Rehmat Babar

Date: 7/31/18

Goal: Protein expression of recombinant HCG beta subunit.

Materials

HCG in pGEX colony 5 glycerol stock

LB + Amp

IPTG

Protocol

10 mL of HCG in pGEX colony 5 glycerol stock

1. 10 mL of LB and 10 µL of Amp was added to a 50 mL falcon tube
2. A p10 tip was used to scrape a little bit of the ice off of the glycerol stock and the tip was ejected into the falcon tube.
3. This was incubated for 18 hours at 37° and 200 rpm on 7/30/18

500 mL Mass Culture

1. The grown up 10 mL overnight culture from the day before was dumped into 500 mL of LB + Amp and was incubated at 37° and 150 rpm for 3 hours.
2. The absorbance at OD 600 was taken for the culture to ensure it was between 1 and 0.6.
3. Once the absorbance was in that range, 500 µL of 1M IPTG was added to the 500 mL culture at an absorbance of 0.807 and was incubated at 30° and 150 rpm for 18 hours.

Results

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

Conclusion

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

Name: Christina Clodomir

Goal: Amplify Beta 3 Loop for pGEX

Materials

RD B3 Loop for pGEX(PCR cleanup) cut w/ NotI+ EcoI 7-18-18 RB

B3 Loop for pGEX PCR cleaunup 7-18-18 R.B.

pGEX R.D. Cleanup 105 ng/ml. 7-6-17 c.j.

Thermo Scientific PCR Master Mix (2X) Lot 00391316

1:10 Dilution of Beta 3 Loop primer for pGEX F

1:10 Dilution of Beta 3 Loop Biobrick pGEX R

Protocol

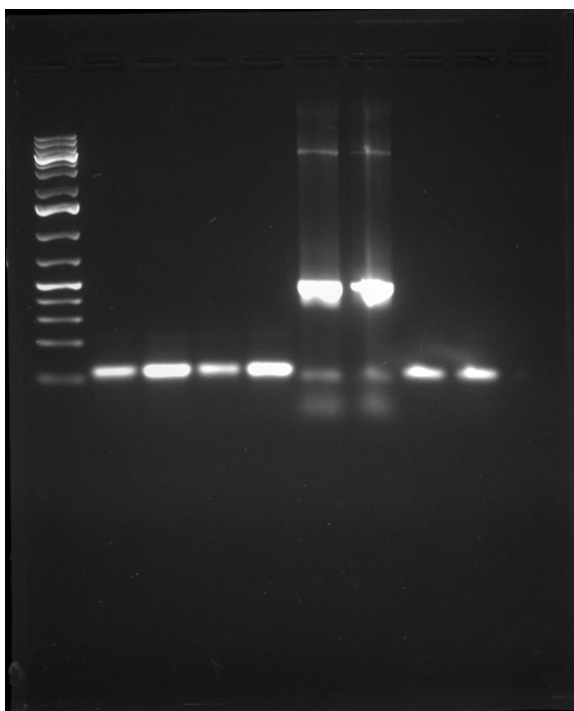
Beta 3 Loop PCR

1. A PCR tube was filled with 35 µL of water, 50 µL of PCR Master Mix (2X), 5µL of 1:10 Dilution of Beta 3 Loop primer for pGEX F, 5µL 1:10 Dilution of Beta 3 Loop Biobrick pGEX R, and 5 µL of DNA sample.
2. The PCR tubes were placed in the thermocycler at the following settings:

    1. 94° C for 3:00 minutes
    2. 94° C for 1:00 minute
    3. 56° C for 0:45 seconds
    4. 72° C for 1:00 minute
    5. 35X (Go to Step 2)
    6. 72° C for 2:00 minutes

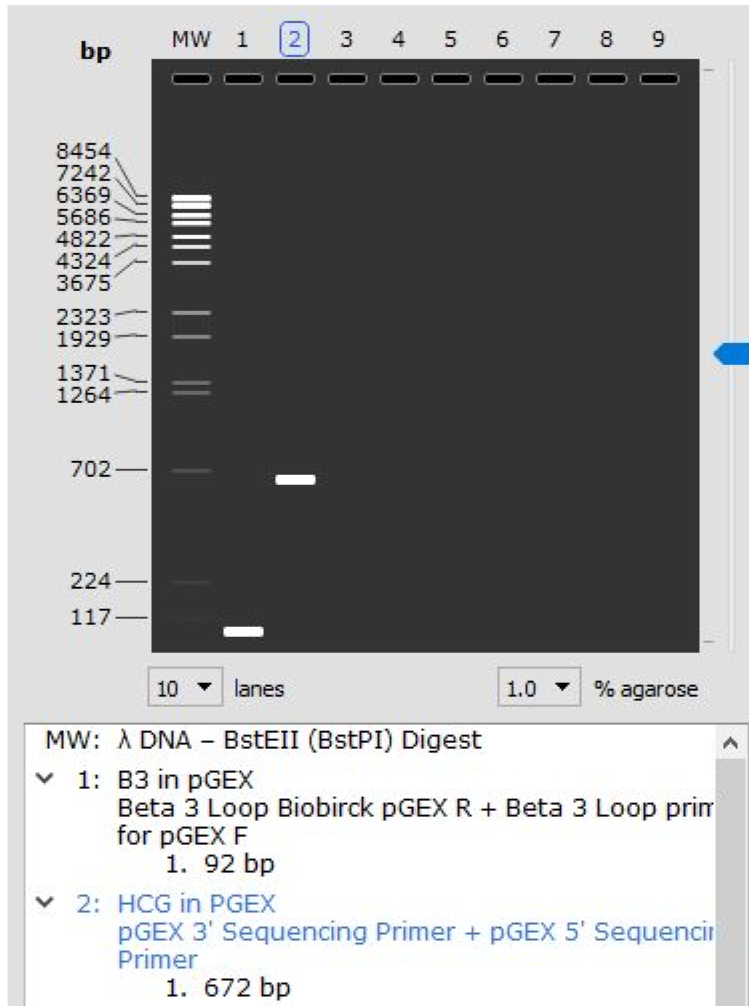
    Lid Temperature: 105° C

Results



Key
Lane 1: Beta 3 Loop for pGEX 7-31-18 from tube labeled PCR cleanup cut with EcoRI and NotI
Lane 2: Beta 3 Loop for pGEX 7-31-18 from tube labeled PCR cleanup cut with EcoRI and NotI
Lane 3: Beta 3 Loop for pGEX 7-31-18 cut with EcoRI and NotI
Lane 4: Beta 3 Loop for pGEX 7-31-18 cut with EcoRI and NotI
Lane 5: pGEX from CJ’s 6-17 RD clean up
Land 6: pGEX from CJ’s 6-17 RD clean up
Lane 7: Beta 3 Loop (for pGEX PCR cleanup) + 7-31-18 c.c
Lane 8: Beta 3 Loop (for pGEX PCR cleanup) + 7-31-18 c.c

Expected Results Simulated in SnapGene



Note: for the second entry there should be a band at 4962 bp.

Conclusion

The Beta 3 Loop for pGEX bands are placed correctly, however we have concerns for the pGEX PCR, it is smaller than expected considering it should be the entire vector at about 4962 bp and this one is smaller than that. Perhaps incorrect primers were used and the entire vector was not amplified and instead just a small piece. We will use another source of the vector for another ligation.