Name: Rehmat Babar

Date: 6/5/18

Goal: Want to amplify some existing DNA labeled as HCG in PSB1C3 and HCG in pGEX to see if there is DNA in these samples and to see if they produce bands at the correct size

Materials

HCG in pSB1C3 plasmid DNA
HCG in pGEX plasmid DNA
1:10 Dilution of HCG Block 5 reverse primer
1:10 Dilution of HCG Block 5 fwd primer
1:10 Dilution of HCG for pGEX F
1:10 Dilution of HCG for pGEX R
Glycerol Stocks Labeled “HCG in pGEX 4,” “HCG in pGEX 6,” “HCG in pGEX 8,” and “HCG in pSB1C3.”

Protocol

PCR on HCG in pSB1C3 and HCG in pGEX plasmid DNA

1. PCR for HCG in pSB1C3
    1. A PCR tube was filled with 7 µL of water, 10 µL HiFi Supermix, 1 µL 1:10 Dilution of HCG Block 5 fwd primer, 1 µL 1:10 Dilution of HCG Block 5 reverse primer, 1 µL of HCG in pSB1C3 plasmid DNA, respectively.
2. PCR for HCG in pGEX
    1. A PCR tube was filled with 7 µL of water, 10 µL HiFi Supermix, 1 µL 1:10 Dilution of HCG for PGEX R, 1 µL 1:10 Dilution of HCG for pGEX F, 1 µL of HCG in pGEX plasmid DNA, respectively.
3. The PCR tubes were placed in the thermocycler at the following settings:

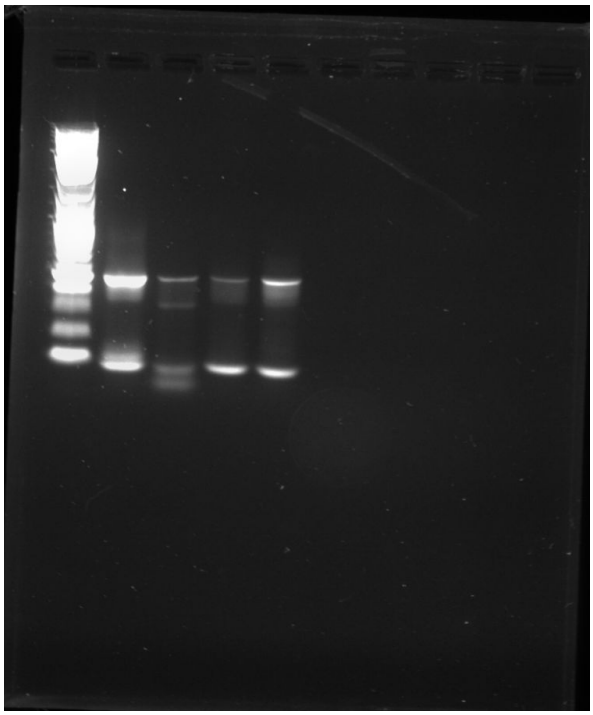
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 52° C for 1:00 minute
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

Overnight Cultures of 4 Glycerol Stocks Labeled “HCG in pGEX 4,” “HCG in pGEX 6,” “HCG in pGEX 8,” and “HCG in pSB1C3.”

1. 4 25 mL falcon tubes were obtained and filled with 5 mL of LB + chloramphenicol.
2. Then, using a p10 micropipette tip, some ice was scraped off each glycerol and was ejected into one of the four falcon tubes.
3. These falcon tubes were incubated at 37° C for 18 hours in a shaker incubator.

Results



Key
Lane 1: GeneRuler 1 kb Ladder
Lane 2: PCR for HCG in pSB1C3 GK
Lane 3: PCR for HCG in pGEX GK
Lane 4: PCR for HCG in pSB1C3 RB
Lane 5: PCR for HCG in pGEX RB

Conclusion: Although there is DNA present, the expected results should have been one band all across the gel at the same band length, however this shows two bands at different places.

Name: Rehmat Babar

Date: 6/6/18

Goal: Want to recover plasmid DNA from the overnight cultures done on 6/5/18 from the glycerol stocks by doing a mini prep and isolating the DNA.

Materials

Overnight cultures from the glycerol stocks prepared on 6/5/18
Mini prep Qiagen kit

Protocol

Mini Prep of Overnight Cultures

1. 3 mL of the overnight cultures was used
    a. 1.5 mL of each overnight culture was pipetted into two separate eppendorf tubes, making a pair for each glycerol stock
2. Each of the 6 tubes was centrifuged for 3 minutes at 8,000 rpm at 25° C
3. The supernatant was discarded from each tube
4. 250 µL of P1 buffer was only added to one eppendorf tube from each pair, it was pipette mixed, transferred all to one tube, and then pipette mixed again so that only 4 tubes containing solution remain
5. 350 µL of N3 was added after waiting one minute and the tubes were inverted 5 times
6. The tubes were centrifuged for 10 minutes at 13,000 rpm
7. The remaining clear solution in each of the tubes was pipetted into separate spin columns
8. They were centrifuged again for 30-60 seconds and th excess liquid was discarded
9. Each of the tubes were washed with 250 µL of PE buffer and centrifuged again for 60 seconds.
10. The flow through was discarded and the spin columns were centrifuged again for 60 seconds and the remaining flow through was discarded.
11. The concentrations of each of the 4 mini preps corresponding to the glycerol stocks previously mentioned were measured.

Gel

1. Ran a gel using the mini prep plasmid DNA, 10 µL of each sample was loaded.

Results

Mini Prep Sample	Concentration (ng/µL)

HCG in pGEX 4	17.5 ng/μL
HCG in pGEX 6	15 ng/μL
HCG in pGEX 8	25 ng/μL
HCG in pSB1C3	32.5 ng/μL

Even though the concentration of the recovered DNA is too low, it will still be imaged on a gel.

Key
Lane 1: Ladder
Lane 2: HCG in pGEX 4 mini prep
Lane 3: HCG in pGEX 6 mini prep
Lane 4: HCG in pGEX 8 mini prep
Lane 5: HCG in pSB1C3 mini prep

Conclusion

Even though the concentration of the recovered DNA is too low, it will still be used to do restriction digests.

Name: Rehmat Babar

Date: 6/14/18

Goal: Want to analyze additional PCR samples in the freezer as well as a restriction digest on the mini preps prepared on 6/618.

Materials

- EcoRI
- NotI
- PstI
- Fast Digest Buffer

Protocol

Restriction Digest on Mini Preps from 6/6/18 for HCG in pGEX

1. 3 eppendorf tubes were filled with 7 µL of water, 10 µL of Fast Digest Buffer, 1 µL of EcoRI, 1 µL of NotI.
2. Then 1 µL of HCG in pGEX 4 mini prep was pipetted into one tube, 1 µL of HCG in PGEX 6 mini prep in another, and 1 µL of HCG in pGEX 8 mini prep in the remaining one.

Restriction Digest on Mini Preps from 6/6/18 for HCG in pSB1C3

1. 1 eppendorf tube was filled with 7 µL of water, 10 µL of Fast Digest Buffer, 1 µL of EcoRI, 1 µL of PstI.

Gel

1. 10 µL of the restriction digests and 5 µL of the PCR product found in the freezers was loaded onto the gels and imaged.

Results

Key
Lane 1: KB Ladder 1000 bp
Lane 2: HCG in pSB1C3 RD
Lane 3: HCG in pGEX 4 RD
Lane 4: HCG in pGEX 6 RD
Lane 5: HCG in pGEX 8 RD
Lane 6: Ladder 100 bp
Lane 7: HCG in pSBC (orange)
Lane 8: HCG in pSBC (green)
Lane 9: HCG in pSBC (blue)
Lane 10: HCG in pGEX (pink)

Conclusion

Name: Rehmat Babar

Date: 6/18/18

Goal: Want to redo the restriction digests done on the mini preps from 6/6/18 since the gel did not show the correct bands. Instead of using EcoRI and PstI for HCG in pSB1C3 I will make use of a different restriction site and use EcoRI and XbaI. For HCG in pGEX instead of using EcoRI and NotI, I will use just XhoI which will cut at two different restriction sites.

Materials

- EcoRI
- XbaI
- XhoI
- Fast Digest Buffer

Protocol

Restriction Digest on Mini Preps from 6/6/18 for HCG in pGEX

1. 3 eppendorf tubes were filled with 7 µL of water, 10 µL of Fast Digest Buffer, 1 µL of XhoI.
2. Then 1 µL of HCG in pGEX 4 mini prep was pipetted into one tube, 1 µL of HCG in PGEX 6 mini prep in another, and 1 µL of HCG in pGEX 8 mini prep in the remaining one.

Restriction Digest on Mini Preps from 6/6/18 for HCG in pSB1C3

1. 1 eppendorf tube was filled with 7 µL of water, 10 µL of Fast Digest Buffer, 1 µL of EcoRI, 1 µL of XbaI, and 1 µL of HCG in pSB1C3 plasmid DNA.

Gel

1. 10 µL of the restriction digests and 5 µL of the PCR product found in the freezers was loaded onto the gels and imaged.

Results

Key
Lane 1: KB Ladder 1000 bp
Lane 2: HCG in pSB1C3 RD
Lane 3: HCG in pGEX 4 RD
Lane 4: HCG in pGEX 6 RD
Lane 5: HCG in pGEX 8 RD
Lane 6: Ladder 100 bp
Lane 7: HCG in pSBC (orange)
Lane 8: HCG in pSBC (green)
Lane 9: HCG in pSBC (blue)
Lane 10: HCG in pGEX (pink)

Conclusion

Name: Ahmad Mohammad

Date: 6/20/18

Goal: To complete a PCR and PCR purification of “miniprep plasmid Hcg in Psb1c3” to verify contents via sequencing

Materials

Components	Concentration (start)	Concentration (final)	50 uL
5X HF Buffer Lot #: 0041608	5X	1X	10 uL
DNTP’s Lot #: 00303784	10 mM	200 uM	1 uL
VF Primer	10 uM	0.5 uM	2.5 uL
VR Primer	10 uM	0.5 uM	2.5 uL
HF Phusion DNA polymerase Lot #: 0051503	2000 u/uL	1.0 / 50 uL pcr	0.5 uL
Template DNA	250 ng/uL	2.5 ng	1 uL
H2O			32.5 uL

Protocol

PCR Protocol:

Initial denaturation: 98℃ 30 seconds

Denaturation: 98℃ 10 seconds

Annealing: 56.5℃ 30 seconds

Extension 72℃ 30 seconds

Final Extension 72℃ 10 minutes

Results

Results inconclusive due to incorrect primers used.

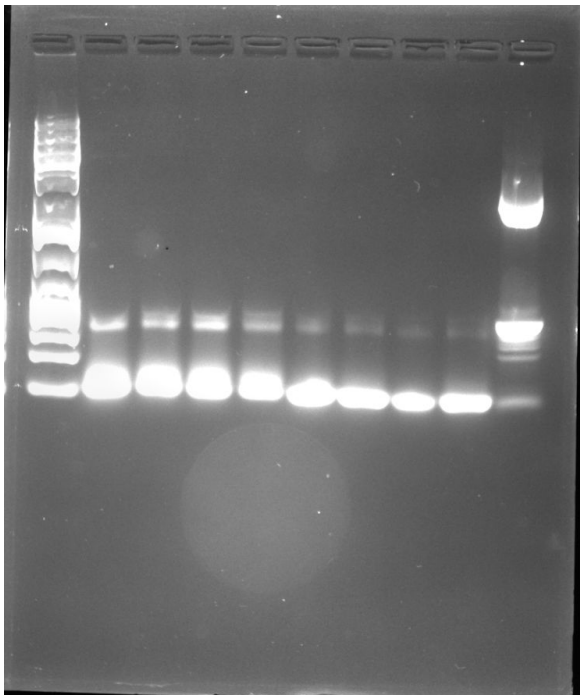
Conclusion

Unable to determine if dna in tube is hcg in psb1c3, will start from scratch to obtain proper insert in vector(s).

Name: Christina Clodomir
Date: 6/25/18
Goal: Run a PCR on tubes for HCG for PSB and HCG for pGEX. We want to see if the PCR product yielded will be the right size so that way we may move on to the next step in our experiment (restriction digest)

Materials
The ladder that was used to run this gel was Thermoscientific Gene Ruler 1kb PLUS DNA ladder

Results: Lane 2-5 are HCG for PSB1C3 and 6-9 were HCG for pGEX. Lane 10 was performed by a student that needed a diagnostic gel for another HCG experiment.



Conclusion: Ran a gel on the PCR prdts from Friday that contained Dr. Brewer's PCR products (HCG for PSB and HCG for PGEX)(HCG sample looked good). However bands on the bottom of the gel were very thick and about 75 bp long. Likely explanation was that they were primer dimers. Primers can stick to each other even if it is non specific to each other (as long as they have some affinity for each other and the temperature is low enough they will stick to each other).
Solution: raise the annealing temperature a bit to the optimal temperature performed in the temperature gradient last week.

Name: Mary Gonzalez/Christina Clodomir

Date: 6/26/18

Goal: Re-run of the previous procedure (PCR on Dr. Brewer's HCG) was done. The PCR tubes were labeled 1-4 (HCG for pGEX) and 5-8 (HCG for PSB1C3). The new annealing temperature was raised/adjusted to 57°C.

Materials

Specific names including the brand and other important information

Protocol

1. Add 1XTBE buffer to fill line
2. add 5 microlliters of 1KBT thermoscientific ladder to the first well.
3. Retrieve 5 microliters of DNA
4. Mix 5 microliters of DNA with 1 microliter of gel loading dye (purple dye)
5. load samples in wells

Results

We obtained the same/very similar results as yesterday. The PCR products of Dr' B's tube only produced a tiny amount of pcr product of HCG for psb and HCG for pgex. The bands were not the right size.

Conclusion

After checking snapgene, and checking the annealing temperature for each of the primers (both for pgex and psb) we found that the suffix and prefix both had different temperatures that differed about 8-10 degrees. The forward and reverse primers also had a temperature difference of about 8-10 degrees. We had to create new primers in snapgene and have to order them so that we can run another PCR.

Name: Ahmad Mohammad

Date: 6/26/18

Goal: To utilize overlap extension PCR to synthesize Factor C from 4 smaller DNA blocks.

Materials

Components	Concentration (start)	Concentration (final)	25 uL	8.8 rxns
5X HF Buffer Lot #: 0041608	5X	1X	5 uL	44 uL
DNTP's Lot #: 00303784	10 mM	200 uM	0.5 uL	4.4 uL
Forward Block Primers (1-4)	10 uM	0.5 uM	1.25 uL	n/a
Reverse Block Primers (1-4)	10 uM	0.5 uM	1.25 uL	n/a
HF Phusion DNA polymerase Lot #: 0051503	2000 u/uL	1.0 / 50 uL pcr	0.25 uL	2.2 uL
Template DNA (Blocks 1-4)	1 ng/uL	1 ng	1 uL	n/a
H2O			15.75 uL	138.6 uL

Protocol

PCR Protocol:

Initial denaturation: 98℃ 30 seconds

Denaturation: 98℃ 10 seconds

Annealing: 65℃ 30 seconds

Extension 72℃ 30 seconds

Final Extension 72℃ 10 minutes

Results

Results, along with proper analysis of primers using snapgene, indicated that the previously designed primers had wildly different annealing temperatures, and so new primers were designed for the overlap extension PCR.

Conclusion

New primers were designed and ordered for the overlap extension PCR of Factor C blocks 1-4.

Name: Ahmed and Rehmat/Laura

Date: 6/27/18

Goal: Ahmed will begin the Overlap Extension PCR to put together all 5 blocks of Factor C. Rehmat and Laura want to extract the plasmid DNA from the overnight cultures of RFP in pSB1C3 and GFP in pSB1C3 that had been incubating for 16 hours prior.

Materials

Forward and reverse primers were used for each block for a total of 8 primers. Block stocks 1-4 were also used.

QIAGEN Spin Miniprep Kit

For Block 1: Reverse and Forward Primer

For Block 2: Reverse and Forward Primer

For Block 3 : Reverse and Forward Primer

For Block 4: Reverse and Forward Primer

BioLabs Purple loading dye

GeneRuler DNA ladder 1kb plus

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

The mini prep was repeated twice because the first time the Buffer PE used did not contain ethanol when it should have.

Results

The plasmid DNA was successfully isolated and the concentrations are as follows:

GFP 1 (trial 1) = 10 ng/µL

GFP 2 (trial 1) = 7.5 ng/µL

GFP 3 (trial 1) = 15 ng/µL

GFP 4 (trial 1) = 10 ng/µL

RFP 1 (trial 1) = 5 ng/µL

RFP 3 (trial 1) = 7.5 ng/µL

GFP 1 (trial 2) = 32.5 ng/µL

GFP 2 (trial 2) = 27.5 ng/µL

GFP 3 (trial 2) = 27.5 ng/µL

GFP 4 (trial 2) = 37.5 ng/µL

RFP 1 (trial 2) = 12.5 ng/µL

RFP 3 (trial 2) = 27.5 ng/µL

Conclusion

The concentrations for the mini preps were on the low side but I will continue to do a restriction digest and eventually a gel extraction.

Name: Rehmat Babar

Date: 6/28/18

Goal: Want to do a restriction digest to isolate the vector from the insert and perform a gel extraction so the vector is ready for ligation

Materials

Thermo Scientific 10X Fast Digest Buffer Lot 00136165

Thermo Scientific Fast Digest EcoRI Lot 00246951

Thermo Scientific Fast Digest PstI Lot 00472716

dI H2O

Plasmid DNA of RFP in pSB1C3 and GFP in pSB1C3

Protocol

Restriction Digest on pSB1C3 containing GFP and RFP

1. A 13X cocktail was prepared since there were 4 samples of plasmid DNA of RFP in pSB1C3 and 8 samples of plasmid DNA of GFP in pSB1C3. The cocktail was prepared as follows: 13 µL EcoRI, 13 µL of PstI, 39 µL of Thermo Scientific 10X Fast Digest Buffer, and 195 µL of dI H2O.

1. 30 µL restriction digest reaction RFP in pSB1C3
    1. An eppendorf tube was filled with 20 µL of the concentration cocktail previously prepared and 10 µL of plasmid DNA of RFP in pSB1C3 for 30 µL total.
2. 30 µL restriction digest reaction GFP in pSB1C3
    1. An eppendorf tube was filled with 20 µL of the concentration cocktail previously prepared and 10 µL of plasmid DNA of GFP in pSB1C3 for 30 µL total.

Results

Well 1

Well 2

Well 3

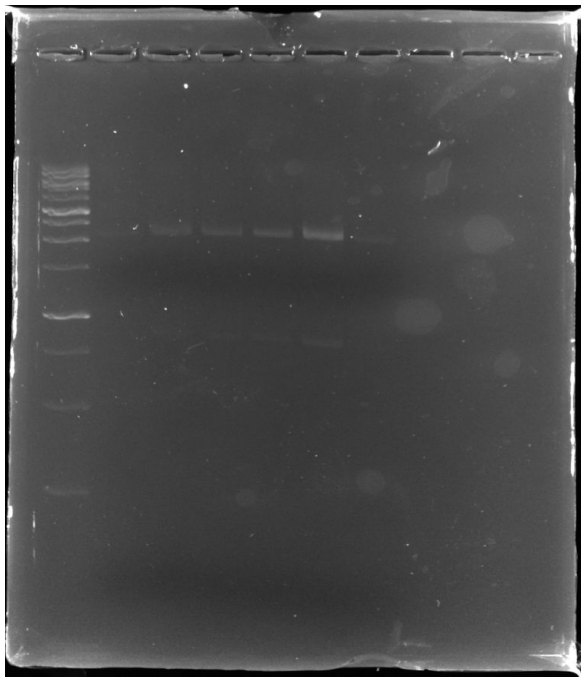
Well 4

Well 5

Well 6

Well 7

Well 1



Well 2

Well 3

Well 4

Well 5

Well 6

Well 7

Well 8

Well 9

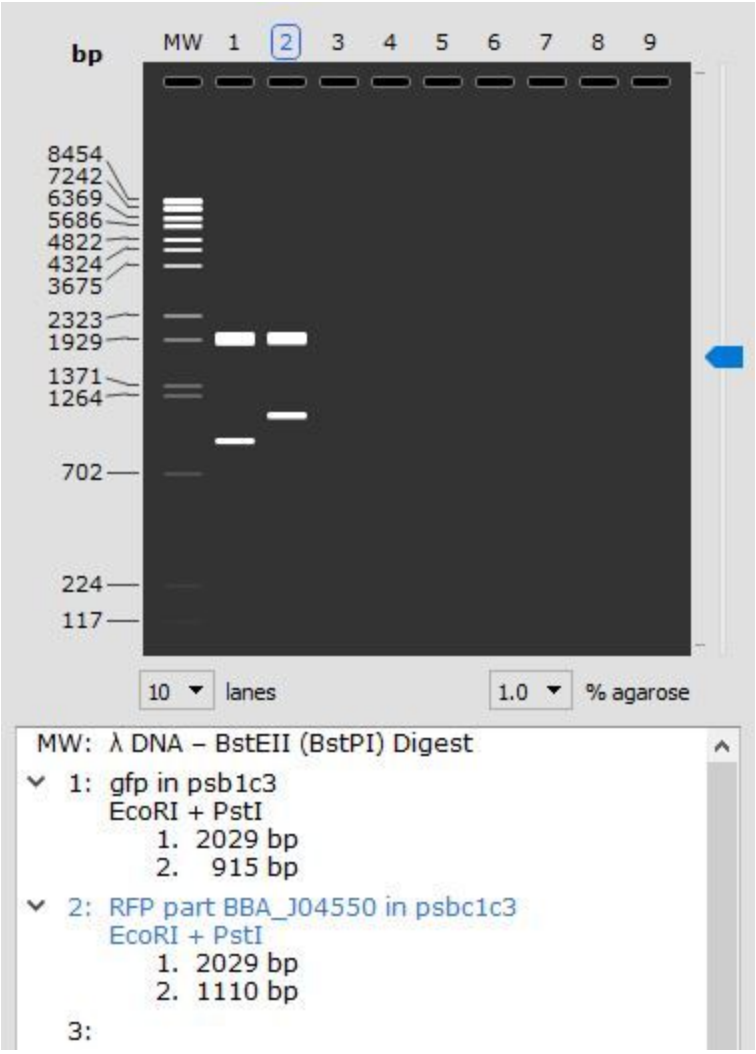
Well 10

Well 11

Well 12

Well 13

Expected Results simulated in SnapGene



Conclusion

Yes the bands appear to be at the correct band size and the there are two bands for each well indicating that the insert was removed by the restriction digest. This gel will be used to do a gel extraction where all of the pSB1C3 will be taken out and prepared for ligation.