Name: Rehmat Babar

Date: 5/23/18

Goal: Want to see if the existing tubes in the freezer from last year contain any DNA and if so, is it labeled correctly. I will do this by amplifying the DNA by doing PCR and by doing a restriction digest. Essentially the restriction digest will serve to isolate the pSB1C3 backbone from its RFP and GFP inserts and the pGEX from its mambalgin insert so we can insert HCG in a ligation later.

Materials
RFP in pSB1C3 DNA
GFP in pSB1C3 DNA
Mambalgin in pGEX 200 ng/μL DNA
Mambalgin in pGEX 275 ng/μL DNA
HiFi Supermix
HCG biobrick prefix
HCG biobrick suffix
HCG PGEX prefix
HCG PGEX suffix
Thermo Fisher Fast Digest Buffer
BioLabs EcoRI
BioLabs NotI
BioLabs SpeI

Protocol

PCR on HCG in pSB1C3 and pGEX

1. 20 μL PCR Reaction of HCG in pSB1C3
    1. A PCR tube was filled with 7 μL of water, 10 μL of HiFi Supermix, 1 μL of HCG biobrick suffix, 1 μL of HCG biobrick prefix, and 1 μL of HCG G-Block DNA, respectively. The DNA was diluted due to a small amount of liquid.
2. 20 μL PCR Reaction of HCG in pGEX
    1. A PCR tube was filled with 7 μL of water, 10 μL of HiFi Supermix, 1 μL of HCG pGEX suffix, 1 μL of HCG pGEX prefix, and 1 μL of HCG G-Block DNA, respectively. The DNA was diluted due to a small amount of liquid.
3. The PCR reaction was run 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

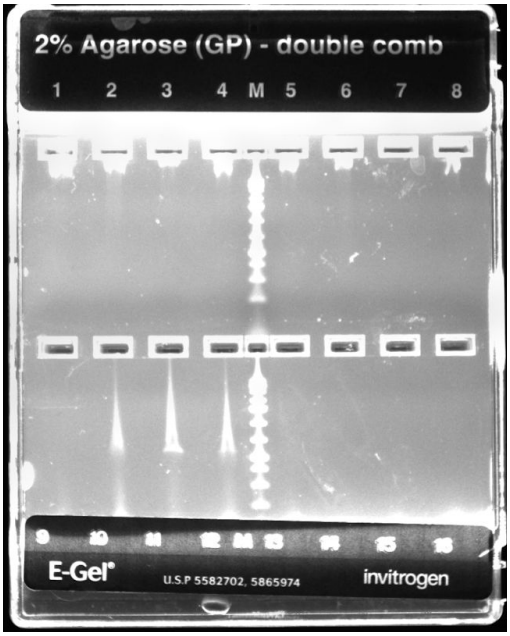
5. 30X (Go to Step 2)
6. 72° C for 5:00 minutes

Lid Temperature: 105° C

Restriction Digest on pSB1C3 containing GFP and RFP and pGEX containing mambalgin

1. 30 µL restriction digest reaction RFP in pSB1C3
    1. Calculated the amount of DNA needed based on the concentration of the DNA using C=m/v.
    2. An eppendorf tube was filled with 1.5 µL of water, 3 µL of fast digest buffer, 1 µL of SpeI, 1 µL of EcoRI, and 23.5 µL of DNA RFP in pSB1C3.
2. 30 µL restriction digest reaction GFP in pSB1C3
    1. Since the concentration of this DNA sample was unknown, 10 µL were used.
    2. An eppendorf tube was filled with 15 µL of water, 3 µL of fast digest buffer, 1 µL of SpeI, 1 µL of EcoRI, and 10 µL of DNA GFP in PSB1C3.
3. 30 µL restriction digest reaction mambalgin in pGEX 200 ng/µL
    1. Calculated the amount of DNA needed based on the concentration of the DNA using C=m/v.
    2. An eppendorf tube was filled with 20 µL of water, 3 µL of fast digest buffer, 1 µL of NotI, 1 µL of EcoRI, and 5 µL of 200 ng/µL mambalgin in pGEX DNA.
4. 30 µL restriction digest reaction mambalgin in pGEX 275 ng/µL
    1. Calculated the amount of DNA needed based on the concentration of the DNA using C=m/v.
    2. An eppendorf tube was filled with 21.4 µL of water, 3 µL of fast digest buffer, 1 µL of NotI, 1 µL of EcoRI, and 3.6 µL of 275 ng/µL mambalgin in pGEX DNA.
5. All 4 tubes for the restriction digest were incubated at 37° C for 30 minutes
6. The PCR product the restriction digests were run on a gel and analyzed for DNA present.

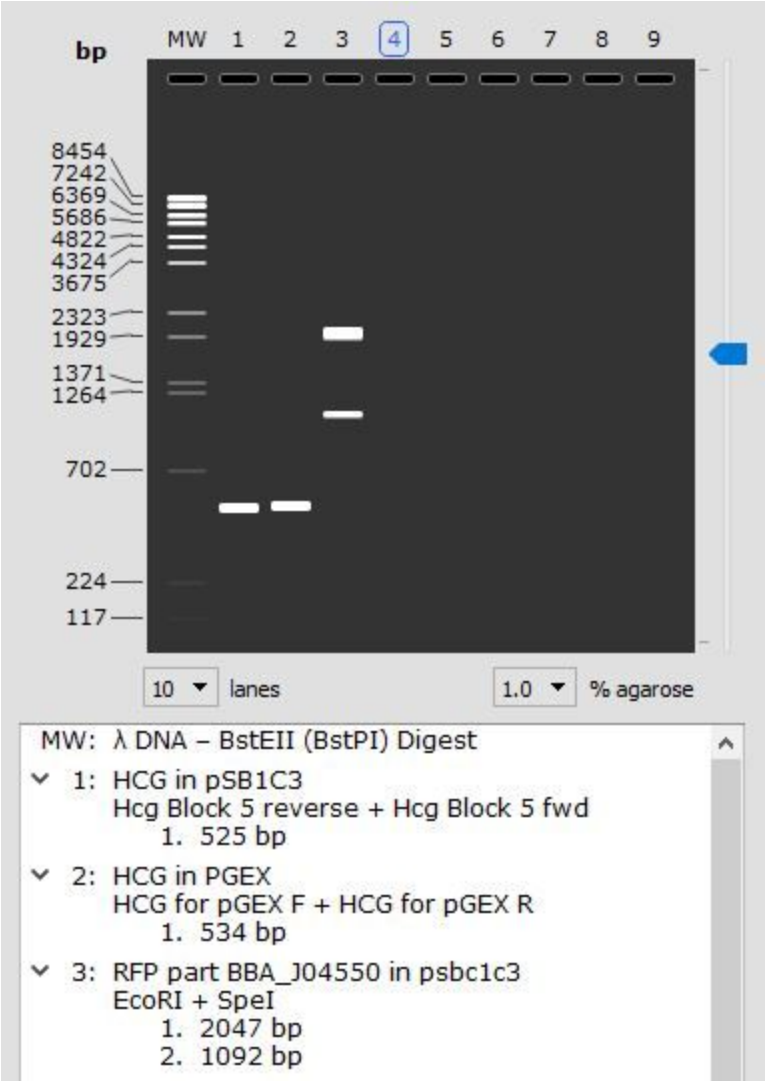
Results



Gel Key

Lane 1: HCG in pSCB1C3 PCR GK
Lane 2: HCG in pGEX PCR GK
Lane 3: HCG in pSCB1C3 PCR LDN
Lane 4: HCG in pGEX PCR LDN
Lane M: Ladder
Lane 5: HCG in pSCB1C3 PCR CC
Lane 6: HCG in pGEX PCR CC
Lane 7: HCG in pSCB1C3 PCR RB
Lane 8: HCG in pGEX PCR RB
Lane 9: RD RFP in pSB1C3

Lane 10: RD GFP in pSB1C3
Lane 11: RD 200 ng/µL mambalgin in pGEX
Lane 12: RD 275 ng/µL mambalgin in pGEX



Conclusion

Restriction digest seems to have been done ineffectively or since the DNA being worked with is over a year old, the DNA must be degraded and not showing the proper band length. The PCR is not at the correct length either and seems to be stuck towards the top of the gel.

Name: Rehmat Babar

Date: 5/25/18

Goal: Want to see if the existing tubes in the freezer from last year contain any DNA and if so, is it labeled correctly. I will do this by running the random samples of DNA labeled with pSB1C3 or pGEX found within in the freezer to see if these backbones are present so they may be useful in the future.

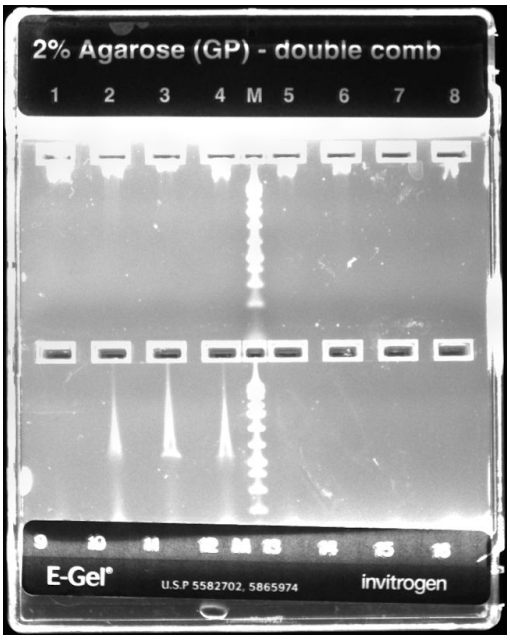
Materials

Various DNA samples containing pSB1C3 and pGEX
Invitrogen E-Gel Agarose 2% (GP)
BioLabs Purple Loading Dye (6X)

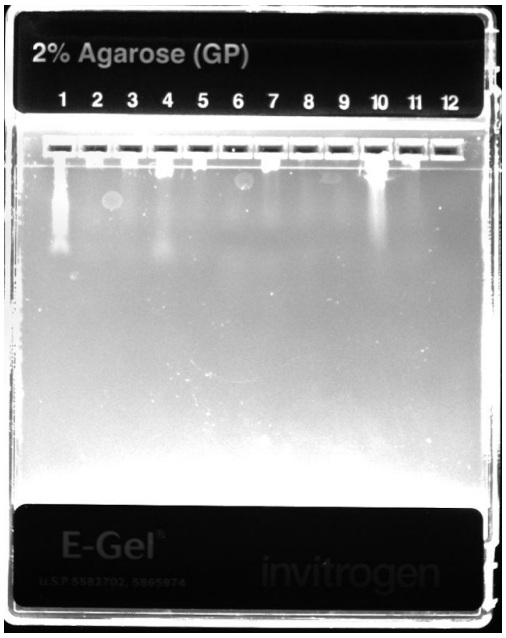
Protocol

1. The samples found were run on 2 separate E-gels with 1 µL of loading dye and 2 µL of the various DNA samples.

Results



Key
E-Gel #1
Lane 1 Top: Midi prep mamba PGEX 275 ng/µL
Bottom: PSCB1C3 PCR GK
Lane 2 Top: Midi prep mamba PGEX 9/16 200 ng/µL
Bottom: PGEX PCR GK
Lane 3 Top: GFP Test Device Interlab in PSB1C3
Bottom:PSCB1C3 PCR RB
Lane 4 Top: GFP Positive Control Interlab in PSB1C3 95 ng/µL
Bottom: PGEX PCR RB
Lane 5 Top: RFP in PSB1C3 mini prep 2 55 ng/µL
Bottom: PSCB1C3 PCR CC
Lane 6 Top: A RFP in PSB 42.5 ng/µL 7/12/16
Bottom: PGEX PCR CC
Lane 7 Top: RFP in PSB1 5/17 NEW mini
Bottom: PSCB1C3 PCR LDN
Lane 8 Top: 23 CBD PSB1C3 Lvb 6/16/17 40 ng/µL
Bottom: PGEX PCR LDN



E-Gel #2
Lane 1: DNA Ladder 1 Kb
Lane 2: LT10 Pro in PSB 80 ng/µL Seqr 9/29/16 CJ
Lane 3: Miniprep EcfP MWB 45 X (DB #1 9/5)
Lane 4:Mamba Yeast in PSB1C3 (MH12 f18 JW) 158 ng/µL
Lane 5: “24” CBB in nPSB1C3 (LOP) 6/16/17 60.0 ng/µL
Lane 6: ECFP 3 Ase Mini Prep stock
Lane 7: PE RFP
Lane 8: “PGEX 6”
Lane 9: “PGEX 4”
Lane 10:PGEX in E.coli; Midi prep 1.0 mL; 245 ng/µL Buffer; 6/21/17 CJ
Lane 11: “PGEX 8”

Conclusion

The DNA seemed to be stuck in the wells and did not travel while the gels ran, some of the PCR product in E-gel #1 produced bands however they were not placed correctly. E-Gel #2 produced the incorrect sized bands and the DNA streaked down the gel. It appears that all of the DNA from last year has either been degraded or this is another underlying issue.