8/3 Double Restriction Enzyme Digest

Two double restriction digests were performed to create a PSB1C3 backbone so the hCG (with prefix and suffix) can be added to create a whole part. The ECFP must be cut out to form the backbone. We digested both the ECFP in PSB1C3 and hCG (with prefix and suffix) with EcoRI and PstI. They both must be cut with the same enzymes to add the parts together. Since the enzymes only cut two base pairs (bp) off of the hCG, the band size difference was indistinguishable. So we performed another double digest with XbaI and SpeI. This created a noticeable size difference. However, when we cut with the XbaI and SpeI, the enzymes produce the same sticky ends. This creates the possibility of an inverted hCG sequence which is why we had to cut with EcoRI and PstI as well.

Materials:

- DNA of ECFP-PCB1C3 (102 ng/ul) and hCG PCR product
- Restriction enzymes: NotI, EcoRI, XBaI, PstI,SPEI
- Green Fastdigest Buffer (10x)
- Nuclease free H20

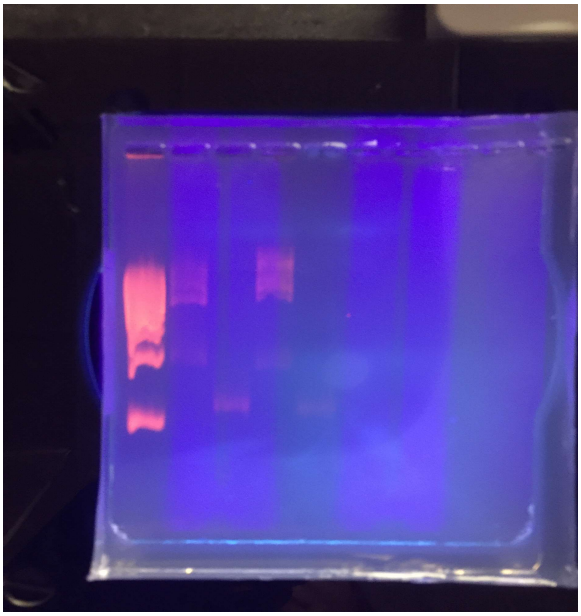
Protocol :
100 ul sample: 2ug of DNA, 20-40 unit of enzyme
The following table was created and used to organize the amounts of each ingredient that were added to each tube
Place in the thermocycler and run at 37□ for 1 hour then deactivate enzymes for 10min at 80□

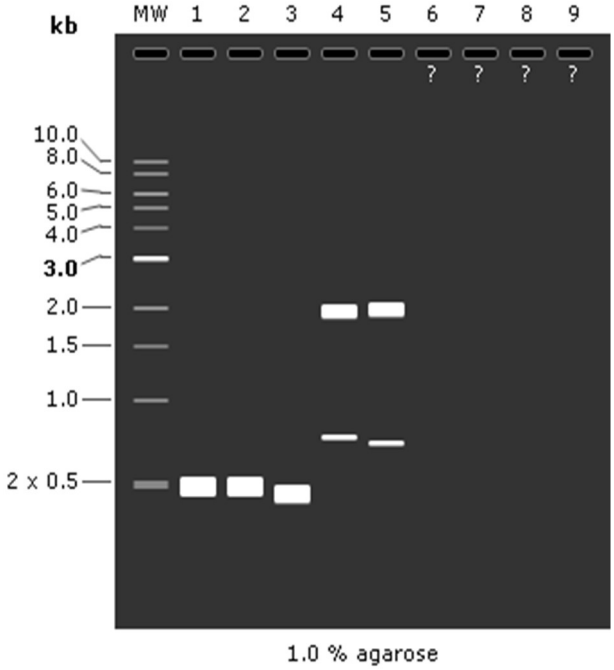
DNA	Buffer	Enzyme	H20
ECFP 20ul	10ul	2ul ECORI 2ul Pst1	66ul
Sample 8 10ul	10ul	2ul ECORI 2ul Pst1	76ul
Sample 9 10ul	10ul	2ul Xba 2ul Spe1	76ul
ECFP 20ul	10ul	2ul Xba 2ul Spe1	66ul

(only sampels 8 and 9 were chosen at random, since all the samples are the same there is no need to cut all of them)

Results:

The Wells Contain the following:
1 1Kb New England Biolabs Ladder
2. hCG uncut
3. hCG cut with EcoR1 and Pst1
4. hCG cut with Xba1 and Spe1
5. ECFP cut with EcoR1 and Pst1
6. ECFP cut with Xba1 and Spe1





The gel was not as clear as expected so
another digest and gel is going to be run using the same numbers and sample numbers.

8/5: Repeat of restriction digest
Since the previous gel did not look as expected. The double digest was repeated using the same protocol. Lt10 in PSB1C3 was used in place of ECFP because there was no more ECFP in the lab.

Materials:

- DNA of Lt10 (80 ng/ul) and hCG PCR product
- Restriction enzymes: Not1, EcoR1, XBa1, Pst1,SPE1
- Green Fastdigest Buffer (10x)
- Nuclease free H20

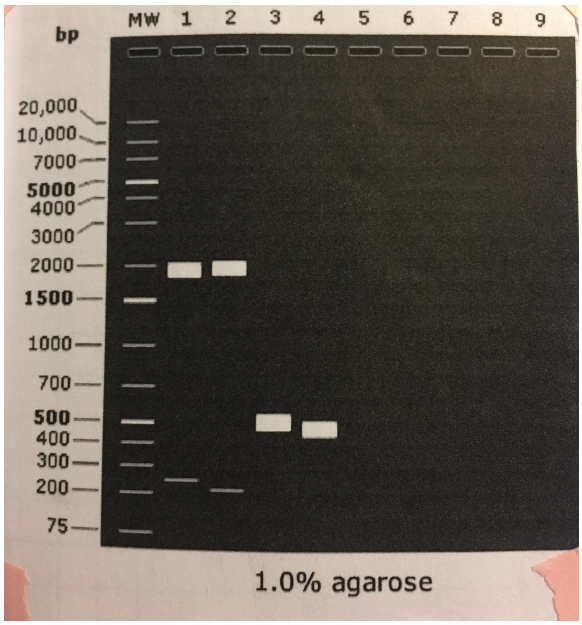
Protocol:
100 ul sample: 2ug of DNA, 20-40 unit of enzyme

The following table was created and used to organize the amounts of each ingredient that were added to each tube
Place in the thermocycler and run at 37□ for 1 hour then deactivate enzymes for 10min at 80□

DNA	Buffer	Enzyme	H20
Lt10 30ul	10ul	2ul ECORI 2ul Pst1	56ul
Sample 8 10ul	10ul	2ul ECORI 2ul Pst1	76ul
Sample 9 10ul	10ul	2ul Xba 2ul Spe1	76ul
Lt 10 30ul	10ul	2ul Xba 2ul Spe1	56ul

(only sampels 8 and 9 were chosen at random, since all the samples are the same there is no need to cut all of them)

Results:



Since the diagnostic gel was a success the PSB1C backbone was extracted from the Lt 10 plasmid using an E-gel.