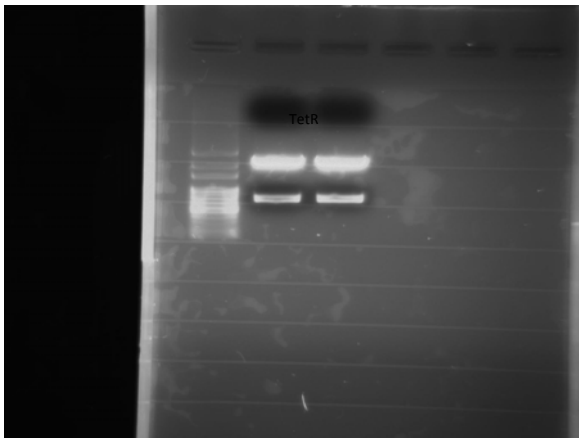
1

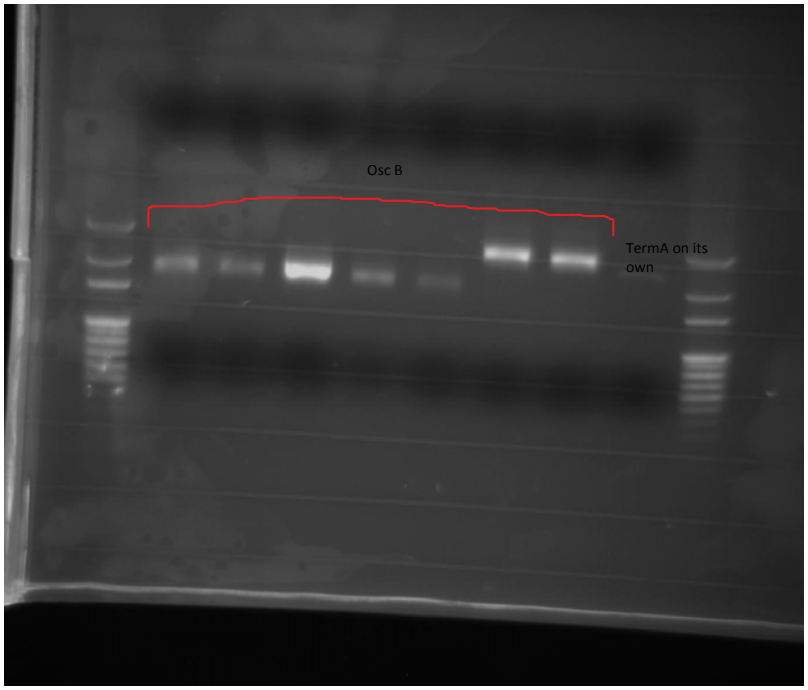
Thursday, May 01, 2014
3:54 PM



This is cut at XP.

When doing transformations, always run a control, pUC19

http://en.wikipedia.org/wiki/PUC19



2

Thursday, May 01, 2014
4:07 PM

RBS is still not working so we digested the RBS again today at SP.
The plan is to attach the TetR into the RBS plasmid because we already have TetR in XP already.

We also did plasmid purification for the liquid cultures from yesterday. They include:

-

Gel purification of TetR cut at ES.

We made liquid cultures for RBS again straight from the stab, we will need to do plasmid purification for Monday.

The goals: create a parts registry of what we have including digested parts that is constantly being updated (every week?) and have the lengths already calculated.

1. To make the final construct, we should just focus on cutting pLac @ SP.
2. We want to ligate RBS with TetR together first so that the circuit is longer. (easier to see in the gel).
3. The RBS and pLac all very short so instead of 10A assembly, we will focus on 3A assembly.
4. After RBS and TetR are ligated together, we can digest them at XP to do another back insert into pLac.
5. Ligate pLac with RBS+TetR and we will finish part 1.

The generators are all on pSB1C3 backbones because that is the standardized backbone that the distribution uses.

Useful numbers:
PCR lengths:
C resistance: 314
A resistance: 238
K resistance: ???

9

Friday, May 02, 2014
2:41 PM

Ligated Parts:
cI + pTet
cI + pTet (4/17)

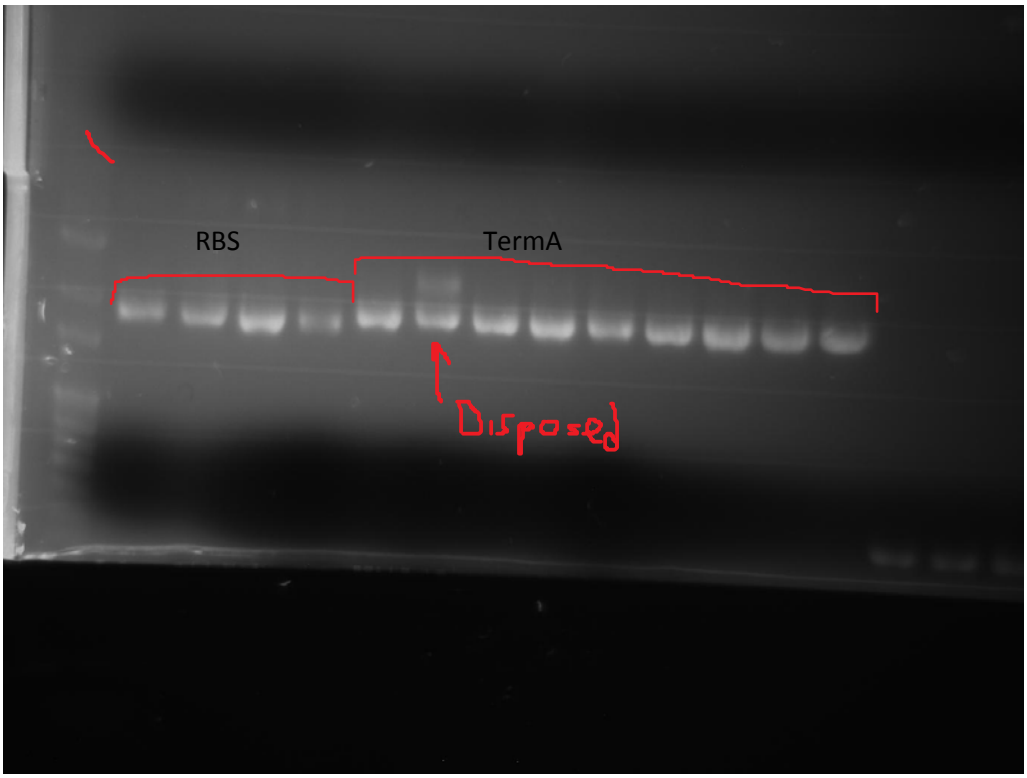
PCR tubes:
cI + pTet (4/17) success
cI + pTet

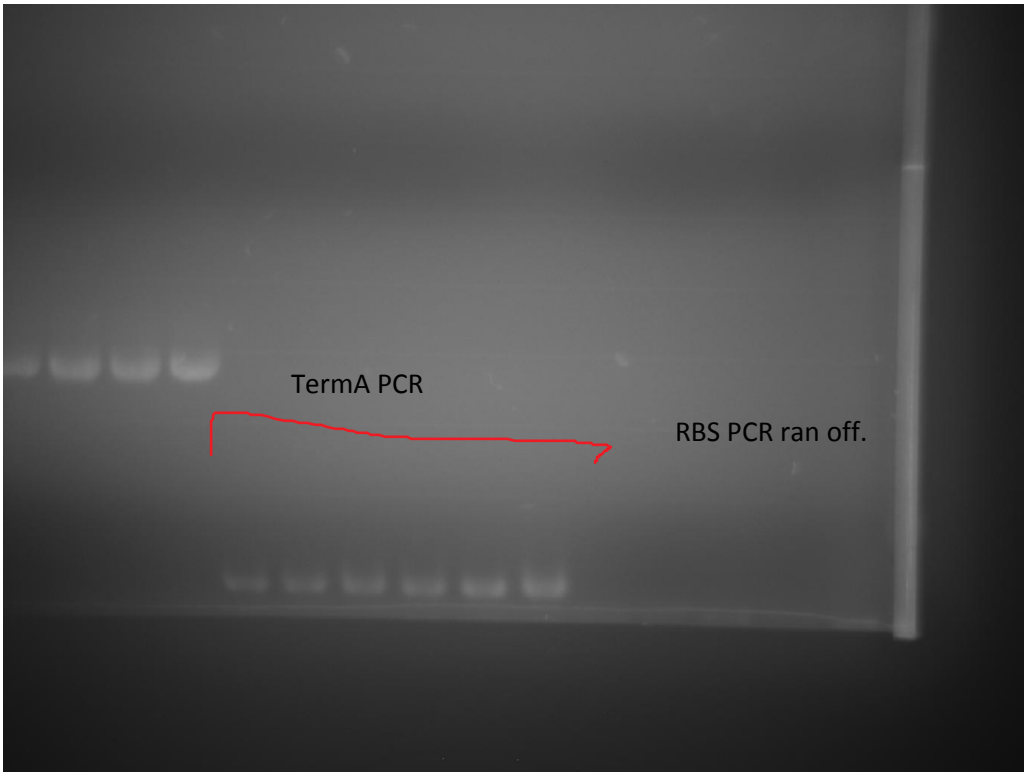
SP
TermA Resistance
TermA

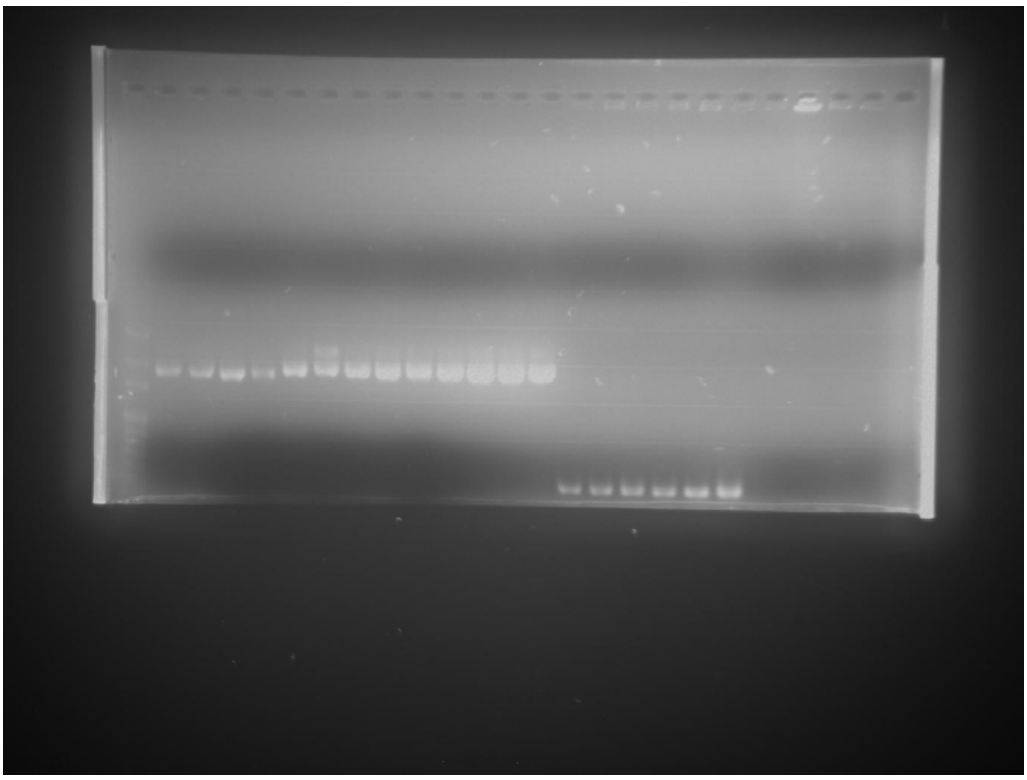
14

Friday, May 09, 2014
3:10 PM

Gel results





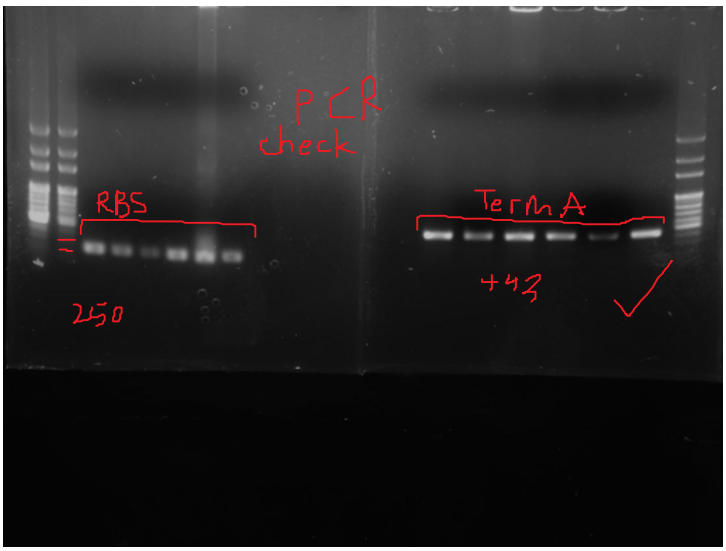


- Designed primers for c-Myc, pSurvivin, TetR/RFP, pSurvivin+GFP, hTERT

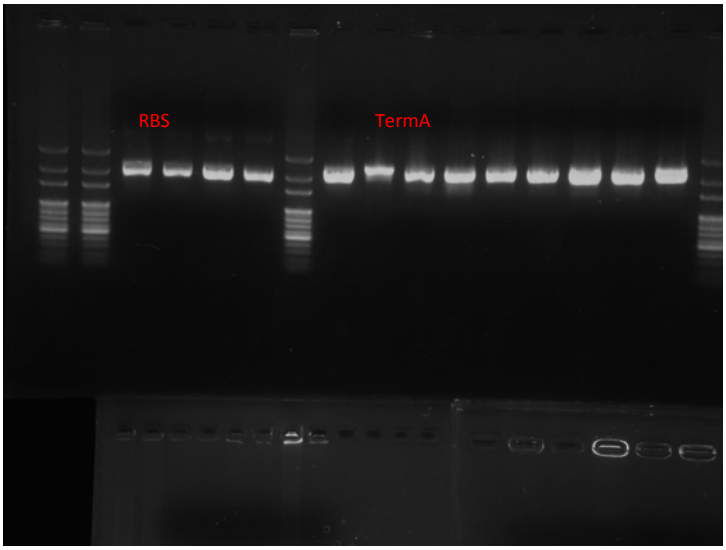
15

Thursday, May 15, 2014
4:34 PM

- Designed primers for c-Myc and pSurvivin



We expect to see 238+12=250 bp for RBS
314+129=443 bp for TermA



Ran gels including the PCR check for RBS and termA.
We expect to see 238+12=250 bp for RBS and 314+129=443.
The purified plasmid for RBS and TermA are alright, around 2k.

PCR

1. Add the length of the part
2. 1kilo bp/minute for the Taq Polymerase
3. Example: we have cI Gen on pSB1C3--> we take cI Gen+ resistance=the part that it will copy and replicate.

PCR temperature precedure.

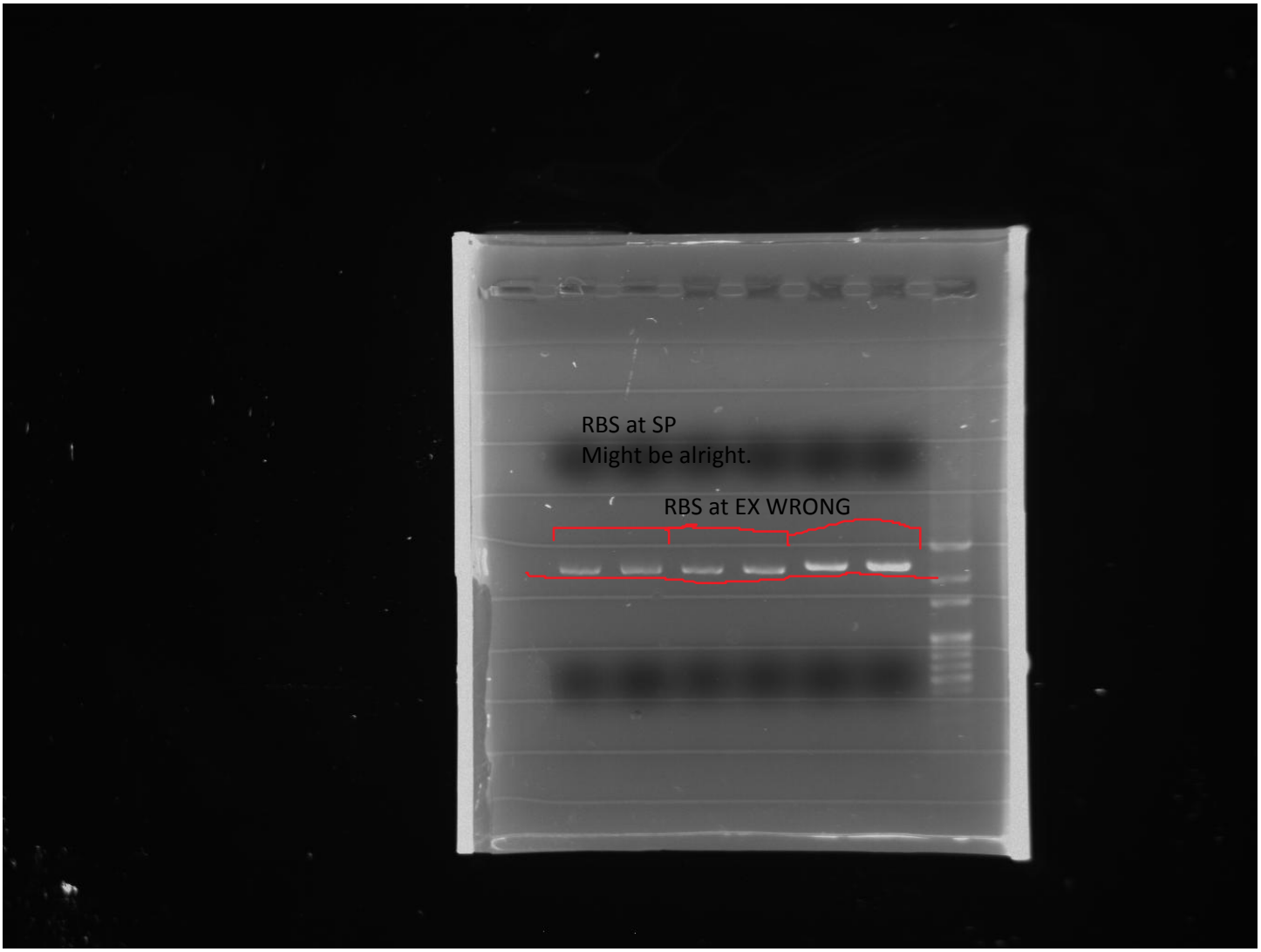
Julie and Beth- 3 in 1 GFP Gen
Mike+Chris - RBS+TermA - plasmids
Andy -make K plates.

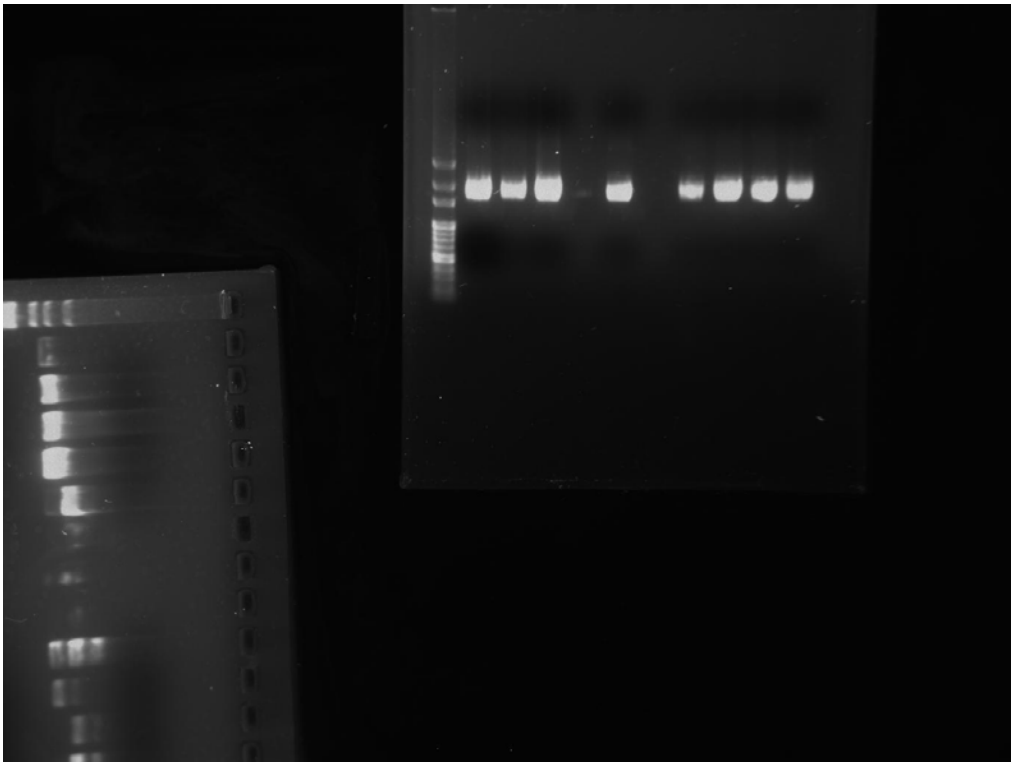
16

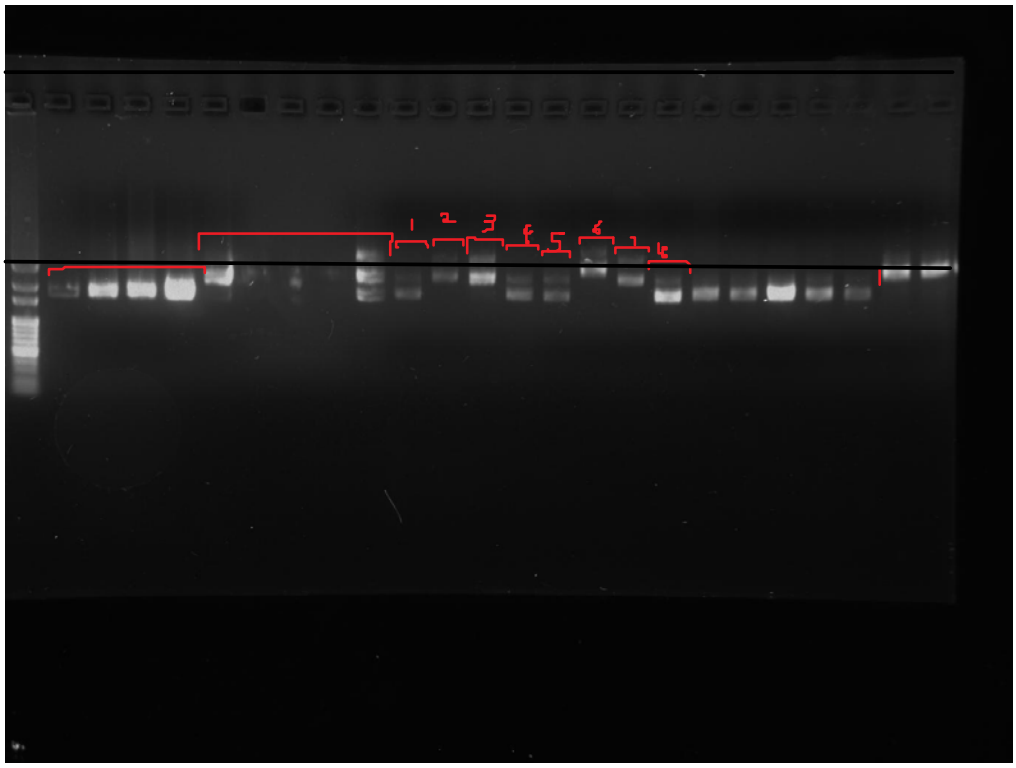
Friday, May 16, 2014
2:30 PM

TermA needs to be cut at EX x2
RBS at SP and ES , both x2

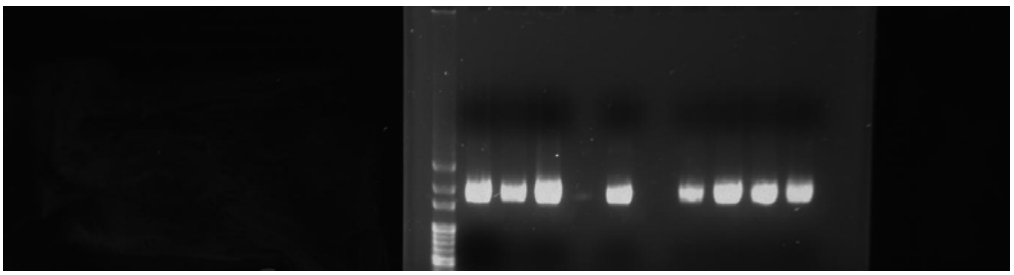
Purified plasmid for GFP Gen
Run gel for PCR for GFP Gen

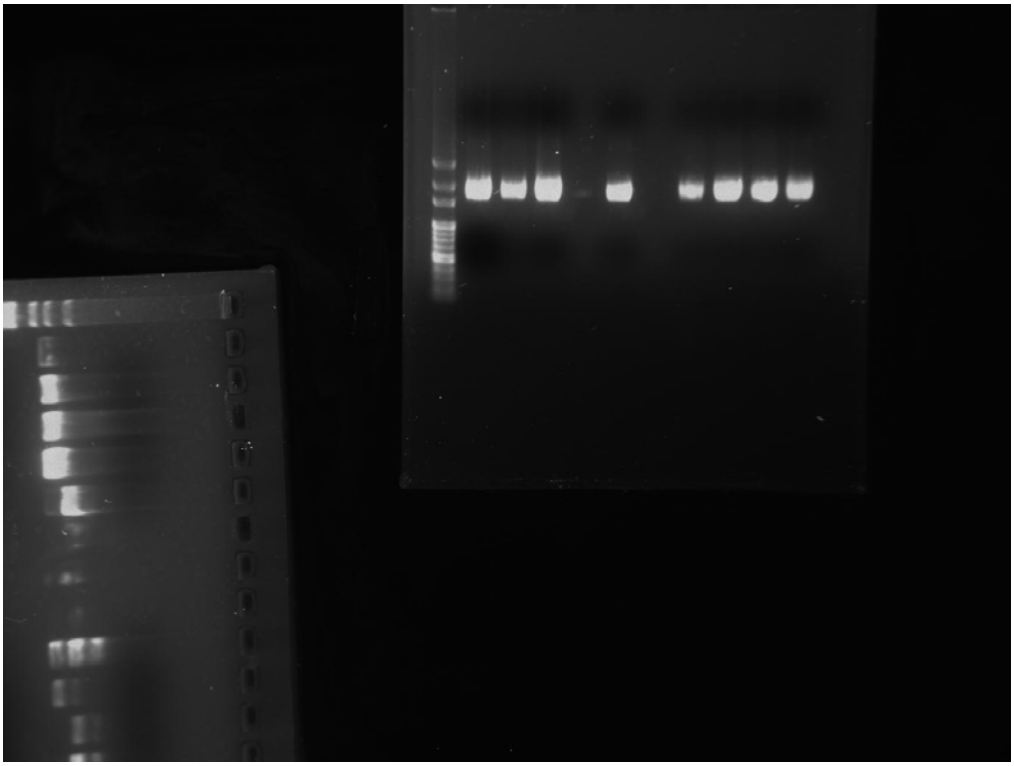






The wrong parts have been thrown out.





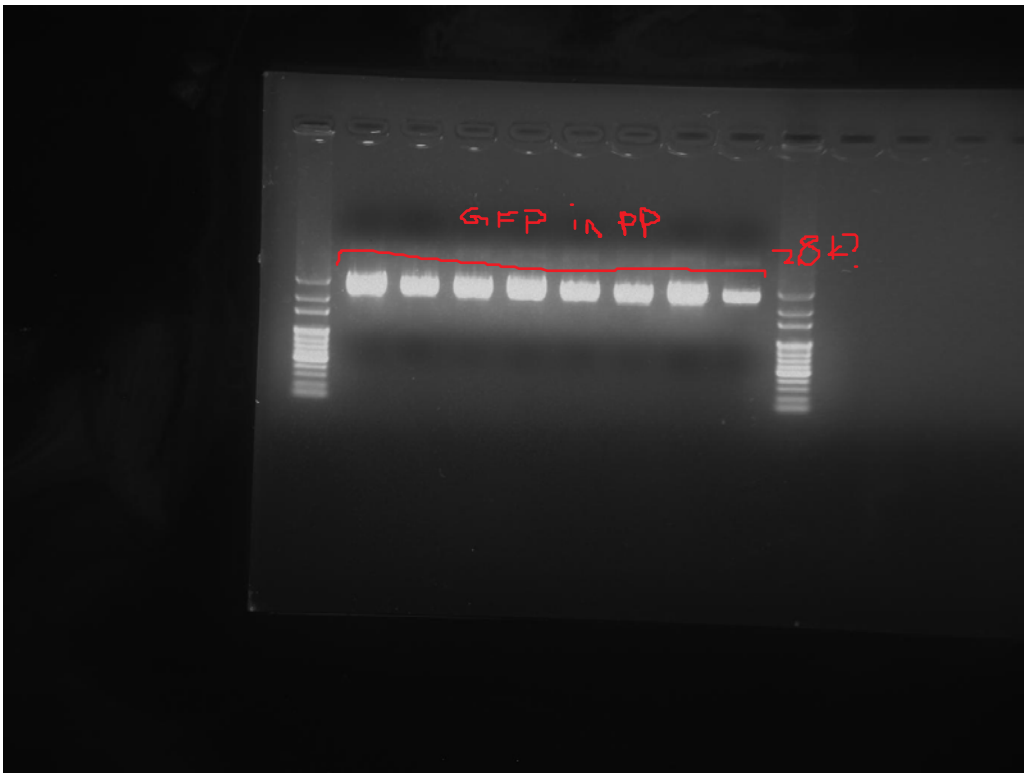
1. Purified plasmid for GFP Gen
2. Run gel for PCR for GFP Gen, is it good?
3. Digest RBS at SP and ES --> Might not show up as expected.
4. TermA at EX both at 15 uL.

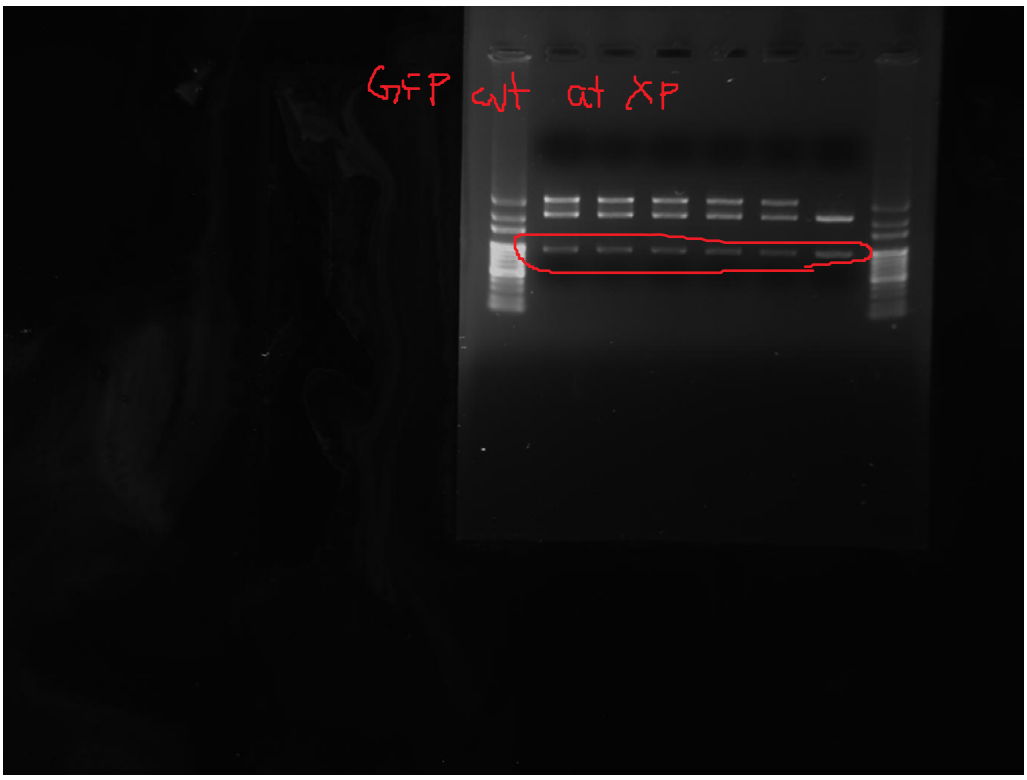
Maybe try PCR clean-up method? This amplifies the DNA.

GFp Gen--> E0840 878+314=1192
2013 Kit Plate 3 23C

19

Monday, May 19, 2014
3:23 PM





GFP Gen should be confirmed --> digest at XP

Cloning Cycle 1

Vector	Insert
pLac	TetR Gen
pTet	GFP Gen
RBS	TermA
pTet	cI Gen
pcI	GFP Gen
pcI	LacI Gen
pLac	GFP Gen

1. UGP
2. Run gels for GFP?

   Confirmed parts
   TetR @Xp 5/24
   pLac @SP 5/24
   pTet+ cI Gen 5/24
   pcI+ LacI Gen at Xp and EX is wrong

PCR Results
cI Gen seems good
pTet seems good
LacI seems good

UGP--Purify RBS and TermA <--GFP Gen

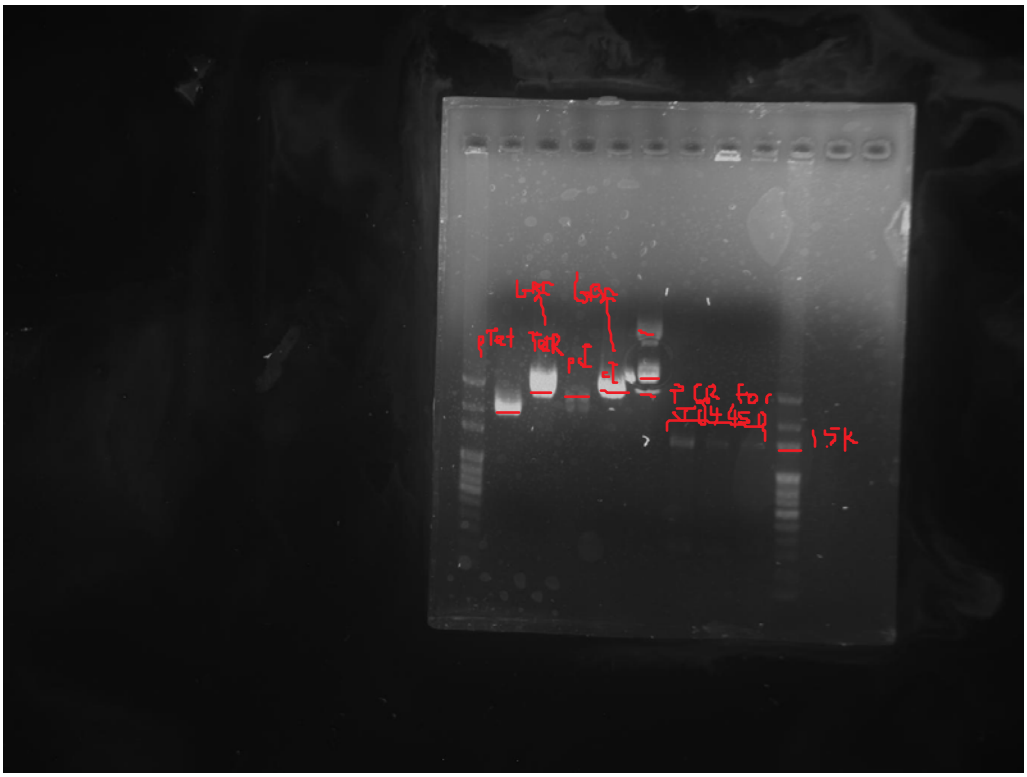
pSB1K3 should be grown.
Digestions RBS+TermA

Ligations:
pcI + LacI Gen
pTet + cI Gen
pLac + TetR Gen

- Prepared cDNA from HeLa for PCR of c-Myc and hTERT and HeLa for pSurvivin

20

Tuesday, May 20, 2014
4:07 PM



5/20/2014
pSB1K3 plasmid purification of pSB1K3 for backbone usage.
3-1
pLac+TetR Gen --> 2 colonies
pTet+cI Gen --> 3 colonies

-Gel purify GFP and go on to ligation

Ligate: pTet+GFP we have yes!
pcI+GFP Gen ©
pLac+GFP Gen (A)
pTet+cI Gen ligation through 10A assembly

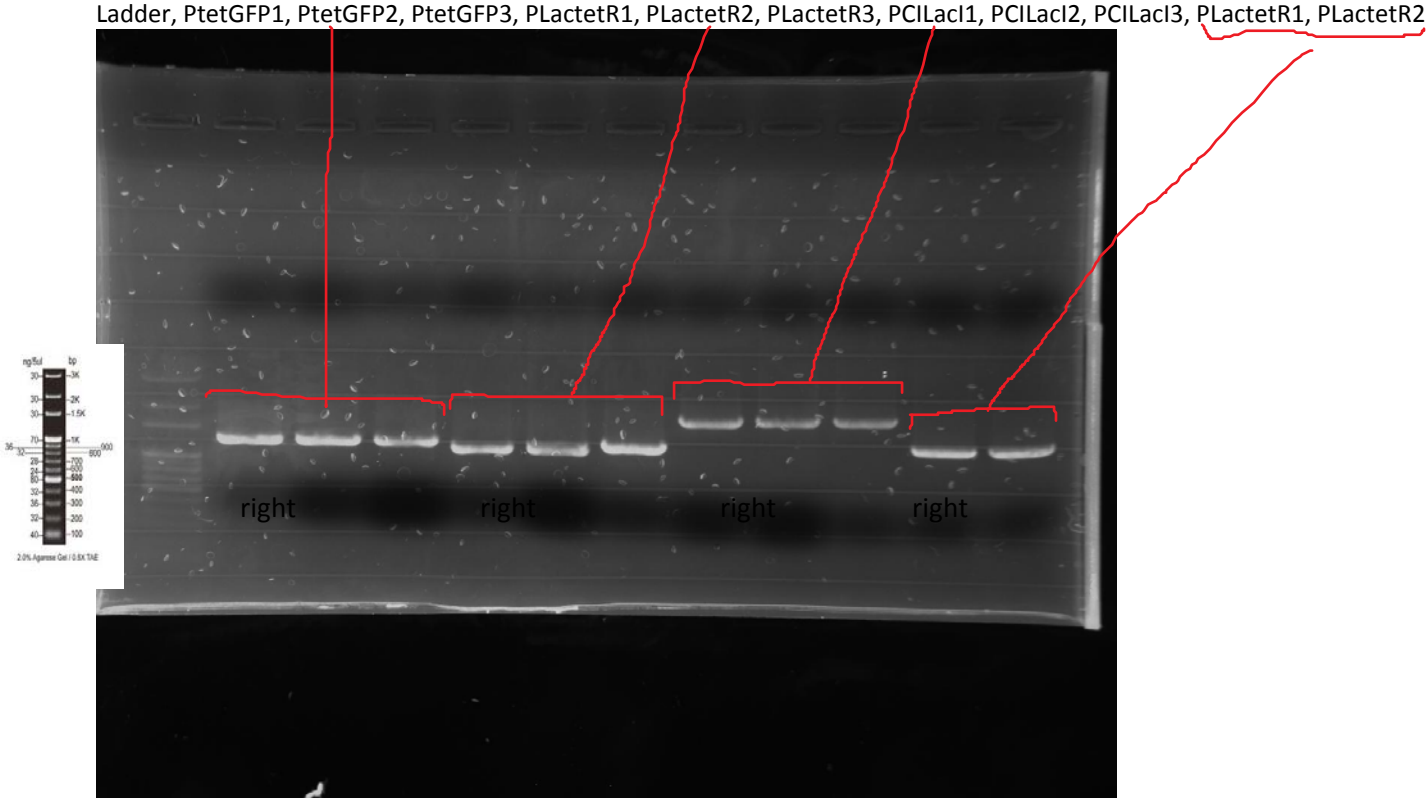
Gel
15 tubes
16 ladder

- Prepared plasmid DNA from biobrick kit for PCR of TetR/RFP

21

Wednesday, May 21, 2014
11:13 AM

Chris ran gel on PCR stuff (results=fine):
Ladder, PtetGFP1, PtetGFP2, PtetGFP3, PLactetR1, PLactetR2, PLactetR3, PCILacI1, PCILacI2, PCILacI3, PLactetR1, PLactetR2



3-1 Rohan's plate done by Giorgio
pLac+GFP 3 colonies.
Retransform pcI from biobrick --> a new plate used to be 50 mg/mL

Plates made by Leon

Plasmid Purification by Annie and Julie

Digestion of Plac, Ptet, PCI at SP by Dean

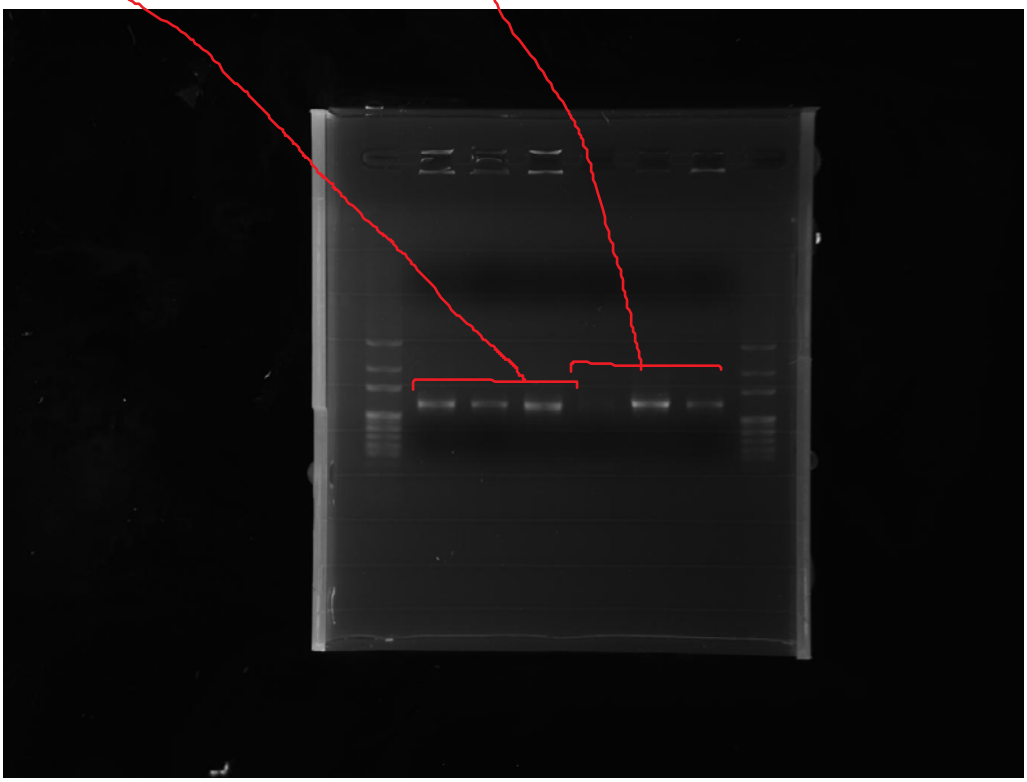
Ligation of PCI+GFPGen and Ptet+CI Gen by Beth

- PCRed out c-Myc, pSurvivin, ran gels on them. Photos are in the powerpoint with gel photos.

22

Thursday, May 22, 2014
3:36 PM

GFP Gen 1, 2, 3 and pLac+GFP Gen 1, 2, 3 PCR Check (number 1 didn't how any results)

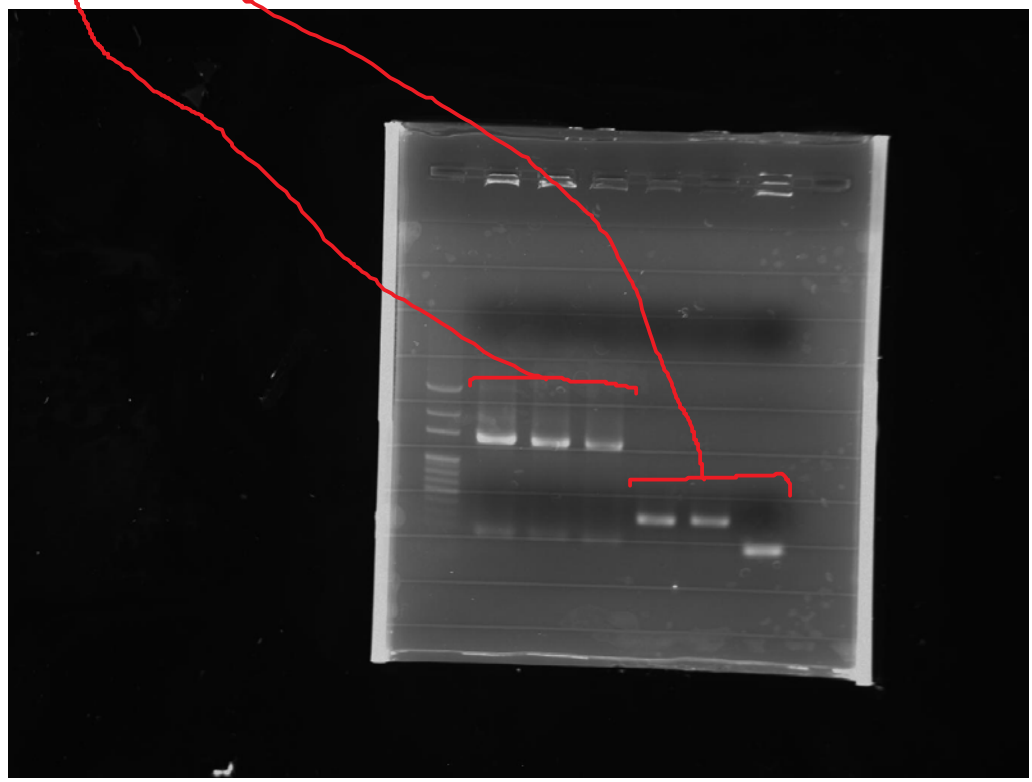


RBS+TermA 3-1 on pSB 1K3 5/21
Some of them were white so they were picked to be juxtaposed to the red ones. Turns out they were not good.
Purified plasmid for pLac+GFP Gen and GFP Gen (2/22)
Gel PCR-white and red cultures

23

Friday, May 23, 2014
3:08 PM

Chris ran gel on:
R1, R2, R3, W1, W2, W3



Hsieh did purification of:
R1, R2, R3, W1, W2, W3

1. Ran gels from the PCR for white and Red RBS +TermA on pSB1K3 backbones. They should tell us if the ligations were successful.
2. Did plasmid purification for 3-1 from the RBS+TermA (K)
3. Looking for things to digest.
4. NYMU--> Roy finished the first part of the coloning cycle (might still need the RBS+TermA because both of the parts are so short.)

Inventory check

1. pLac+TetR Gen
2. pTet+GFP Gen
3. pcI LacI Gen

Digest pLac+terR at ES And pTet+GFP at XP

PCRed out TetR/RFP, ran gels on it, showed it was correct

25

Wednesday, June 18, 2014
4:17 PM

Religated parts from the previous day.

- PCRed out hTERT
- Gel check shows it is incorrect
- Transported TetR/RFP onto BioBrick plasmid, to be used for construction of the 2 component oscillator

26

Wednesday, June 18, 2014
4:20 PM

3-1 parts from NYMU.

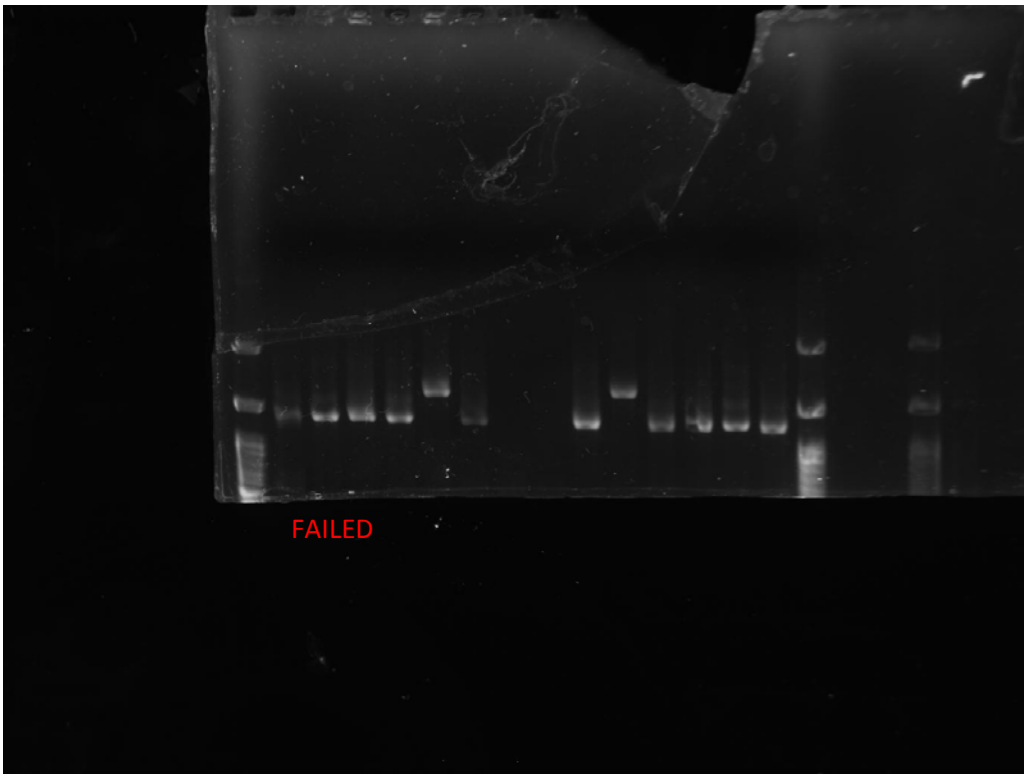
Today we changed the digestion protocol. In order to maximize the plasmid concentration, we are using most of the pure plasmid for digestion and also ligation.

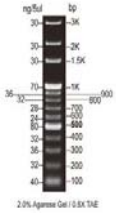
The question comes up, does the elution buffer have any impact in competing with the pH with the enzyme buffer or ligation buffer?

27

Tuesday, May 27, 2014
3:38 PM

Ran PCR gels the 3-1. It overran.
The order





Order from left to right

1. Ladder
2. pTet cI #1
3. pTet cI #2
4. pcI GFP #1
5. pcI GFP #2
6. pcI LacI #1
7. TetR Exp
8. RBS TermA #1
9. RBS TermA #2
10. TetR Exp
11. pcI LacI
12. TetR Exp
13. TetR Exp
14. TetR Exp
15. TetR Exp

Did the processes following 3-1.
This means that 14 tubes (of liquid culture) were purified.
Gels were ran for the PCR check.

Good plasmids.

Digestions

RBS+TermA done at XP 43 uL.

Chloramphenicol backbone at EP (pSB1K3)
More Kanamycin at EP (pSB1K3)
pTet+cI at ES
pcI+LacI at ES
pcI+GFP at XP

All of the promoters are digested at ES and these are on chloramphenicol except for pLac

- PCRed hTERT again using phusion, gel showed failed

28

Wednesday, June 18, 2014
4:23 PM

Ligate

1. pTet +cI at ES with pcI+GFP at XP on C resistanc
2. pLac+RBS+TermA
3. pTet+RBS+TermA
4. pcI+RBS+TermA
   ES XP

Run gels for backbones.
Running backbones on parts cut at EP.

29

Thursday, May 29, 2014
3:26 PM

Chris did 3-1 for pLac+TetR+mRFP

Phillip did transformations for the pSB1C3 backbone and purified gel parts for the backbones for both Amp and Chloramphenicol.

Need to digest the pSB1C3 at EP.

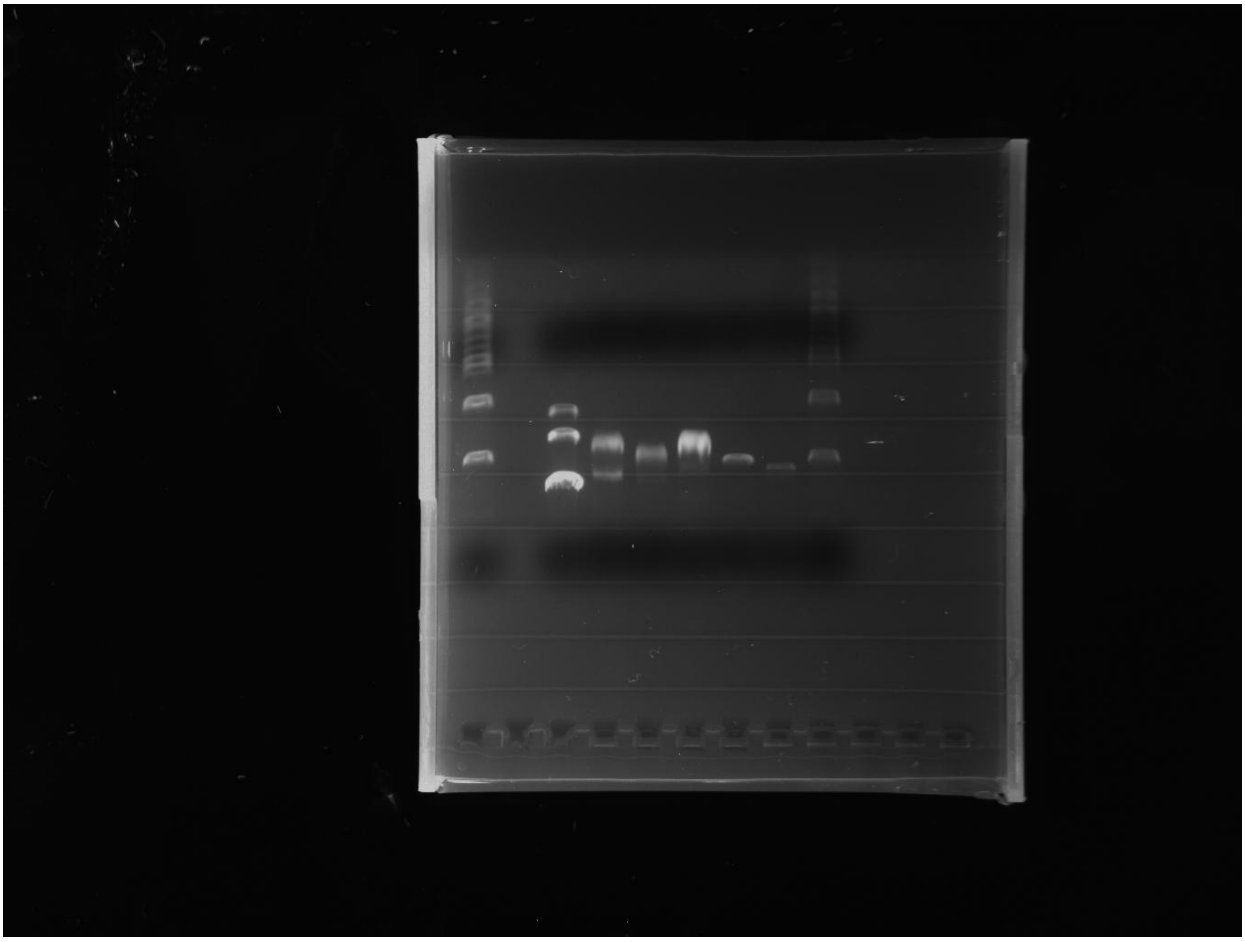
pLac+TetR+mRFP --> the point is to be later used to test if RFP and GFP can be expressed at the same time.

- PCRed hTERT again using phusion and GC buffer, gel showed failed

30

Friday, May 30, 2014
4:59 PM

1	Ladder
2	
3	Plac tetI 1ES RFP DP
4	Ptet cI #2 5/27
5	PCI Lac I #1 5/27
6	PCI GFP #2 5/27
7	PSB1 C3 SP #1
8	PSB1 C3 SP #2
9	Ladder
10	
11	
12	



Plac Tet1 1ES RFP DP
pTet CI

Digested more pSB1C3 backbone
Ligate

1. pLac+TetR+mRFP with pTet+GFP Gen XP which is good
   Should be done together.
   Then ran gels on PP.

1. pTet+cI+pcI+GFP Gen ligation
   a. Finished lgiation of pcI Experiment!
2. pLac+GFP Gen (5/21 had 3-1 from Rohan's plates, they should be purified plasmid form!
3. Purify plasmid for pLac+TetR+mRFP

Digest

We have RBS+TermA ready.

- Need to digest @Xp
- Ligate to the pLac, pTet, and pcI

Final plan: Transform RBS+TermA on K plates.

31

Wednesday, June 18, 2014
4:30 PM

Gel:

1. Ladder
2. pSB1C3
3. pSB1C3
4. pTet cI #1
5. pTet cI #2
6. pcI GFP #1
7. pcI GFP #2
8. pcI LacI #1
9. RBS TermA #1
10. RBS TermA #2
11. LADDER

Run gel purification for pSB1C3 and PCR results for pSB1C3 (RFP)
What about LacI Exp?
LacI+GFP Gen
PCR check for TetR Exp.
3 pSB1C3 backbone grew!
pcI Exp
pTet+cI+pcI+GFP Gen
RBS+ TermA