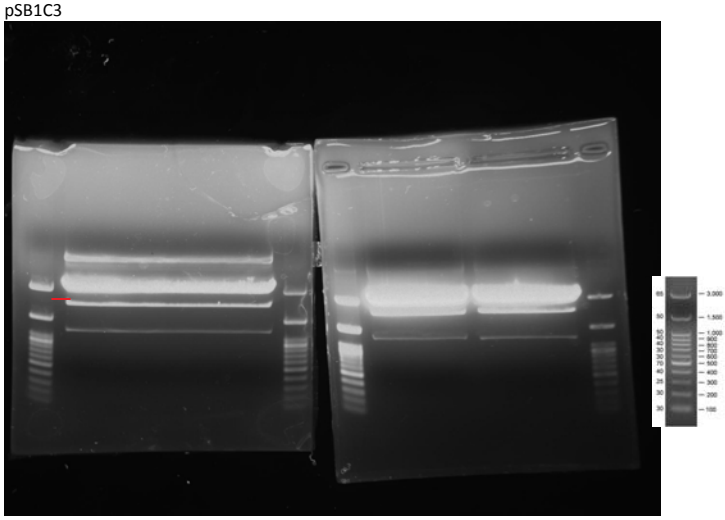
2

Monday, June 02, 2014
4:07 PM

pSB1C3



Purified plasmid for pSB1C3 digested at EP and gel purification to separate out the vector and insert.

1. Did 3-1 for some colonies of RBS+ TermA. However RFP was heavily expressed.

The cI Exp: pTet+cI Gen+pcI +GFP Gen seems to be fine

Two colonies were picked for PCR confirmation.

Total expected base pair length should be around 1959.

Trying ligation with 10 uL total

3

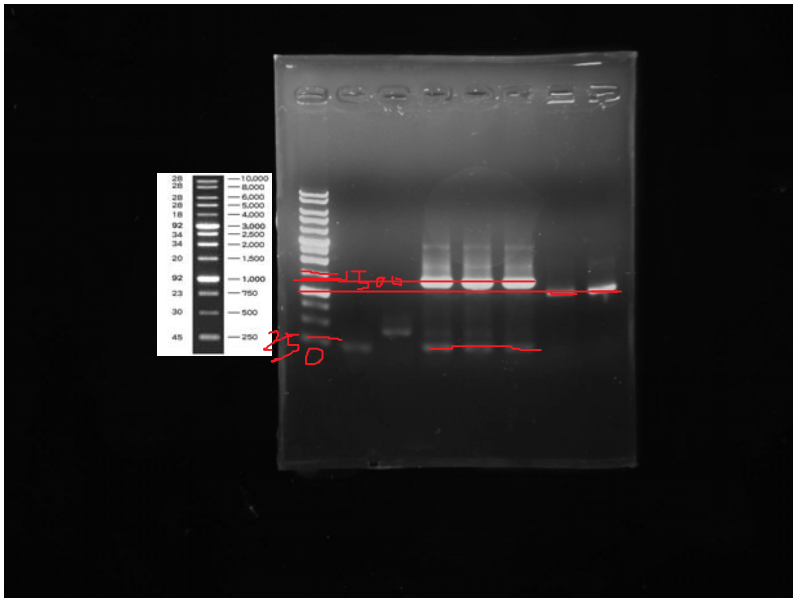
Tuesday, June 03, 2014
1:30 PM

PCR Check:

- 1 - pLac+RBS+TermA
- 2 - pTet+RBS+TermA
- 3 - pCI+RBS+TermA

Gel(Left to Right w/ wells on top)

- RBS Term 1
- RBS Term 2
- PSB1C3 1
- PSB1C3 2
- PSB1C3 3
- CI EXP 1
- CI EXP 2



RBS+Term

Edward did 3-1 on pLac+RBS+DT, pTet+RBS+DT, pcI+RBS+DT and ran gels for PCR from previous experiments.

Gel check
cI Exp showed that it was around 1k bp which is not right because it should be around 2kbp.

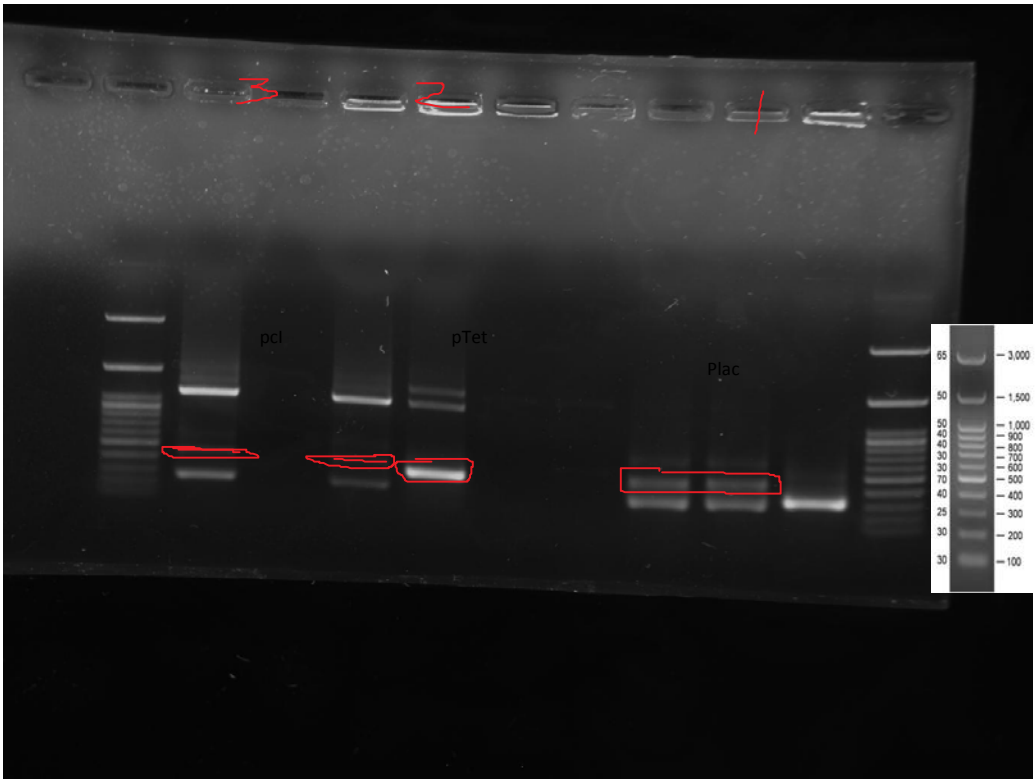
RBS+TermA is wrong because it should be around 12+129=141 and then you add the resistance which makes it around 400.

Can we store PCR checks in the -20 until the next day?

How do you do calculate how much to add to PCR checks in Gel?
For K resistance, it should be around 305 to add.
For C resistance, you add 314.
For A resistance, you add 238.

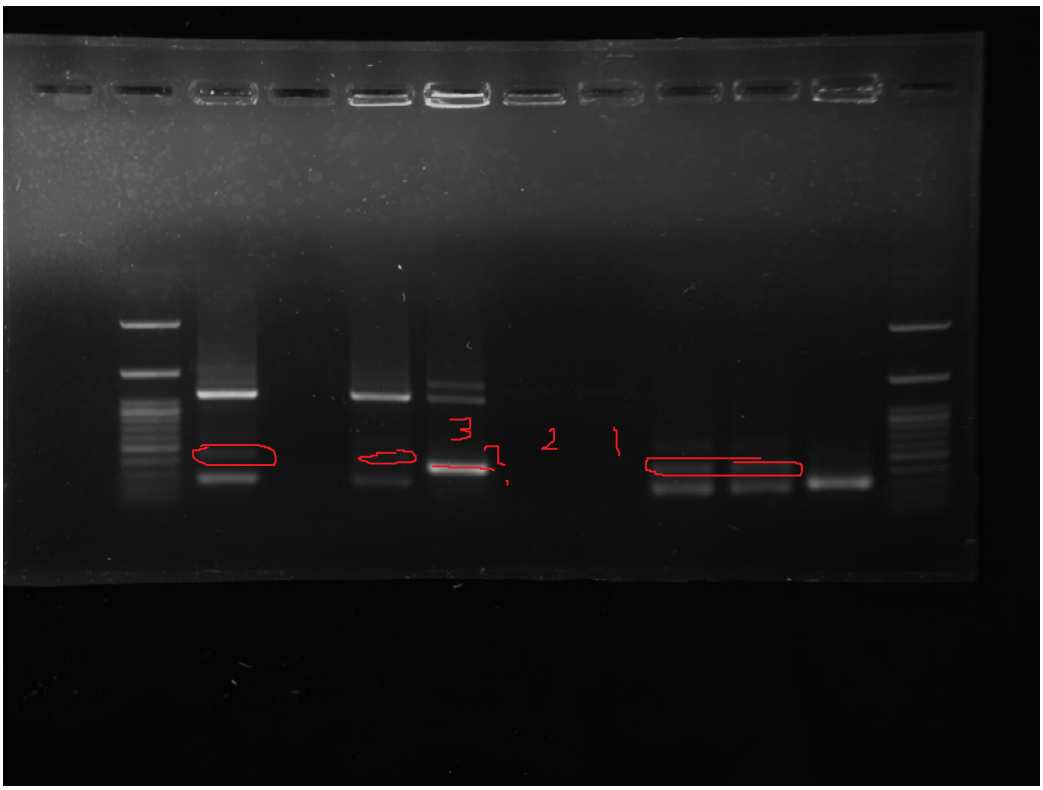
4

Wednesday, June 04, 2014
11:27 AM



PCR Check:

- 1 - pLac+RBS+TermA
- 2 - pTet+RBS+TermA
- 3 - pCI+RBS+TermA



PCR check results.

2. pTet+RBS+DT=195
1. pLac+RBS+DT=196
2. pcI+RBS+DT= 238

Ligations pLac+TetR+mRFP with pTet+GFP Gen

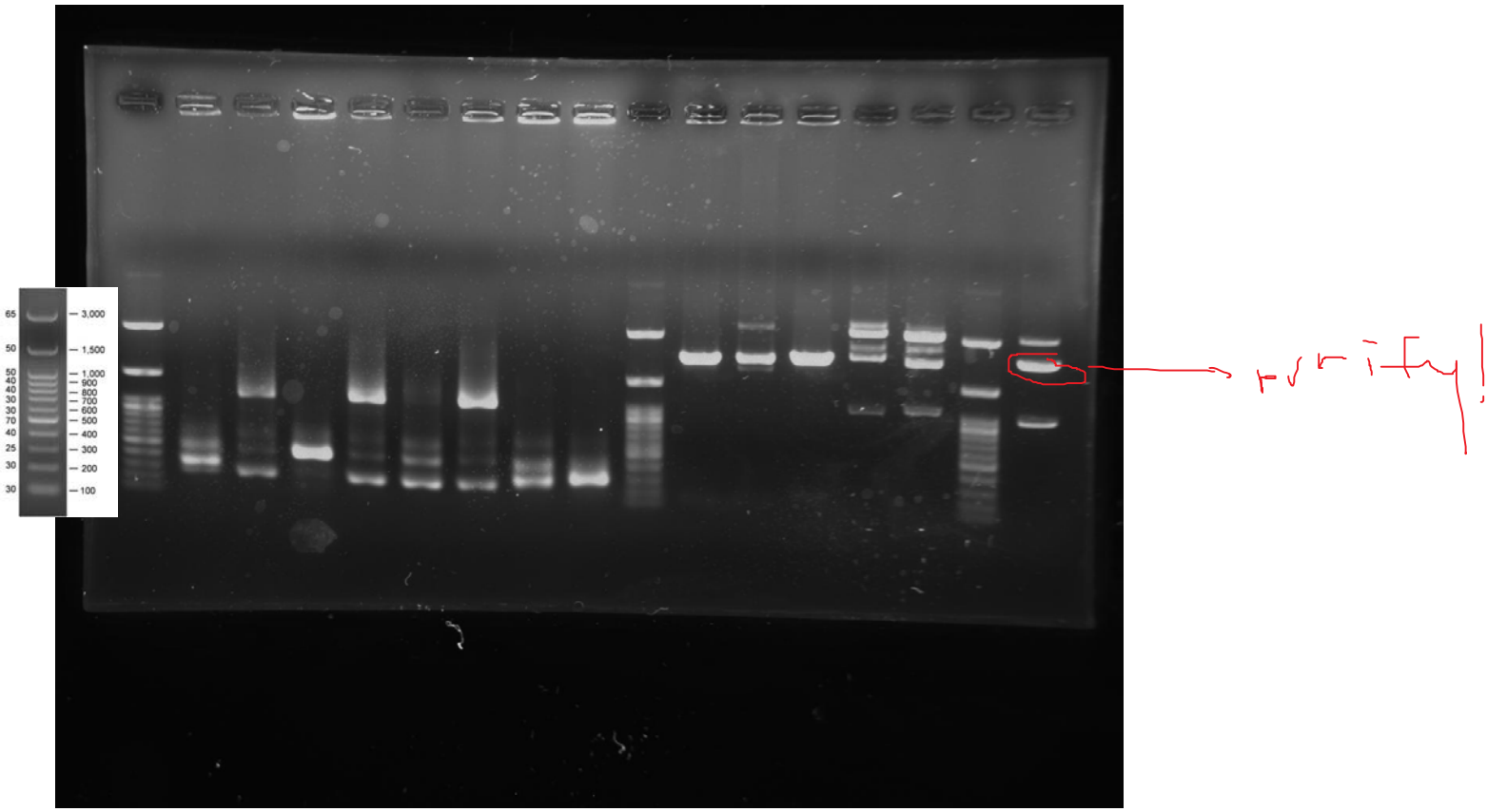
1. pcI Control

2. pTet Control.

Digestions: pLac+GFP Gen is being digested along with pSB1K3 (which needs to gel purified)

5

Thursday, June 05, 2014
11:28 AM



PCR for pLac+TetR Gen +mRFP +pTet+GFP Gen

Length: 55+840+706+54+878=2,533

1. Run gels on PCr results +some digested parts.

Order

1. Ladder
2. pcl control 1
3. "" 2
4. RBS+DT 1
5. pTet Control 1
6. RDT 2
7. pTet Control 2
8. pLac 1
9. "" 2
10. Ladder
11. pTet ES
12. pLac ES
13. pcl ES
14. pTet+GFP 5/23 XP
15. pcl GFP 5/27 XP
16. pLac GFP 6/4 XP
17. Ladder
18. pSB1C3 6/4 EP

Need to run gels for RBS+DT to make sure that it becomes ligated to their respective promoters.

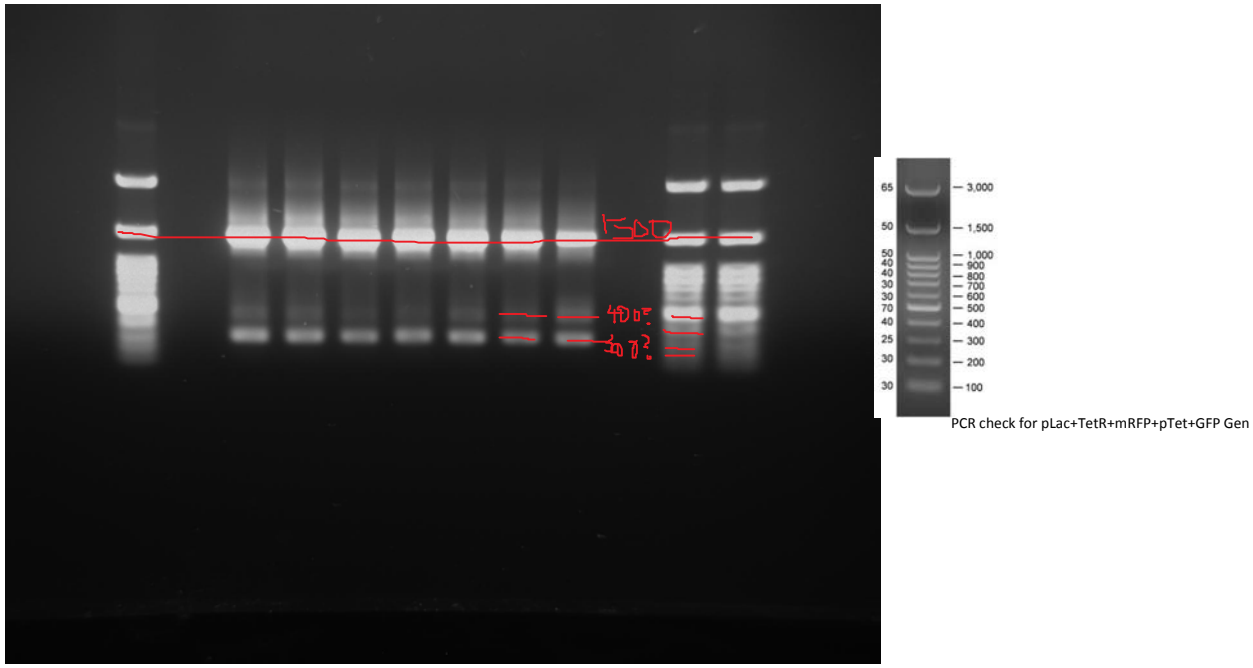
There were many bands, doesn't seem like a lot of the plasmids we have right…

2. Finished gel purification of pSB1K3 which were gel checked yesterday.
3. Purifying plasmids from above.

Rachel pLac+TetR+mRFP+pTet+GFP Gen
Should be 55+840+706+54+878=2,533

6

Thursday, June 05, 2014
1:58 PM

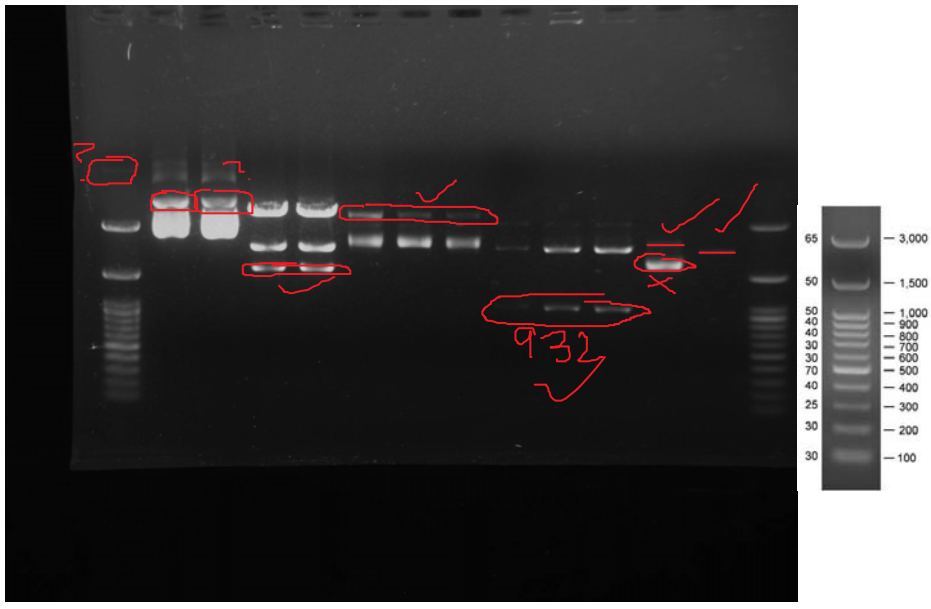


PCR check for pLac+TetR+mRFP+pTet+GFP Gen

Possible hypothesis:
Perhaps the whole construct did not ligate together properly and they were only composed of one specific part.
This means that the pLac+TetR+mRFP could be the only construct within the bacteria.
The expected length of this would then therefore be 55+12+685+706+129=1,587

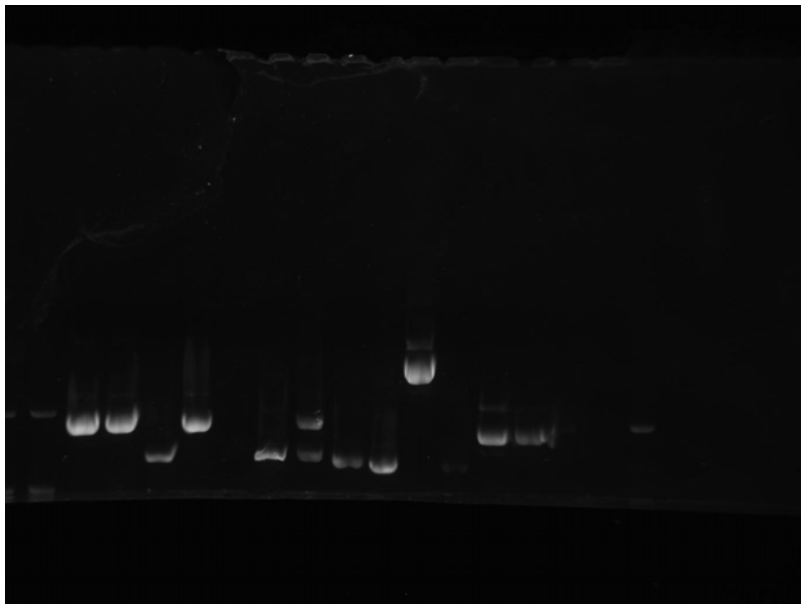
This is Rohan's box, and the results showed that there are multiple bands for the plasmids that were used.
The order is the following from left to right.

1. Ladder
2. K resistance pLac+TetR+mRFP #2 5/30
3. K resistance pLac+TetR+mRFP #3 5/30
4. pLac+TetR+mRFP cut at ES 2
5. pLac+TetR+mRFP cut at ES 3
6. pTet+GFP #1
7. pTet +GFP#2
8. pTet+GFP #3
9. pTet+GFP cut at XP #1
10. pTet+GFP cut at XP #2
11. pTet+GFP cut at XP #3
12. pSB1K3 2 5/27
13. pSB1C3 EP 6/4

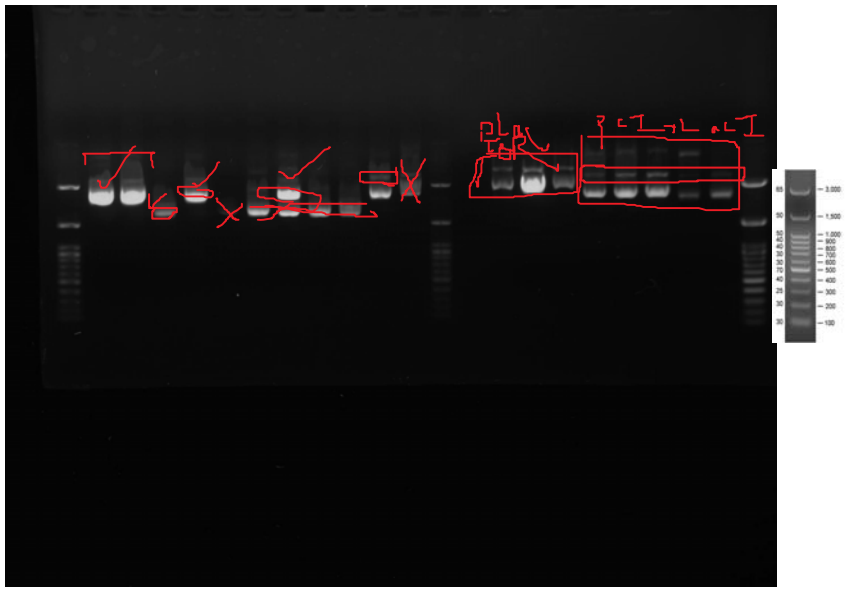


Questions:
Contamination of the ladder because there is sign of a plasmid greater than 3k bp.

pLac+TetR ES seems fine, but the presence of three bands means that the other bands were also cut, and if these were the plasmids that were in the original pLac+TetR+mRFP then doesn't that mean that it could possibly interfere with the plasmid that is actually inserted into the final construct?
In addition, the pTet+GFP plasmids are definitely in there however again, there are 2 other bands that are present in the digested part for pTET+GFP. This is very weird.
Finally, even the pSB1K3 backbone does not seem to have been purified correctly because there is an additional band at around 1.7k or something, which also means that we will need to run gel purification for the backbones.



Ran off rerun on the bottom

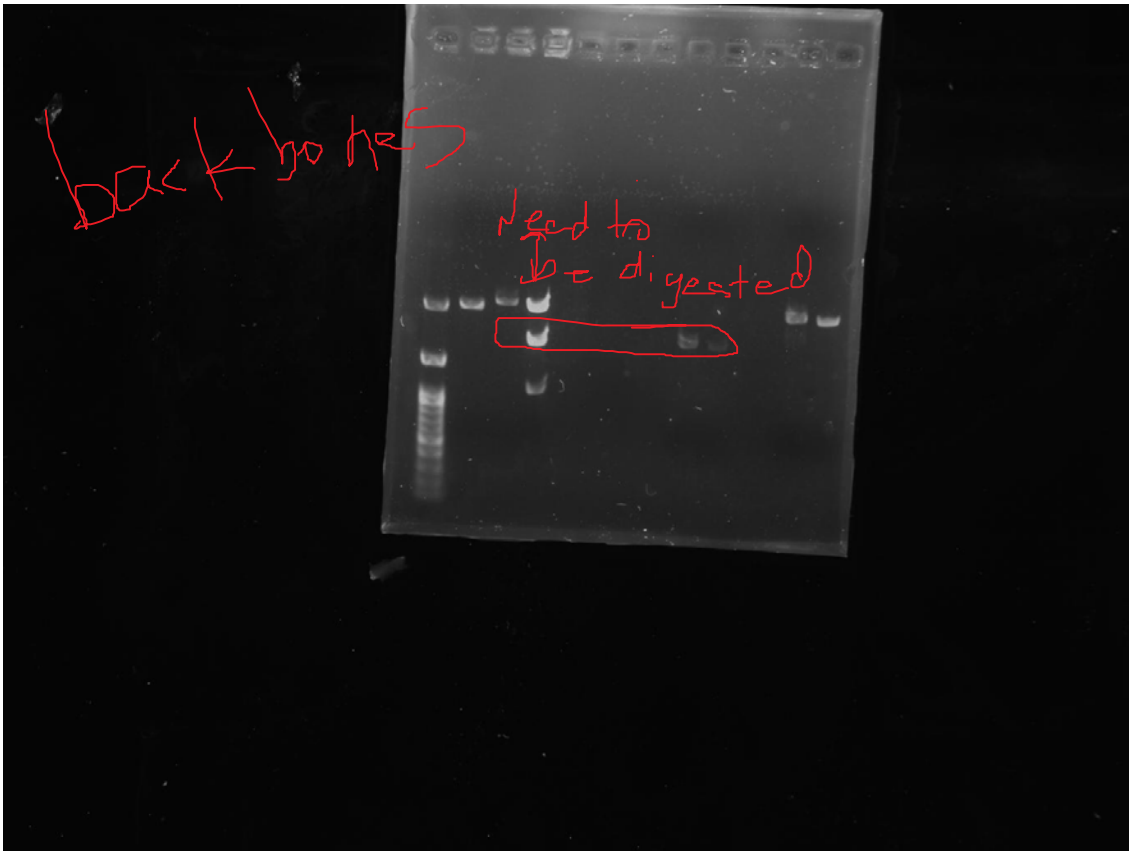


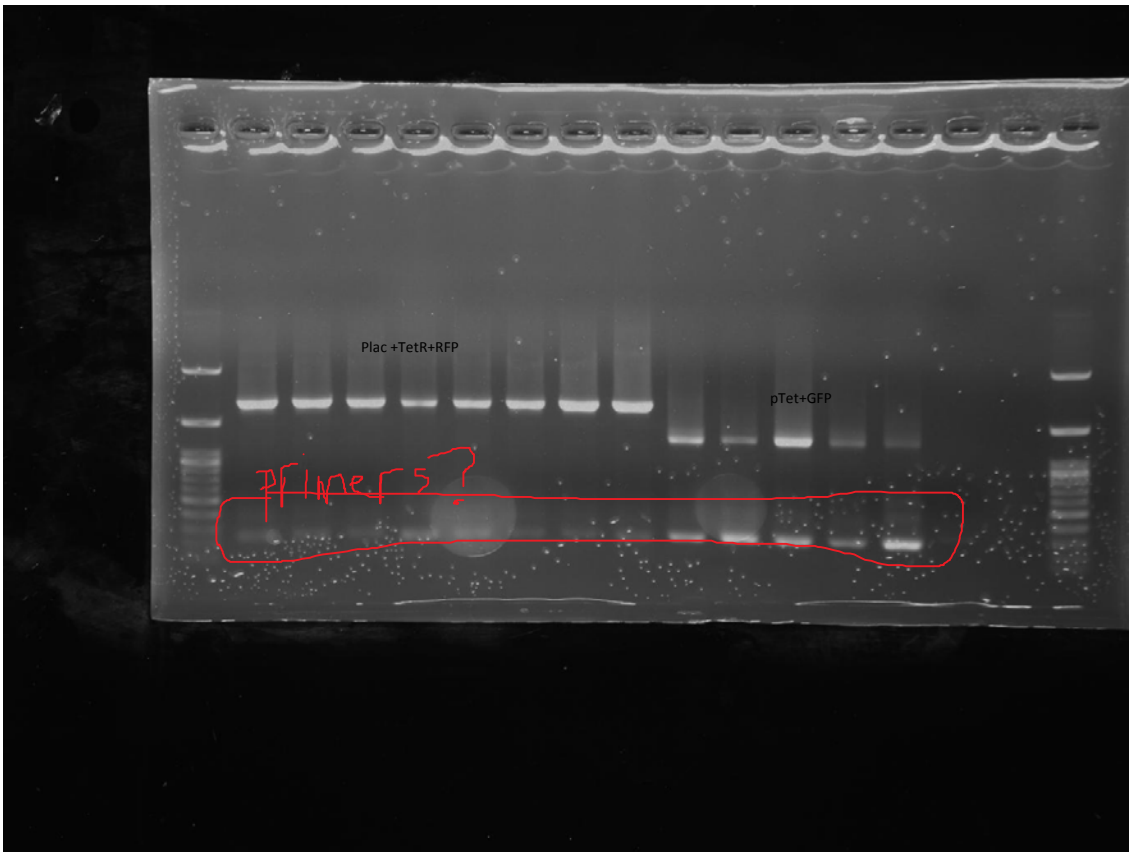
Order from left to right:

1. Ladder
2. pTet+RBS+DT 6/5 1 A
3. pTet+RBS+DT 6/5 2 A
4. pcI+RBS+DT 1 6/59 A
5. pcI+RBS+DT 2 6/5 A
6. pLac+RBS+DT 1 6/5 A
7. ac
8. RBS+DT 2 6/5 K
9. RBS+DT 3 6/5 K
10. RBS+DT 4 6/5 K
11. pcI +GFP Gen C
12. pLac+GFP Gen K
13. Ladder
14. Blank
15. pLac +TetR 1 5/21 K
16. pLac+TetR 2 5/21 K
17. pLac+TetR 3 5/21 K
18. pcI+LacI 1 C
19. pcI+LacI 2 C
20. pcI+LacI 1 5/27 C
21. pcI+LacI 2 5/21 C
22. pcI+LacI 1 5/27 C
23. Ladder

Expected length

1. Ladder
2. 129+54+12+2155=2350
3. Ibid
4. 141+97+2155=2393
5. Ibid
6. 55+129+12+2155=2351
7. 141+2204=2345
8. Ibid
9. Ibid
10. Ibid
11. 97+878+2070=3045
12. 55+878+ ? 55+878+2204=3137
13. Ladder
14. Blank
15. 97+930+2070=3097
16. Ibid
17. Ibid
18. 55+840+2204=3099
19. Ibid
20. Ibid
21. 97+930+2070=3097
22. Ibid
23. Ladder


backbones
Need to
be digested


Plac +TetR+RFP
pTet+GFP
primers?

8

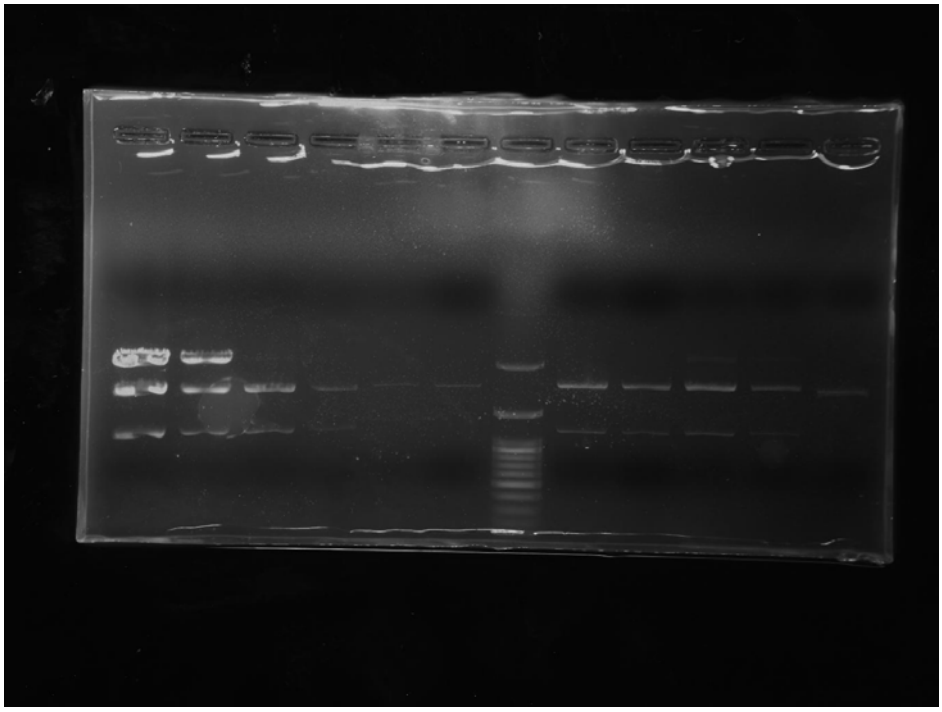
Friday, June 06, 2014
11:08 AM

1. Purified plasmid for pLac+TetR+mRFP --> rachel
2. Did 2-1 for pTet+GFP Gen (PCR check liquid culture)
3. Rohan is trying out pLac+TetR+mRFP ligation to pTet +GFP Gen
4. Checked the resistance for pLac +GFP Gen (K resistance)

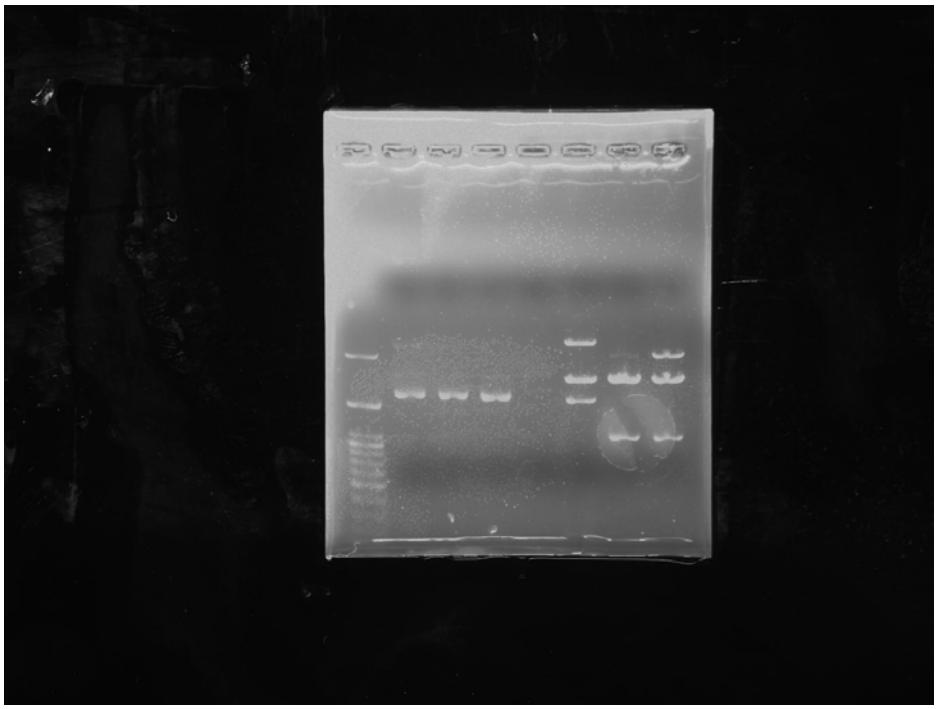
9

Friday, June 06, 2014
5:38 PM

Purified plasmid for pLac+ TetR+ mRFP.
Did 2-1 for pTet Gen (PCr



Backbone check:



1. Ladder
2. pLac4
3. pLac5
4. pLac1
5. pLac2
6. pLac+TetR/RFP Digested Part
7. pTet+GFP Gen 1 6/9 Digested Part
8. pTet+GFP Gen 2 6/9 Digested Part

Purify plasmid for 36 tubes --> Julie, Bethany, Dean, Rachel
30 backbones and 6 tubes of pTet+GFP Gen

Need liquid culture at 4 for pTet x3. pcI x3. pLac x3

Autoclave more centrifuge tubes --> Brian

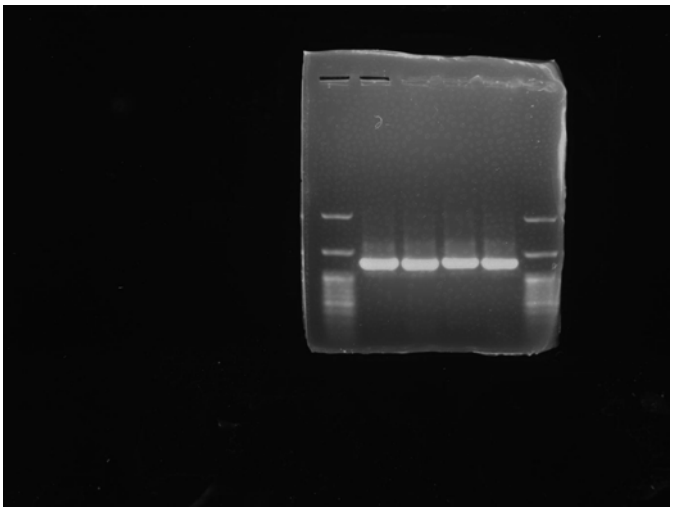
Get primers we are running out!

Need to digest pLac+GFP Gen at XP --> seems good, the transformations grew on K plates therefore the resistance is K!

10

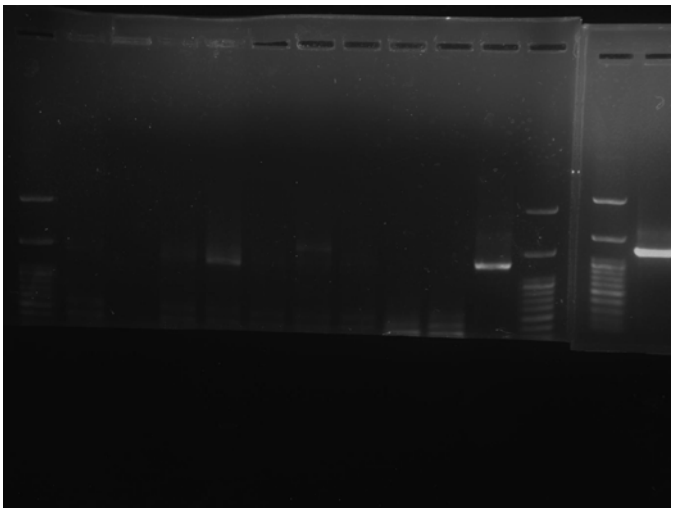
Sunday, June 08, 2014
1:23 PM

Ran gels for PCR items from the 2-1. This is done to confirm if the liquid cultures are correct or not.



PCR CHECK FOR THE 14 LIQUID CULTURE TUBES STRAIGHT FROM NYMU.
THIS STUFF SHOULD BE GOOD.

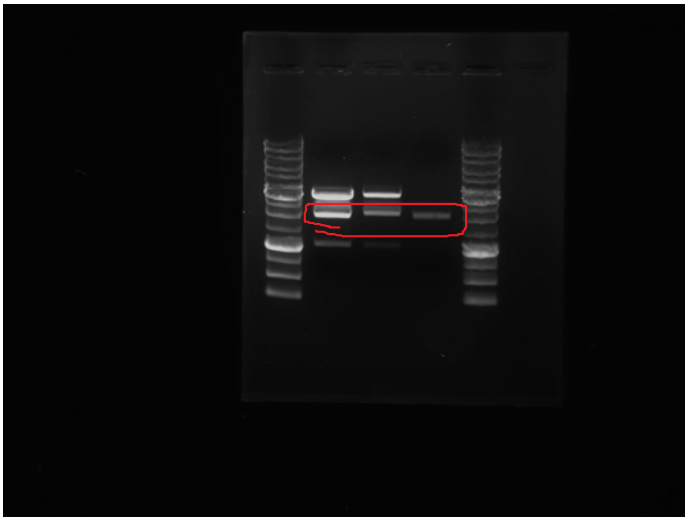
1. Ladder
2. pLac+TetR 1
3. pLac+TetR 2
4. pTet+GFP 1
5. pTet+GFP 2
6. Ladder



The gel ran off because the timer was set for 30 minutes. Next time the timer should be set at 15 minutes and check how far the gel has ran before we add an additional 5 minutes. Sometimes the gel runs faster tan other times because of TAE concentration differences and also current differences. Therefore it is vital that only 15 minute is set for the first time, this ensures that the DNA does not run off the gel.

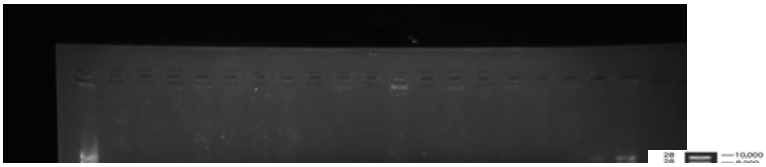
But the order is

1. Ladder
2. pTet+cI 1
3. pLac+GFP 1
4. pTet+cI 2
5. pcI+GFP2
6. pcI+LacI 1
7. pcI+LacI 2
8. RBS+DT 1
9. RBS+DT 2
10. pLac+GFP 2
11. Ladder

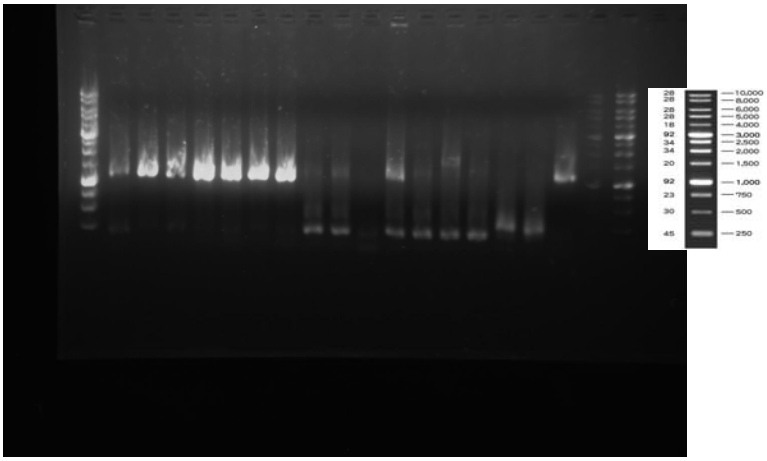


1. Ladder
2. pSB1C3
3. pSB1K3
4. pSB1A3
5. Ladder

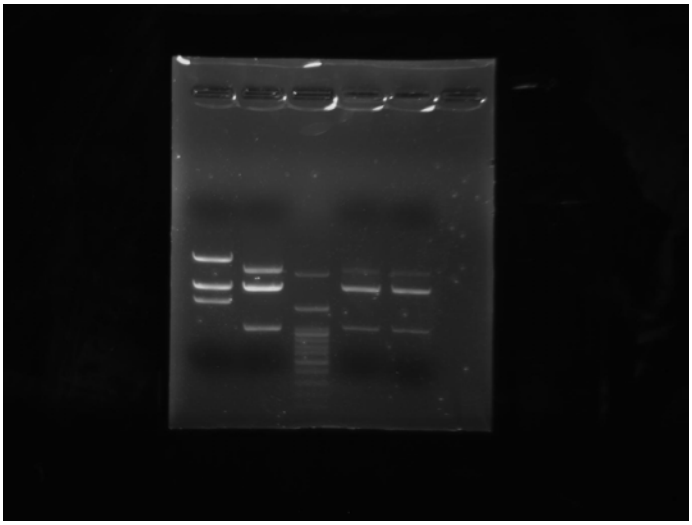
They were all cut and put in the UGP box for gel purification.



1. Ladder
2. pLac+GFP 2
3. pLac+GFP 3
4. pLac+GFP 1
5. pLac+TetR 1



1. Ladder
2. pLac+GFP 2
3. pLac+GFP 3
4. pLac+GFP 1
5. pLac+TetR 1
6. pLac+GFP 1
7. pTet+GFP 2
8. pTet+cI 1
9. pTet+cI 2
10. pLac+RFP (GFP?) 1
11. pcI+GFP 1
12. pcI+GFP 2
13. pcI+LacI 1
14. pcI+LacI 2
15. RBS DT?
16. RBS DT?
17. pLAc+GFP 2
18. Ladder
19. Ladder



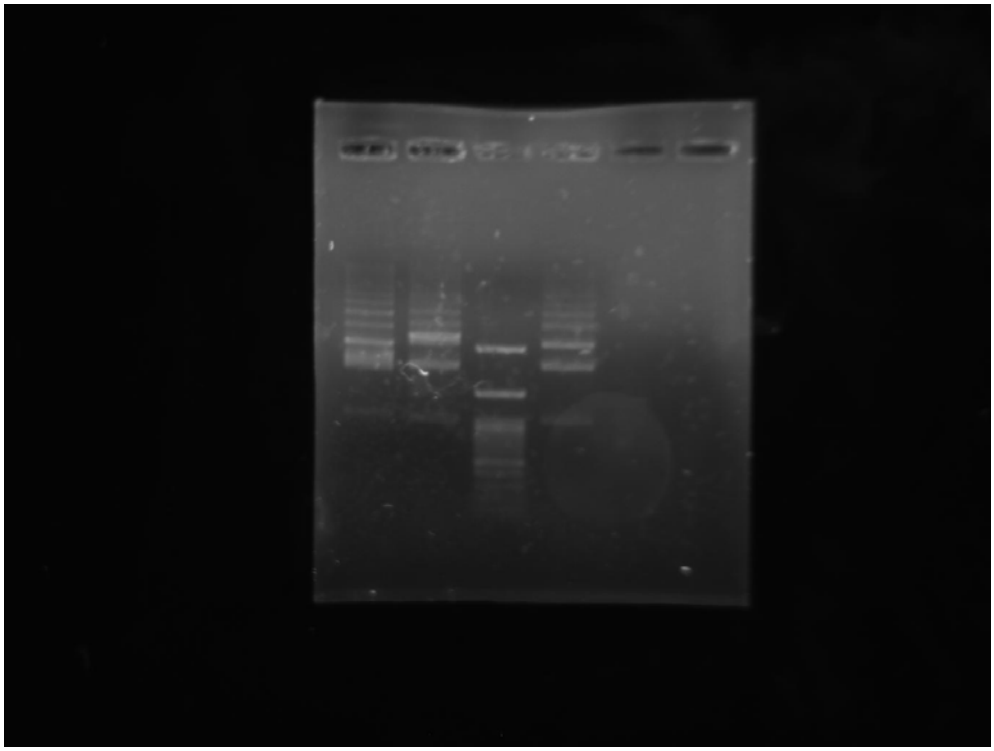
1. pLAc+TetR/RFP ES
2. pTet+GFP XP
3. Ladder
4. pTet+GFP XP
5. pTet+GFP XP

Ultimately we ligated 3 of the experiments.
Results: Morning didn't turn out good.

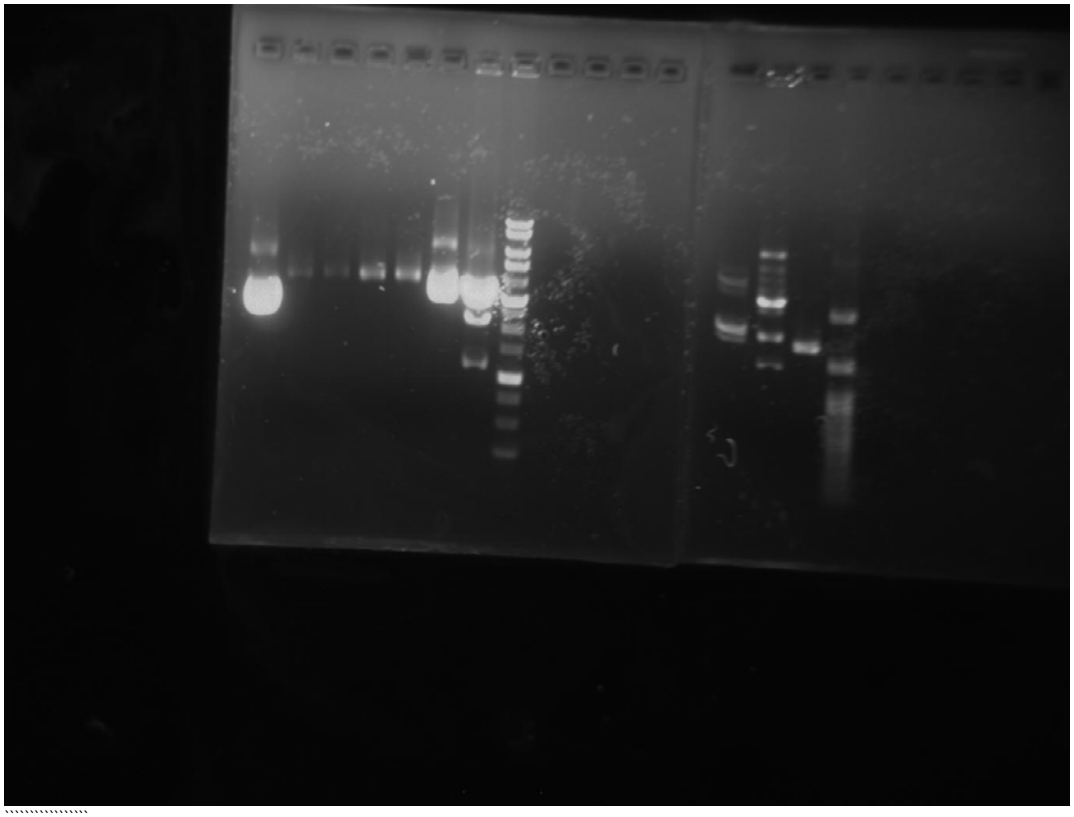
Reattempted the experimental constructs that shows repression of the promoters.
Ran gel check for the ligated part. Seems to have multiple bands greater than 3k. Therefore one of the bands may be the correct band.

11

Monday, June 09, 2014
12:46 PM



1. A pcl EXP ligation: pTet+cl Gen+pcl+GFP Gen=4144
2. A pLac EXP ligation: pcl+LacI Gen+pLac+GFP Gen=4493
3. Ladder
4. A pTet EXP ligation: pLac+TetR Gen+pTet+GFP Gen=3982



1. pcl @SP PDP C
2. LacI Gen XP XP C
3. pcl PP C
4. Ladder

- PCRed out pSurvivin+GFP and pSurvivin again
- Transported pSurvivn and pSurvivin+GFP both to pUC plasmids

12

Tuesday, June 10, 2014
7:37 PM

Purified Plasmids for all the promoters (pLac, pTet, pcI) at both ES and SP.
Gel Results:

We will finish ligation of this product with RBS+TermA with the promoters.

13

Tuesday, June 10, 2014
12:44 PM

Everything that Phillip needs to get done:

1. Ligation of pLac, pTet, and pcl with RBS+TermA (already cut at XP). Do these on pSB1A3 backbone. The protocol for ligation is to create a solution with a total of 20uL inside a PCR tube. We insert 6 upstream (ES), 6 downstream (XP), and 5 backbone (PDP pSB1A3), then add 2 10x ligase buffer and 1 ligase.
2. After the ligations are done we will transform them using 3 uL of the ligated product and add 50 uL of competent cells.
3. Transformation of pLac+TetR+pTet+GFP and pLac+pcl
4.

pLac+TetR Gen	ES	pTet+GFP Gen	XP
pTet+cl Gen	ES	pcl + GFP Gen	XP
pcl+LacI Gen	ES	pLac + GFP Gen	XP

5. Same ligation protol as above, the ES is upstream and the XP is downstream.

If the ligation for the promoters with RBS+TermA are successful (colonies grew) they should be picked (recommend at least 3 colonies) and done 3-1.

3-1 Protocol

Taq Mix RATIO (Mix all the solutions together in a centrifuge tube first and then transfer them into individual PCR tubes).

1. 8 ddH20
2. 10 Taq 2x Mastermix
3. 2 Primers (they should be mix called VF2 and VR)

The antibiotic for the liquid cultures should correspond to the antibiotic resistance of the culture which is Ampicillin for the ligation of promoters with RBS+TermA

1. Add 4.5 mL of LB
2. Add 4.5 uL of Ampicillin (in the -20 box labeled antibiotics)

Streak

After 3-1 is done, wait for 16 hours.

Then do plasmid purification. Then digest the plasmid at ES.

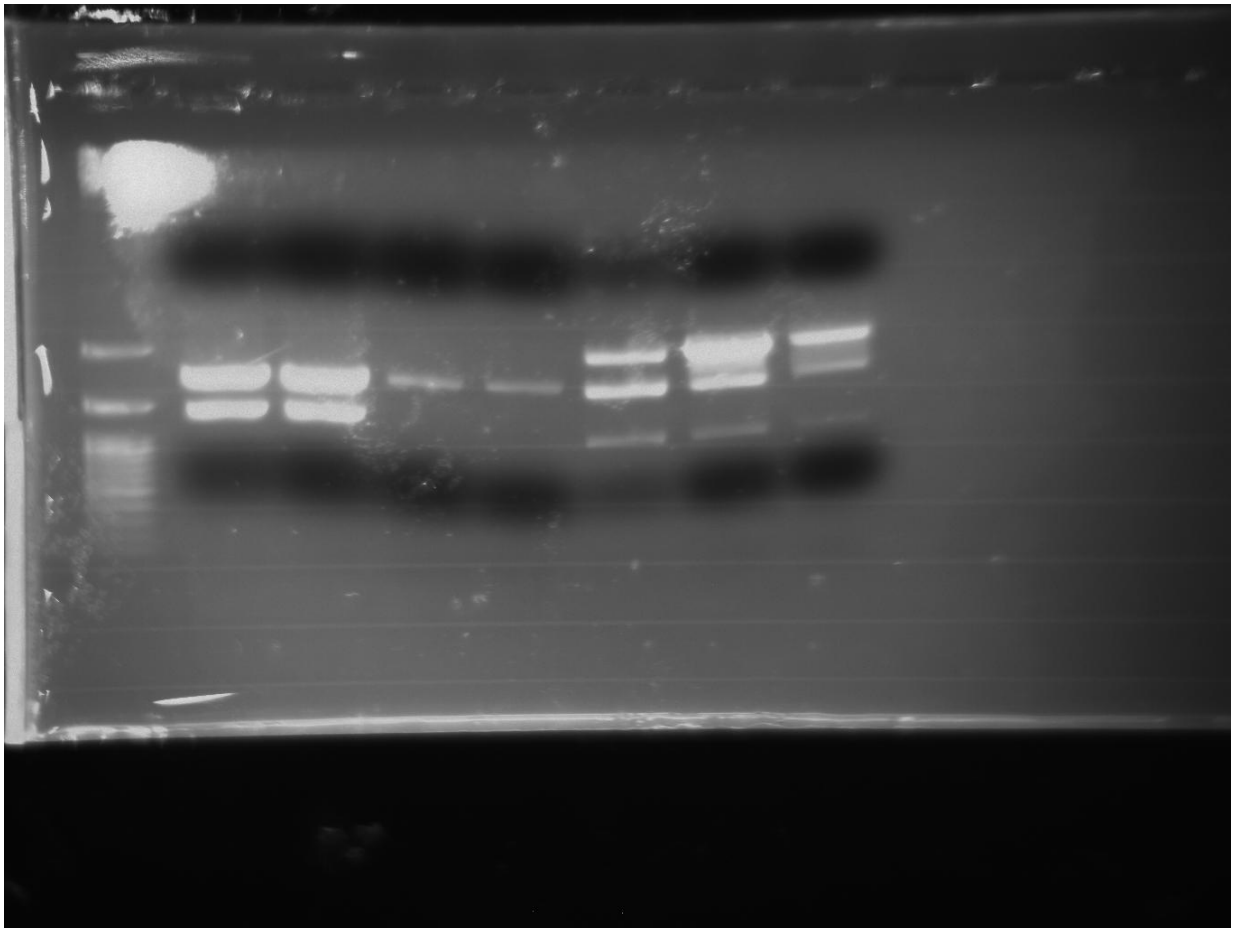
Digestion Protocol

1. Add 43 of the plasmid
2. Add 5 10x 2.1 buffer
3. Add 1 of EcoR-H1 (or something like that)
4. Add 1 uL of SpeI

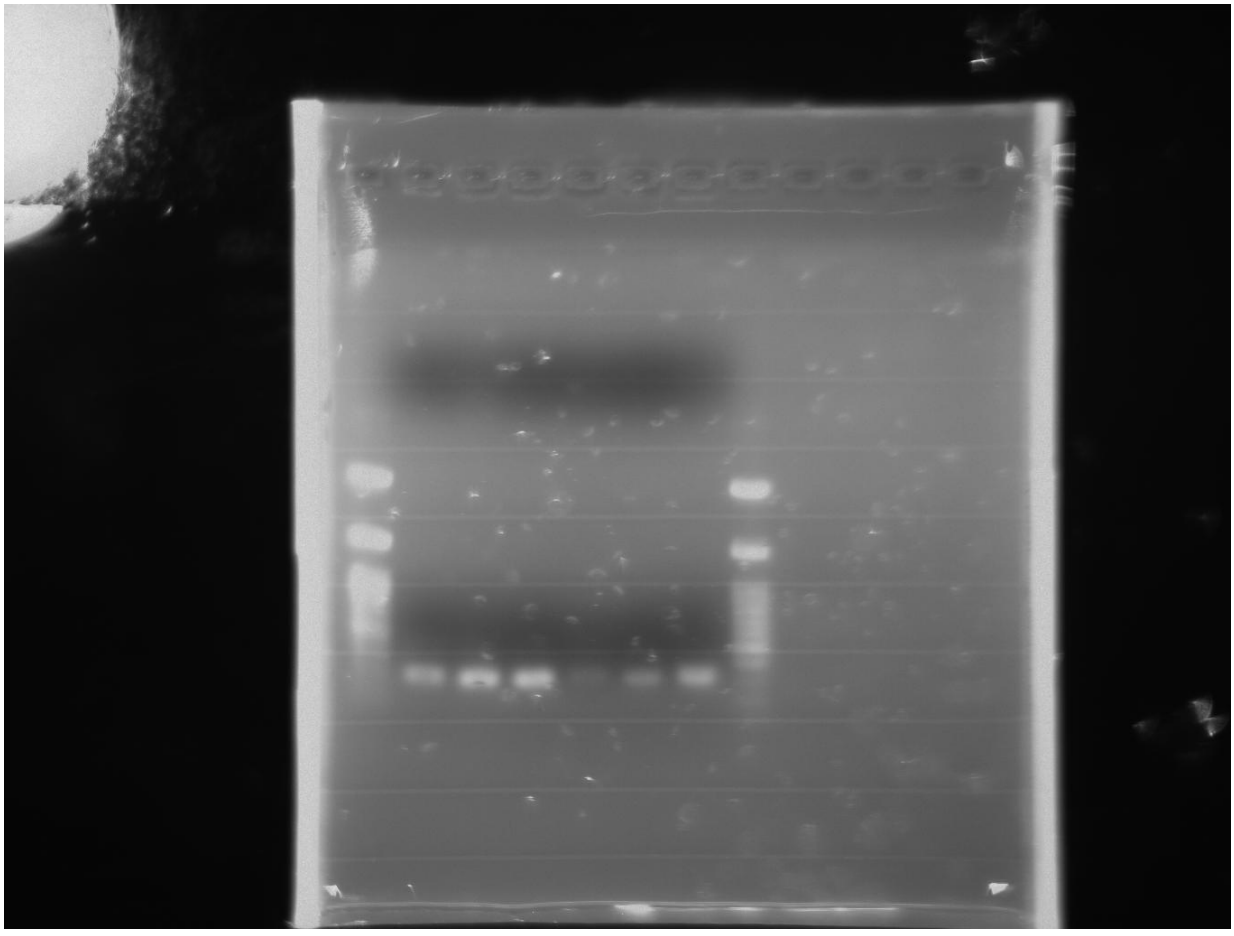
IMPORTANT REMINDER: THE GREAT PART OF THE PIPETTER SHOULD NEVER TOUCH THE INSIDE OF THE CONTAINERS OF THE ENZYMES. ONLY THE AUTOCLAVED PORTION OF THE TIP SHOULD TOUCH.

ROHAN

- Ligate the pLac, pcl, and pTet, which are cut at ES, to the LacI Gen which is currently cut at XP, remember this is 3A assembly. Backbone needs to be cut at EP and then purified
    - Transform the construct
- 3-1 the transformation of pLac+LacI gen, pcl+LacI gen, pTet+LacI gen
- Plasmid extract from the cultures
- Digest the three plasmids at XP, digest K1253000 at ES
- Ligate K1253000 with each of the three plasmids which have been digested, use a different backbone from the two plasmids being ligated.
- Testing time.



Ladder
Laci gen xp c
Laci gen xp c
Psb1 c ep
Psb1 c ep
Psb1 a ep
Psb1 a ep
Psb1 k ep



Ladder
Plac exp 1
Plac exp 2
Plac exp 3
Plac exp 4
Plac exp 5
Ptet exp
ladder

14

Friday, June 13, 2014
1:13 PM

Take out the liquid cultures from yesterday 3-1 for the experiments

2. Run gel check for the PCR tubes.

Primers concentration
100 uM in stock solution after being hydrated.
10 uM can be made by simply adding 9x the original volume of the solution (10x dilution)

So then this solution can be added to the Taq MasterMix with a total of 20 uL. This means that the final concentration will fall under 0.5 uM which is perfect for PCR.

Ran gels for the pTet Experiment and also pLac experiment PCR results from yesterday.

- Tranfected pSurvivin+GFP into MRC-5 cells

15

Friday, June 13, 2014
1:13 PM

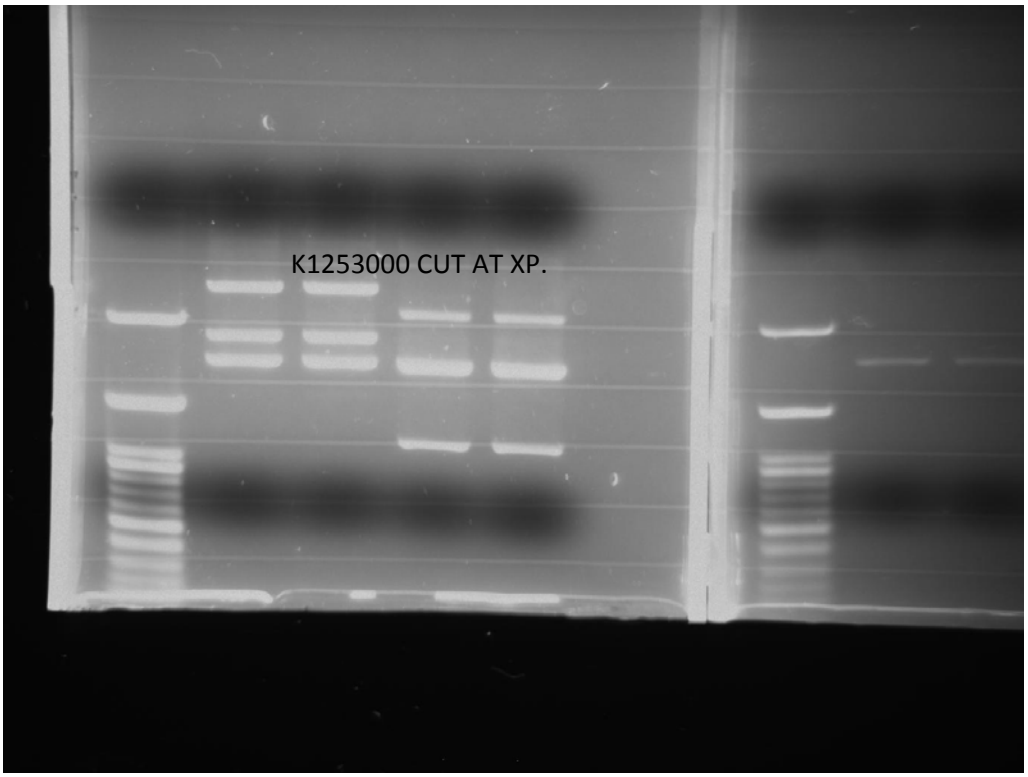
The plates for pcI+RBS+DT, pTet+RBS+DT, pLAc+RBS+DT all grew.

Therefore I needed to 2-1 which includes the Taq mix and the liquid culture for plasmid purification tomorrow.

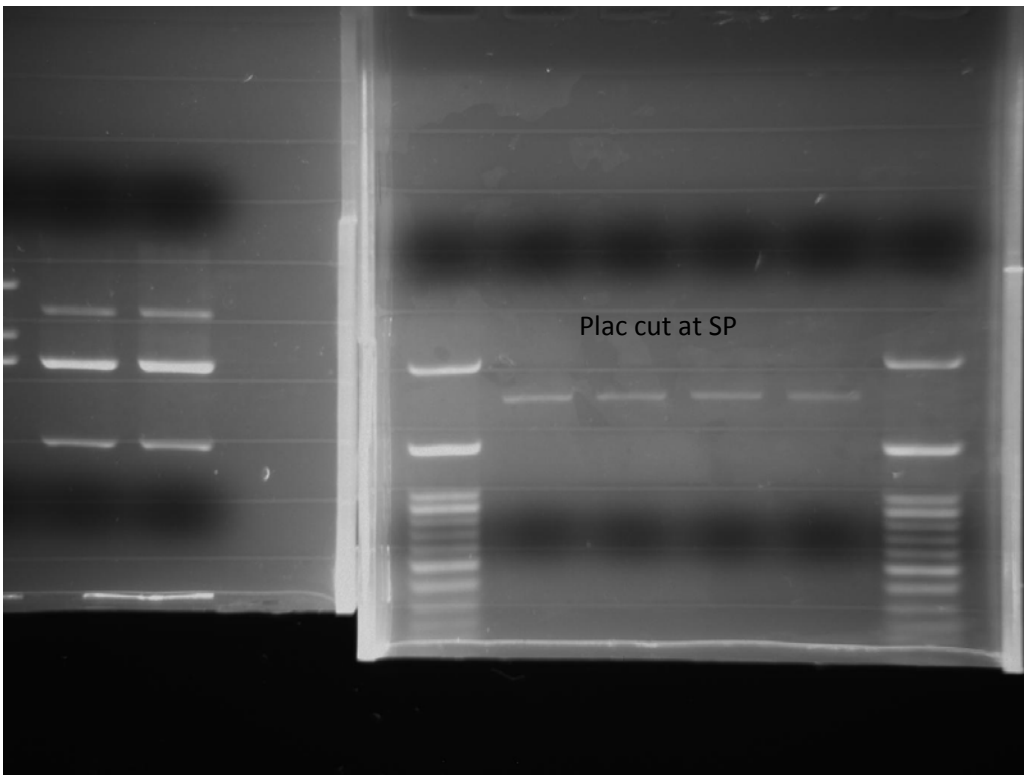
- Results of the transfection of pSurvivin+GFP into MRC-5 cells show that the cells do not glow green, as expected.

16

Friday, June 13, 2014
1:13 PM

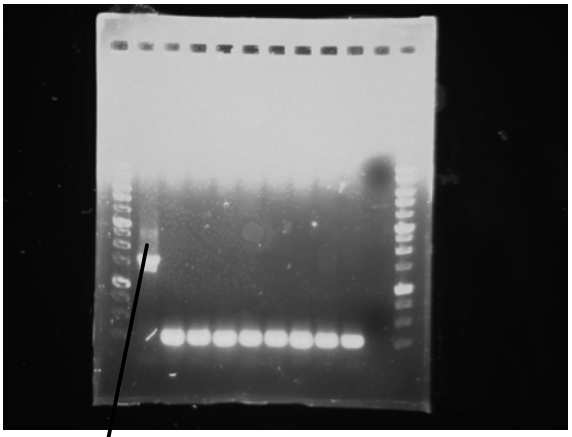


EXPECTED LENGTH:

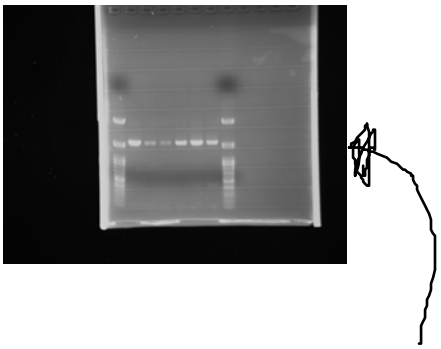


17

Friday, June 13, 2014
1:13 PM

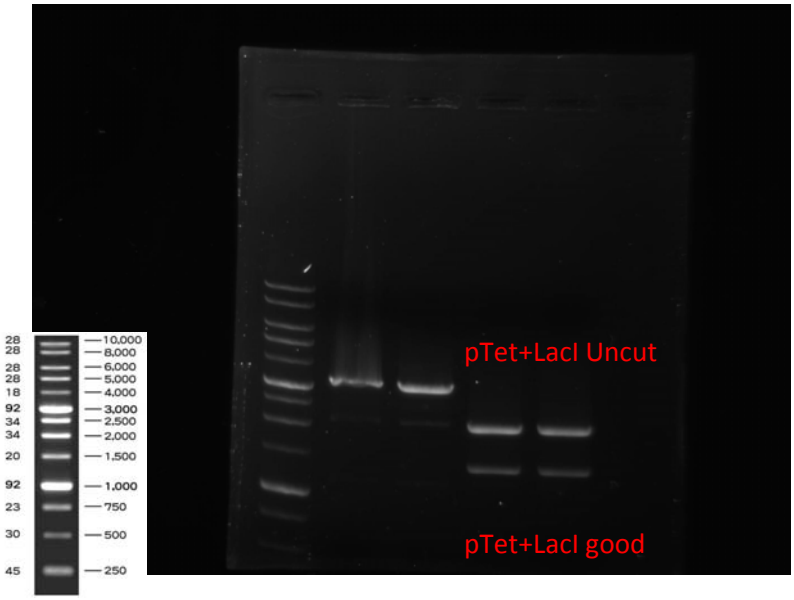


1. 1st Well pTET+LacI Correct
2. 2-9 Well pTet+LacI Wrong
3. 10 Well K1253000 SP PDP Correct



54+

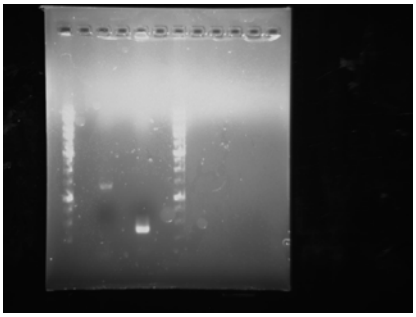
All pLac LacI gen are correct



- Promoter testing of pTet, pcI, pLac
-

18

Wednesday, June 18, 2014
12:35 PM



1. 1kb Ladder
3. K1253000+pcI+LacI Gen #1 Wrong, should be 4200 bp but it is at 1400, which makes absolutely no sense because pcI +LacI PCR by itself should be 1600 bp at the minimum
6. K1253000+pcI+LacI Gen #2 Wrong, should also be at 4200 bp but it is also still wrong, looks like 400 bp. Makes no sense.

Digestion for pTet+LacI Gen overnight at XP. Hopefully, the DNA is completely cut. The protocol for digestion has now been changed to 25 minutes for incubation at 37 degrees.

The order for the gel is
1. Ladder
2. pTet+LacI Gen
3. pTet+LacI Gen
4. pTet+LacI Gen
5. Blank

The expected length was 1362, hopefully this is right?
Then the gel was purified. For higher concentration, all of the three wells were loaded into one single well.
The trial had already been purified but it was the larger band from yesterday.

Redigest pTet+LacI

- IPTG and Tetracycline testing of the half oscillator and full oscillator plasmids

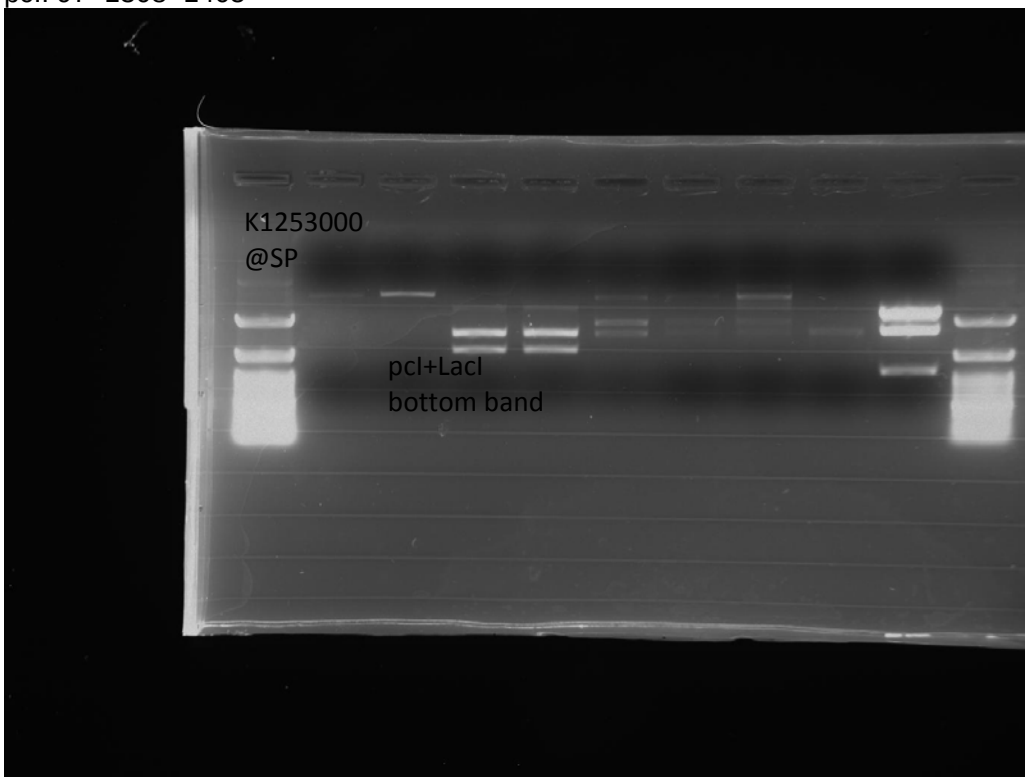
Find gel photos needed and labeled

- K1253000, picture of it fluorescing red on a plate, short discussion of why this occurs =2533
  UVP00317June152014.jpg
  Also picture below

- pTet, pLac, pcI + LacI Gen
  pTet: 54+1308=1362
  - First well of UVP00329June162014

  pLac: 55+1308=1363
  - UVP00331June172014

  pcI: 97+1308=1405



- pLac+TetR/RFP
  55+12+685+12+706+129=1,599

- pLac, pTet, pcI + GFP - bands
  pLac: 55+878=933
  pTet: 54+878=932
  pcI: 97+878=975

- All the promoters + repressors used in the repression circuits - bands
  pTet experiment: 1827
  pcI experiment: 1989
  pLac experiment: 2338

Night

- K1253000 and pLac+LacI, and K1253000 by itself
  - Compare red and green

        - Red K1253000 with tetracycline, green K1253000+pLac+LacI with IPTG
    - pSurvivin+GFP gel picture\

- Digested c-Myc, pSurvivin, and pSurvivin+GFP at EP and transported them to BioBrick plasmids

- Purify all the promoter EXPs that are currently in the shaking incubator
- 3-1 all the promoter EXPs that are currently growing in the incubator
- Ligate the promoters to RBS+TermA, of which I have no clue of its whereabouts

6/14

Part A

- **10:30 am start time stamp for BOTH the CULTURES AND PLATES of the [pLac, pTet, pcI] + GFP**
  - **Do this for 12 hours every hour, so end at 10:30pm**
- **11:30 am time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **12:30 am time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **1:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **2:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **3:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **4:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **4:30 pm take plates of [pLac, pTet, pcI] + GFP plates and 3-1 all of them, but CONTINUE THE TIME STAMPING**
  - **All of these plates AND THE CULTURES we will continue with time stamps until 10:30 pm**
  - **We will only plasmid purify the culture tubes the following morning to get the plasmids. DON'T PLASMID PURIFY THE [pLac, pTet, pcI] + GFP OMG**
- **4:30 pm take c-Myc, pSurvivin, TK gene cultures out of the shaking incubator**
  - **Plasmid purify all of these**
- **5:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **6:30 pm take [pLac, pTet, pcI] + [LacI Gen] plates out of the incubator, and 3-1 them**
  - **Do 6 tubes of culture for each one, even if there are not enough colonies, double up on one colony. We want ALL THE PLASMIDS WE CAN GET.**
- **7:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **8:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**

- **9:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
- **10:30 pm time stamp [pLac, pTet, pcI] + GFP culture and plates**
    - **FINISH THE TIME STAMP HERE**
    - **STORE THE PLATES IN THE FRIDGE IN AN ORGANIZED FASHION PLEASE.**

Part B

- **8:00 am take the EXP culture tubes out of the shaking incubator, and store them in the fridge, to be plasmid purified at 12pm**
- **8:00 am take the EXP plates out of the incubator, and store them in the fridge, to be 3-1 at 6:00 pm**
- **11:00 am take photos of all the EXP plates that are successful**
- **11:00 am 3-1 all the successful EXP plates, let them grow in the incubator/shaking incubator for one hour**
    - **Do at least 6 cultures and restreak repeats, since we will be timestamping these the next day.**
- **12:00 pm take the cultures and restreaks of the EXP plates and store them in the fridge**
- **10:30 am ligate pLac, pTet, pcI to [RBS+Term] and transform them**
- **12 pm plasmid purify the EXP cultures in the tubes**
- **6:30 pm take the cultures and restreaks of the EXP plates that were earlier put in the fridge, and put them in the incubator/shaking incubator to grow**
- **2:30 am (not a typo) take the pLac, pTet, pcI to [RBS+Term] transformations out and 3-1 them**

6/15

PART A

- **8:30 am take out [pLac, pTet, pcI] + GFP restreak plates and cultures, and store them in the fridge**
    - **Plasmid purifications will be done at 12 pm**
- **10:30 am [pLac, pTet, pcI] + [LacI Gen] plasmid purification of all 6 tubes**
- **12:00 pm [pLac, pTet, pcI] + GFP cultures to do their plasmid purification**

- **Part B**
- **10:30 am time stamp [pLac, pTet, pcI] EXP plates and cultures**

- **11:30 am time stamp [pLac, pTet, pcI] EXP plates and cultures**
- **12:30 pm time stamp [pLac, pTet, pcI] EXP plates and cultures**
- **1:30 pm time stamp [pLac, pTet, pcI] EXP plates and cultures**
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- **3:30 pm time stamp [pLac, pTet, pcI] EXP plates and cultures**
- **4:30 pm time stamp [pLac, pTet, pcI] EXP plates and cultures**

Timestamps

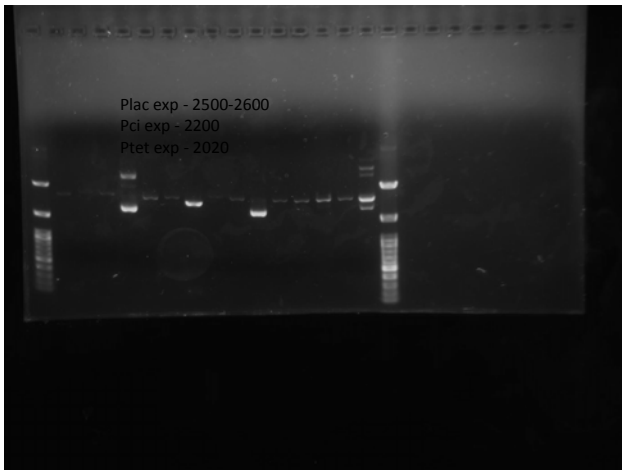
Saturday, June 14, 2014
10:57 AM

1. 11:00 am pLac+GFP 1-1 Plate
2. 11:00 am pLac+GFP 2-1 Culture
3. 11:00 am pTet+GFP 1-1 Plate
4. 11:00 am pTet+GFP 2-1 Culture
5. 11:00 am pcI+GFP 1-1 Plate
6. 11:00 am pcI+GFP 2-1 Culture

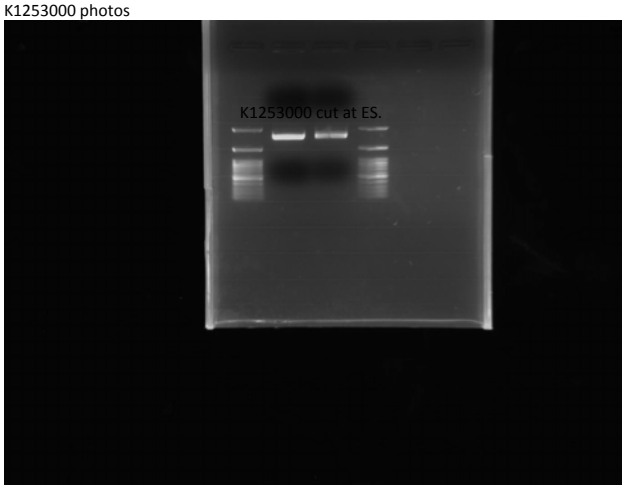
7. 11:00 am pcI+GFP 1-1 Plate
8. 11:00 am pcI+GFP 2-1 Culture
9. 11:00 am pcI+GFP 1-1 Plate
10. 11:00 am pcI+GFP 2-1 Culture
11. 11:00 am pcI+GFP 1-1 Plate
12. 11:00 am pcI+GFP 2-1 Culture
13. 11:00 am pcI+GFP 1-1 Plate
14. 11:00 am pcI+GFP 2-1 Culture
15. 11:00 am pcI+GFP 1-1 Plate
16. 11:00 am pcI+GFP 2-1 Culture
17. 11:00 am pcI+GFP 1-1 Plate
18. 11:00 am pcI+GFP 2-1 Culture

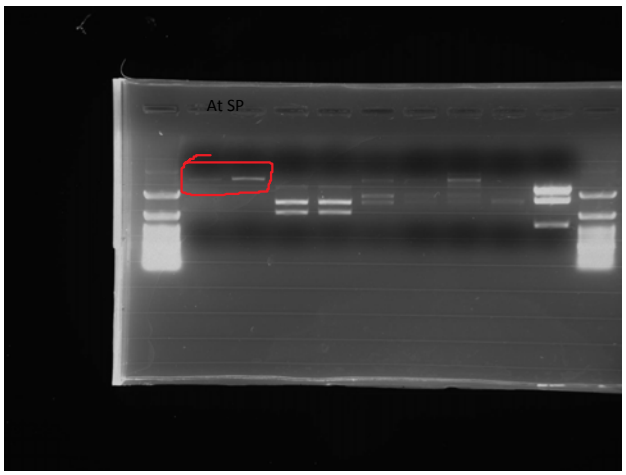
Gel result photos

Thursday, June 19, 2014
12:37 PM

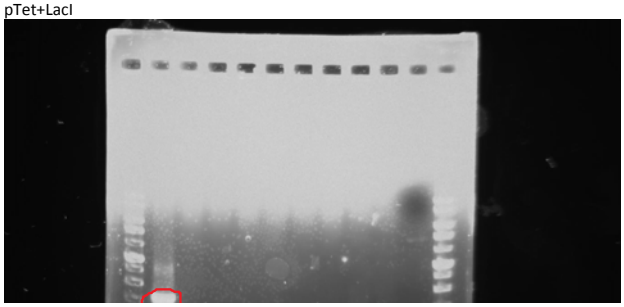


K1253000 photos





pTet+LacI



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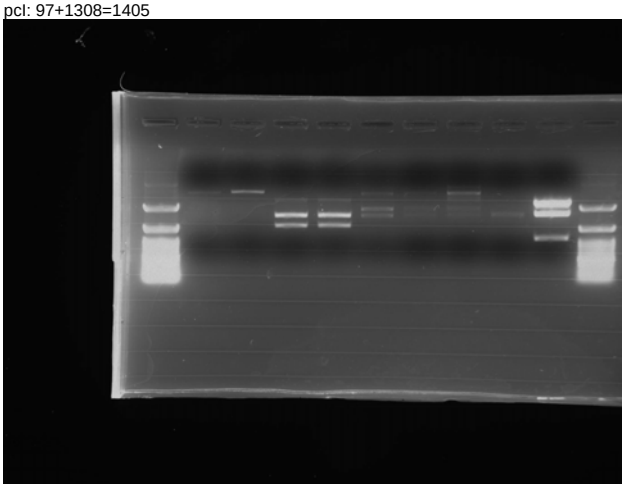

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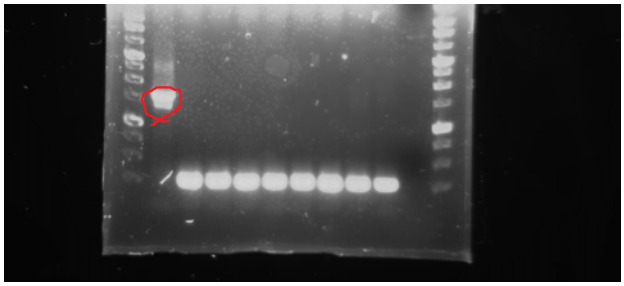
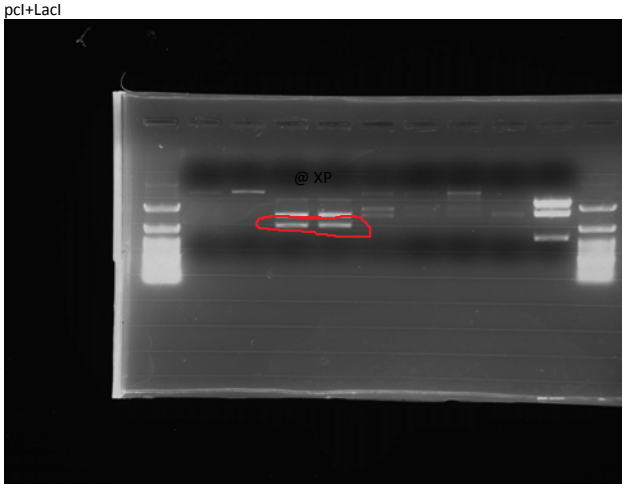
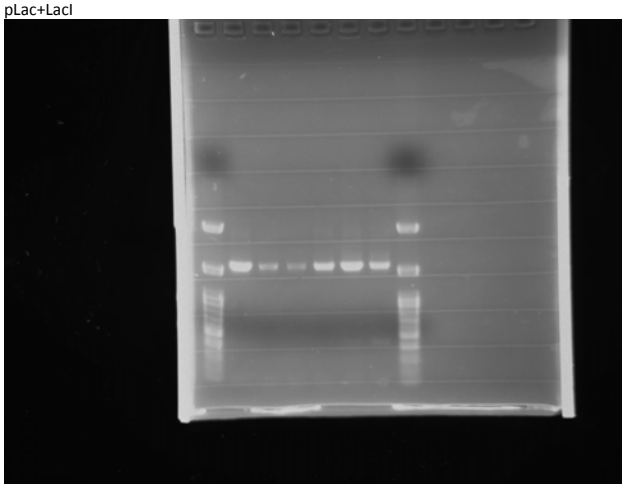


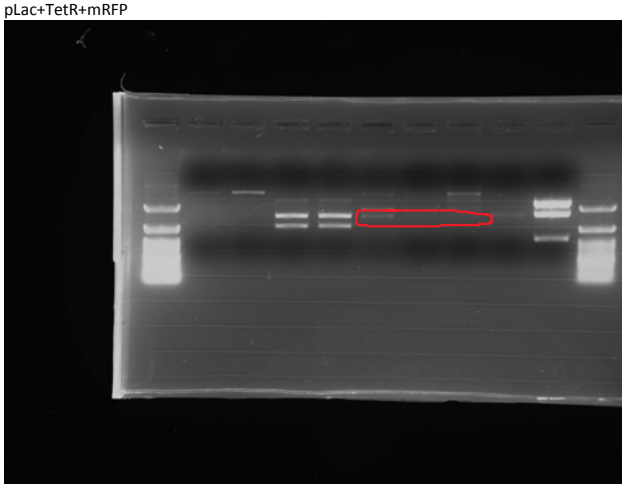
pcI+LacI



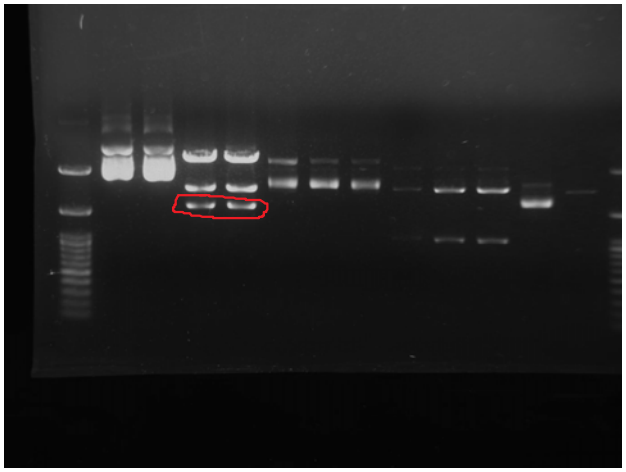
pLac+LacI



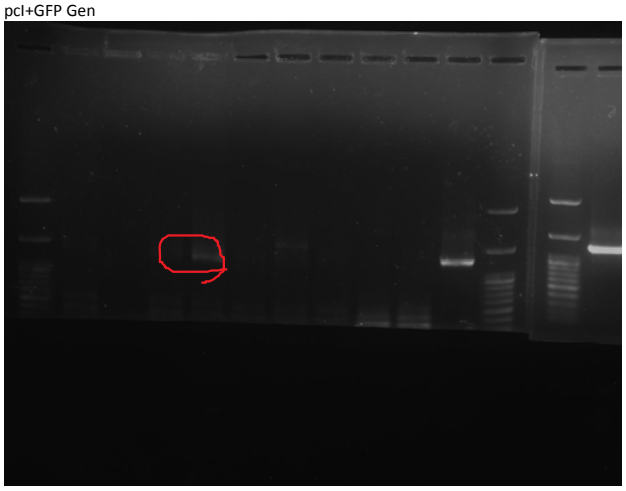
pLac+TetR+mRFP



pTet+GFP Gen



pcI+GFP Gen



pLac+GFP Gen