7

2014年3月25日
下午 08:07

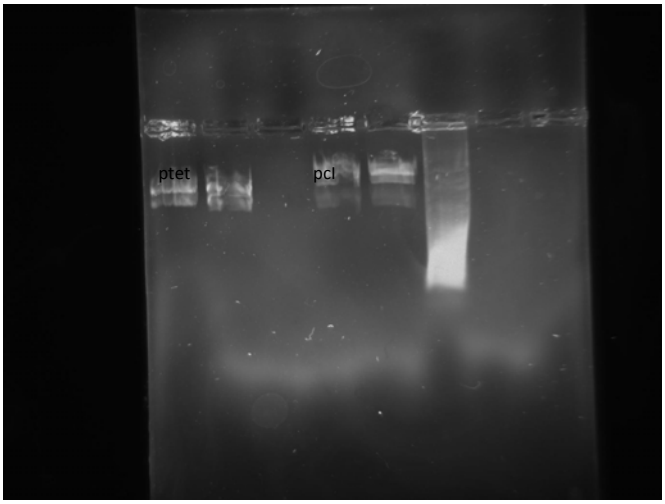
Things that were done today:
TetR Gen #2 was digested at XP
CI Gen #3 was digested at XP
LacI Gen #3 was digested at XP

pLac - we need it at SP
pTet #1 was digested at SP
pCI #3 was digested at SP

-Melody and Rachel

1
2 ptet sp

3 pcI sp
4



Ladder

Phillip's notes:

We have all the parts in the BioBricks Kit:
LacI Gen: BBa_P0412 (Kit Plate 3, 2H, C resistance)
TetR Gen: BBa_P0440 (Kit Plate 3, 9C, C resistance)
CI Gen: BBa_P0451 (Kit Plate 3, 16N, C resistance)
pTet: BBa_R0040 (Kit Plate 3, 5E, C resistance)
pCI: BBa_R0065 (Kit Plate 3, 5K, C resistance)
pLac: We have this part.
The one in the biobrick Kit Plate is wrong. See BBa_R0011

8

Wednesday, April 09, 2014
11:04 AM

Redid the digestion for pcI and pTet.
Gel purified everything else, should be in purified plasmid.

UV results:

9

Wednesday, April 09, 2014
2:24 PM

Redo digestion for pTet and pcI

The gels have to run!

10

Thursday, April 10, 2014
3:12 PM

Things done today:
Made 6 each of 1.0% and 0.8% agarose gels, large combs

11

Friday, April 11, 2014
3:28 PM

A-LacI Gen P0412-1
B-""-2
C-""-3
D-pcI R0065-3
E- ""-2
F- ""-1
---------- We stopped here
G-TetR Gen P0440-1

I- ""-3
J- pLac R0010
K- ""-2
L- ""-3
M- pTet R0040-1
N- ""-2
O- ""-3
P- cI Gen P0451-1
Q- ""-2
R- ""-3

Didn't finish 3 in 1 !

14

Monday, April 14, 2014
2:46 PM

Things that needs to be done today:
Digest all the parts for the oscillator again

15

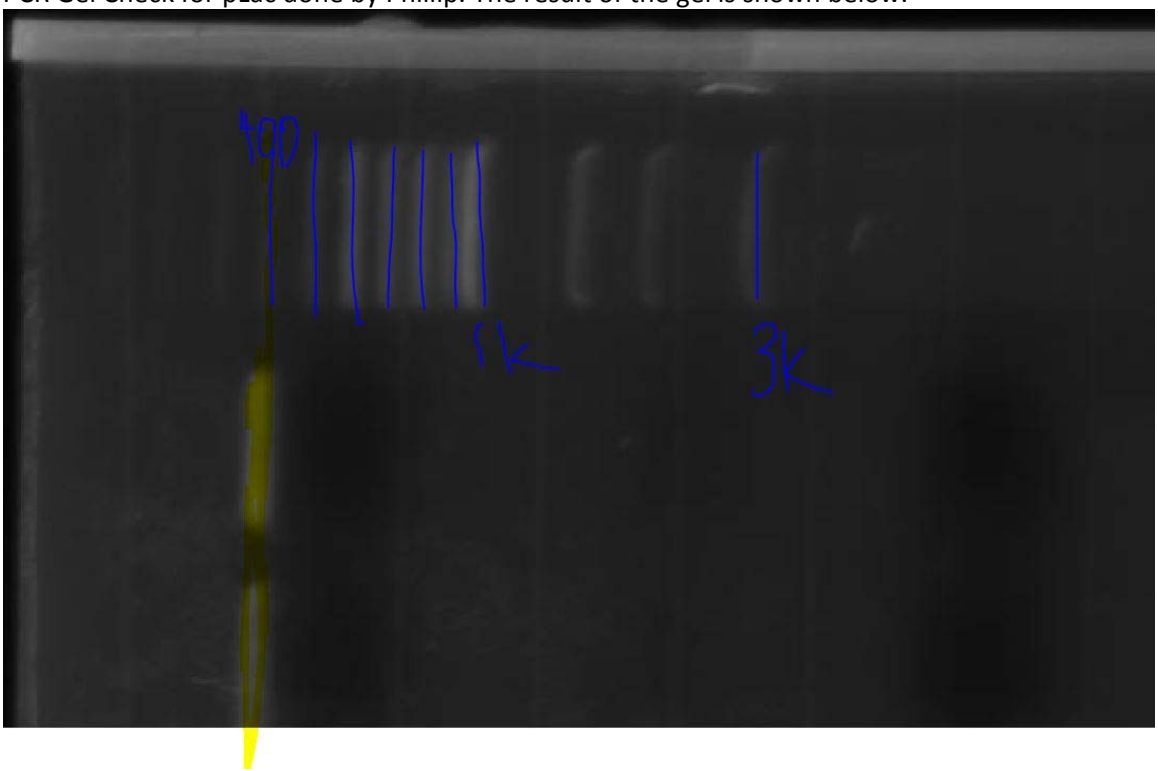
Tuesday, April 15, 2014
3:23 PM

Finished gel purification of all the oscillator parts.
Did 3 in 1 for pLac
Edward made new gels.

16

Wednesday, April 16, 2014
3:08 PM

Things done today:
Plasmid Purification of the lacI is done by Lily and Michael.
PCR Gel Check for pLac done by Phillip. The result of the gel is shown below.

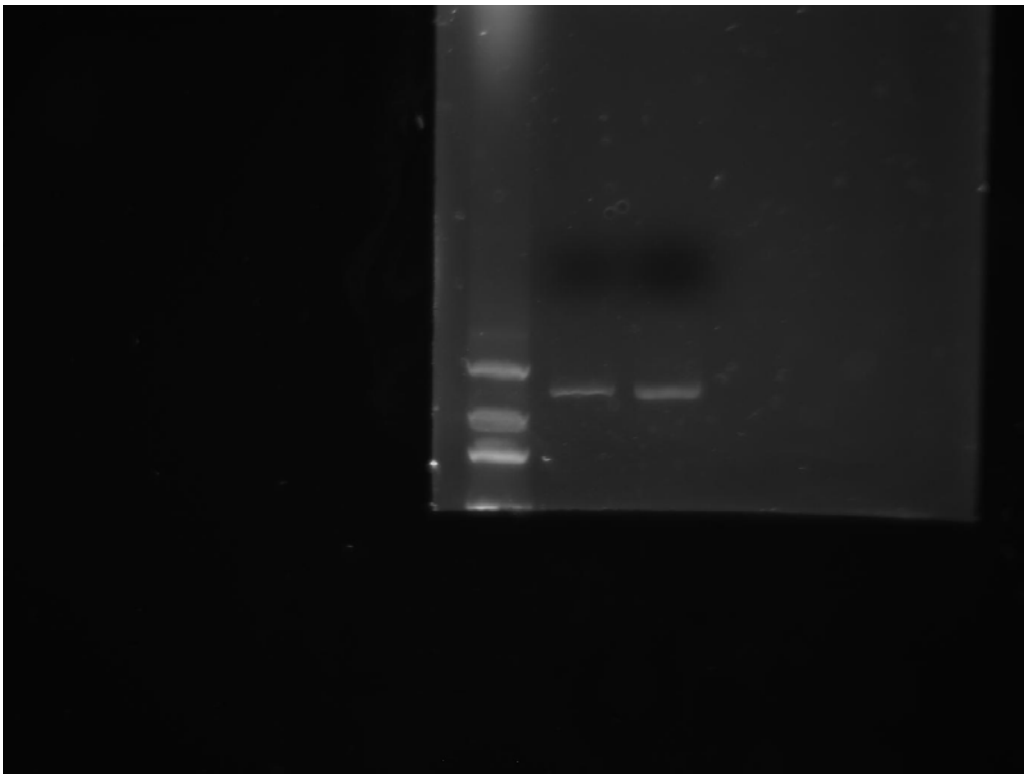


Ligation for pcI and lacI is done by Dean and Bethany
Ligation for pTetR and cI is done by Julie and Annie.
New plates were made by ~~Greg~~ Edward
Edward and Christopher continue to brainstorm for modeling.
For future reference: put 250 lambda of 50 microgram/mL chloramphenicol when making plates with 500mL of LB+agar

17

Thursday, April 17, 2014
3:29 PM

Phillip and Andy ligated the parts
Melody did digestion for plac



18

Friday, April 18, 2014
2:36 PM

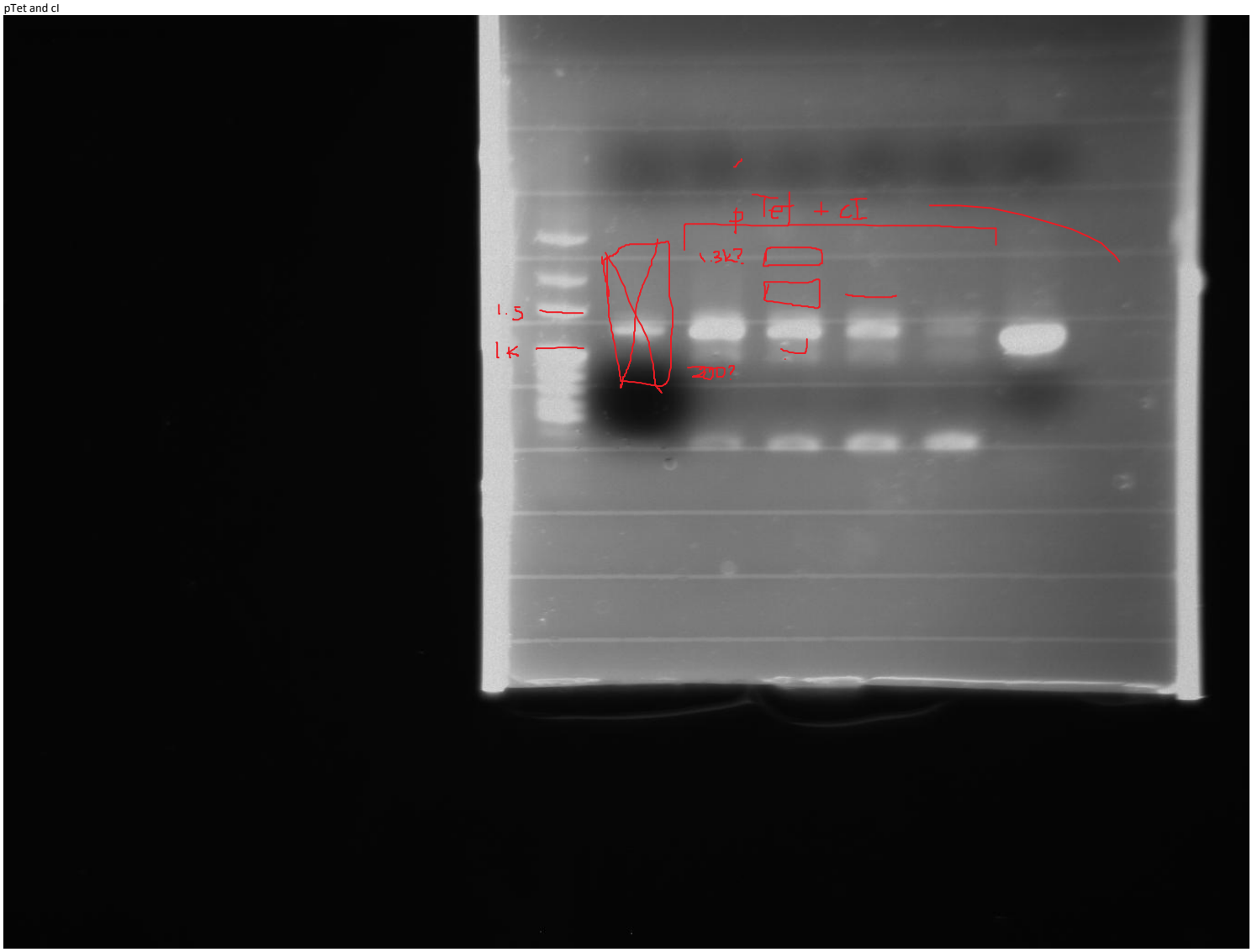
Things done today:
The results for yesterday pcI and LacI

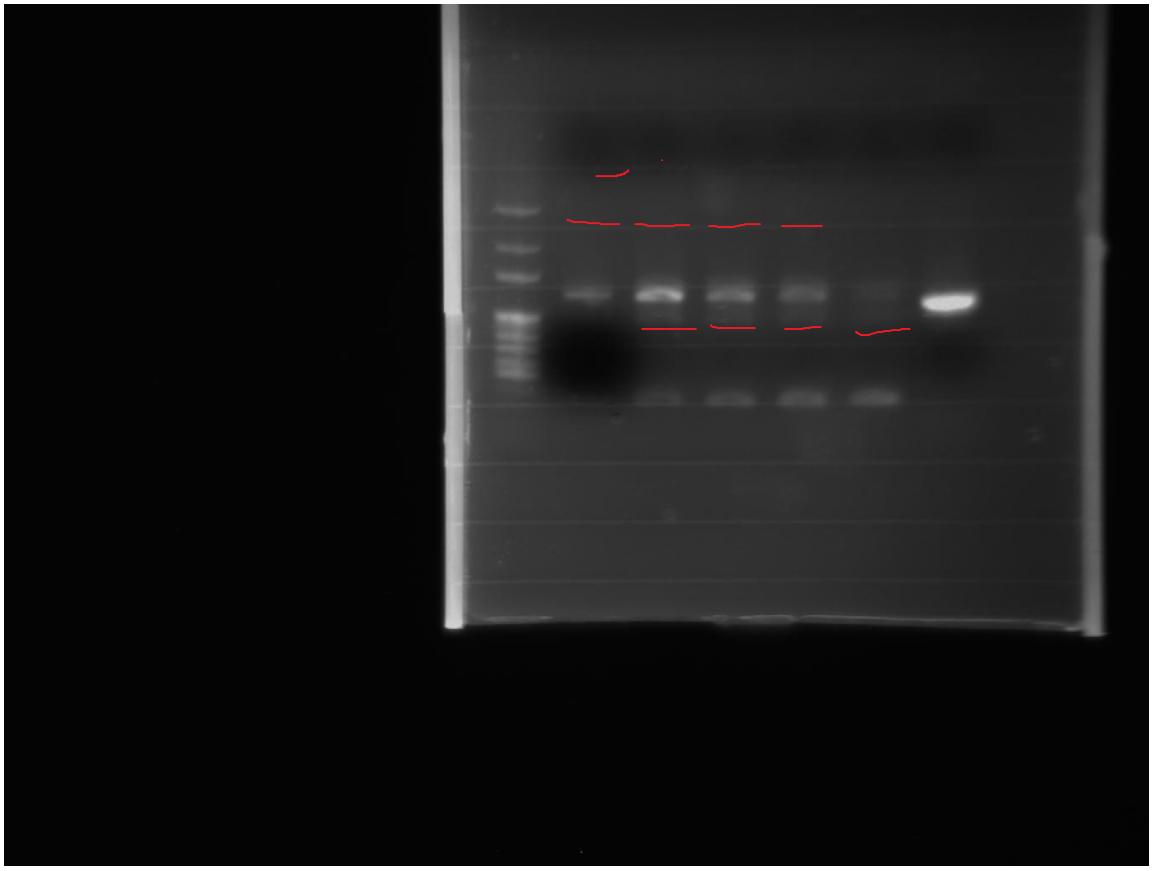
19

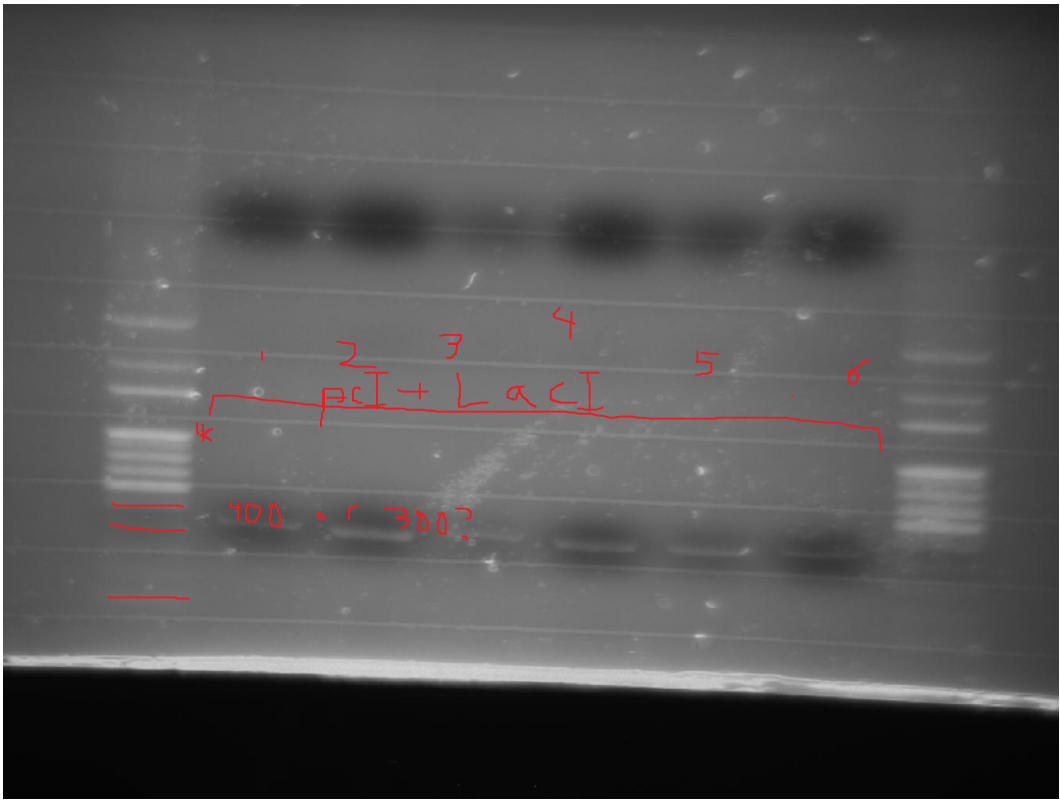
Saturday, April 19, 2014
12:04 PM

Process done today:
3 in one for pTet and cI

pTet and cI



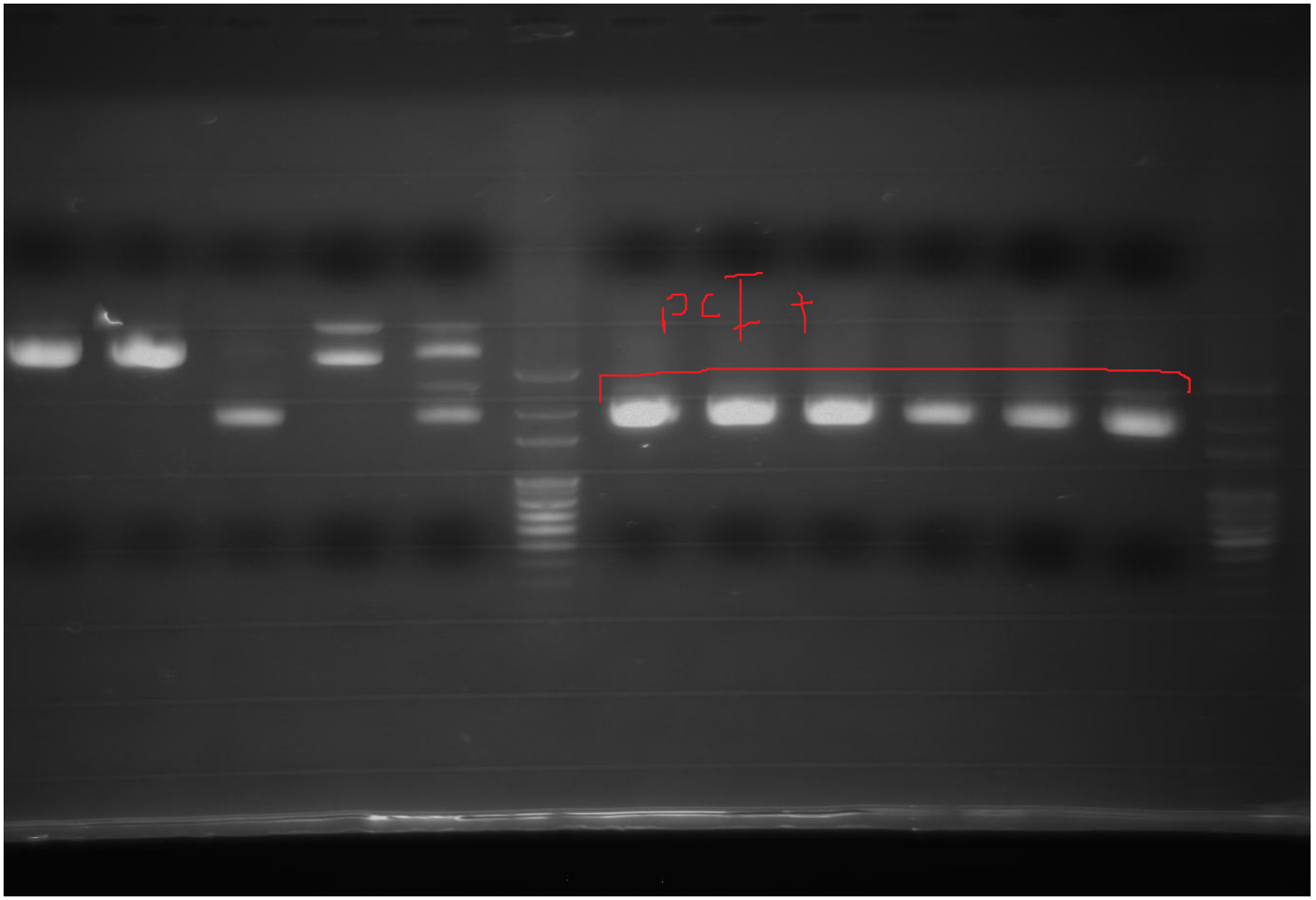


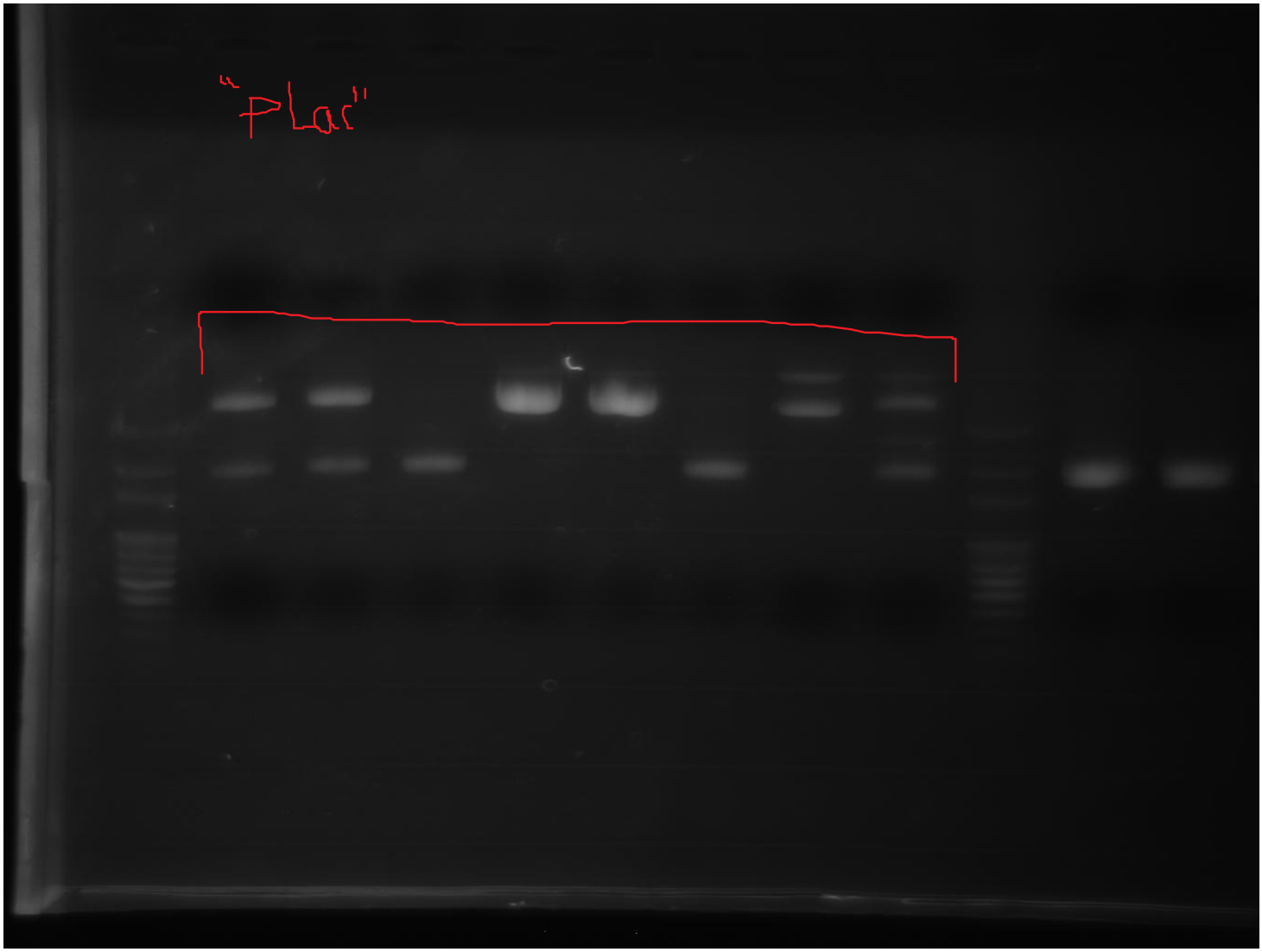

1
2
3
4
5
6
pcI + LacI
400 & 300?

21

Monday, April 21, 2014
11:09 AM

Run all pLac, run




"Plac"

22

Tuesday, April 22, 2014
3:37 PM

3-1 pLac that was transformed by Andy and Phillip yesterday

This is on amp.
Plasmid purify for pLac for the 15mL tubes (liquid culture)

Phillip- run gels of plasmids.

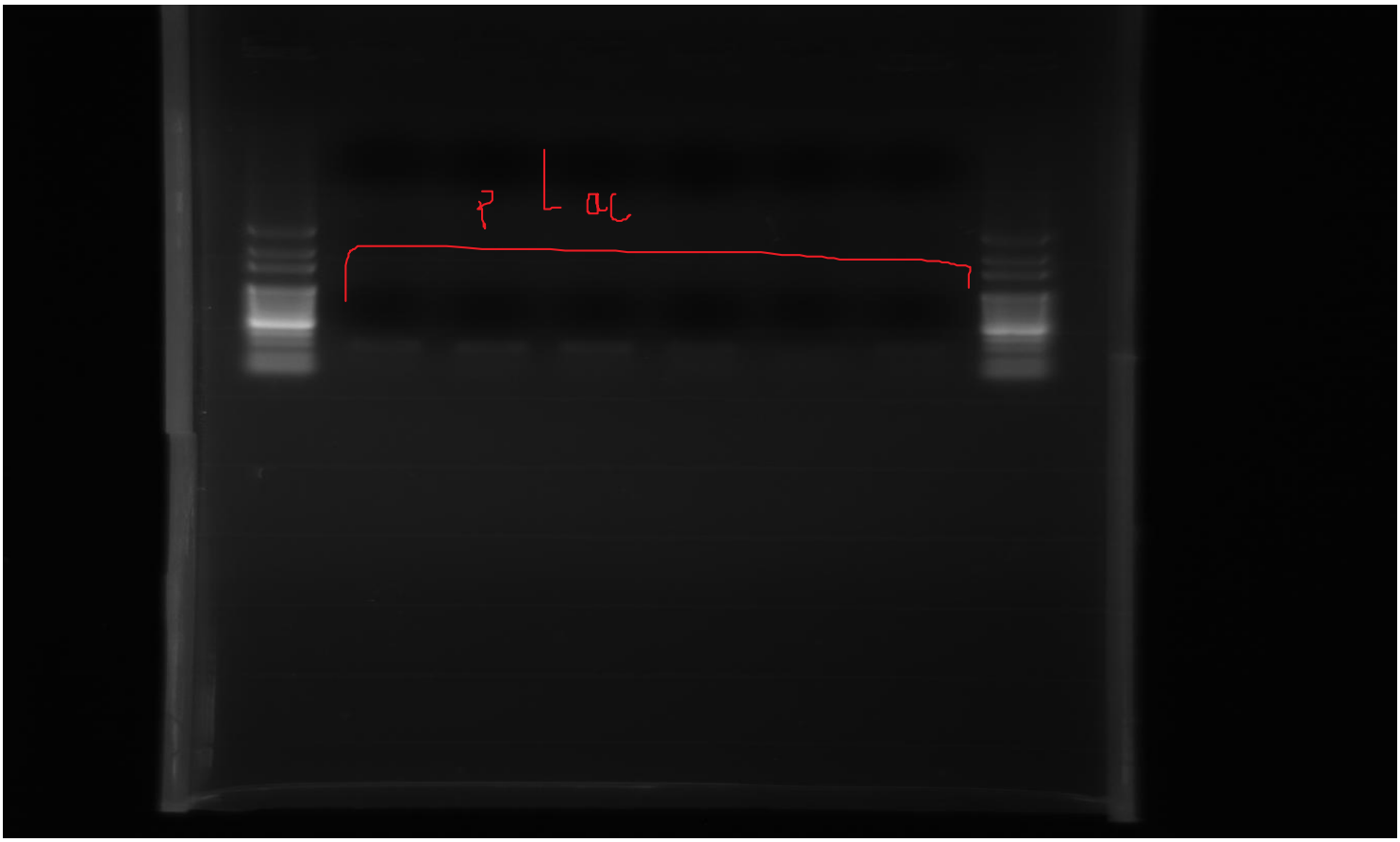
Digestion of 2 ligation products FAILED.
Reason: we do not know what the products.

23

Tuesday, April 22, 2014
4:41 PM

Transform all the parts for the oscillator.

Run PCR on gel to check if the pLac is good.



pLac has been confirmed to work! We expect to see around 293 and we saw around 300 so it seems like the pLac in Amp is good.

Plasmid Purification for liquid cultured pLac.

Reattempt digestion for ligated parts.

pcI +lacI transformation by

24

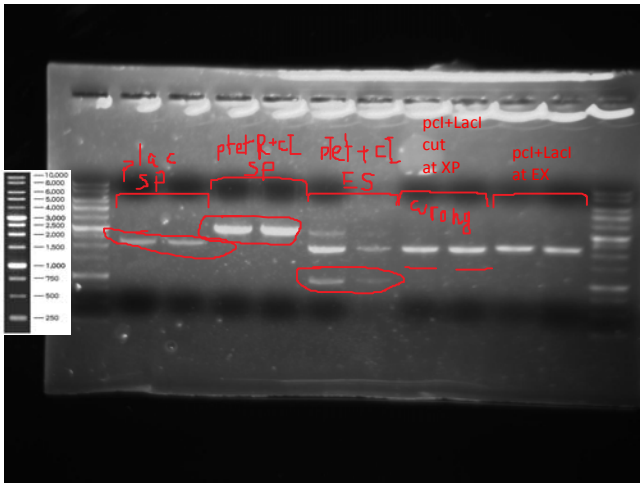
Wednesday, April 23, 2014
4:01 PM

Run PCR for the oscillator parts and also check if the RBS is good because we are going to have to ligate that. HIGH PRIORITY.

Plasmid purification for all the oscillator tubes. NEEDS TO BE DONE BY TODAY.

Digestion of ligated parts. Okay priority.
pcI-LacI should be cut at XP and EX.
pTet-cI should be digested at SP and ES.

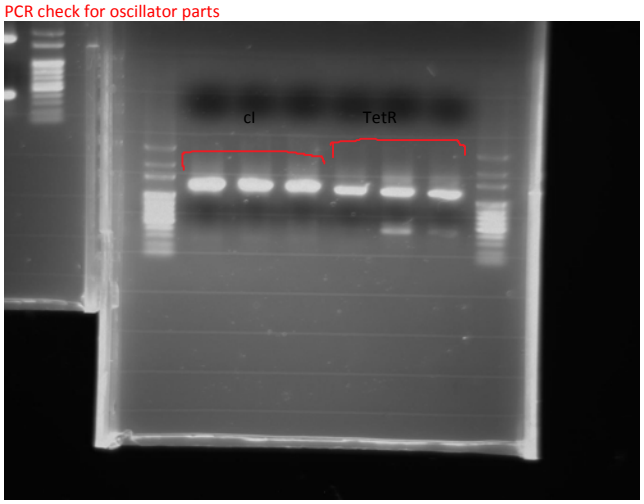
Digest purified plasmid for pLac should be back inserted. Cut at SP. HIGH.



1Kb DNA Ladder III

pLac at SP seems fine, we expect to see 2134 bp and we did.
pTet+cI at SP we expect to see around 984+2070=3054 so that is alright.
pcI + LacI at XP should be around 1408.
pcI +Laci at EX should also be around 1408+2070=3478 so this is wrong too.

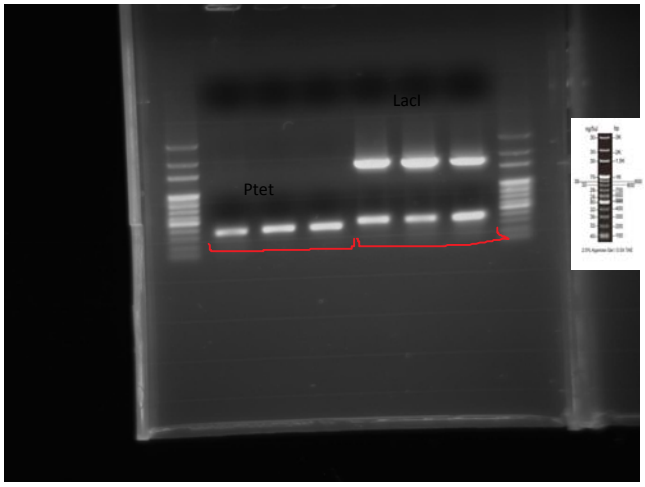
PCR check for oscillator parts



cI is 1138 + C resistance= 1452

TetR+ C resistance= 840 + C resistance = 1154

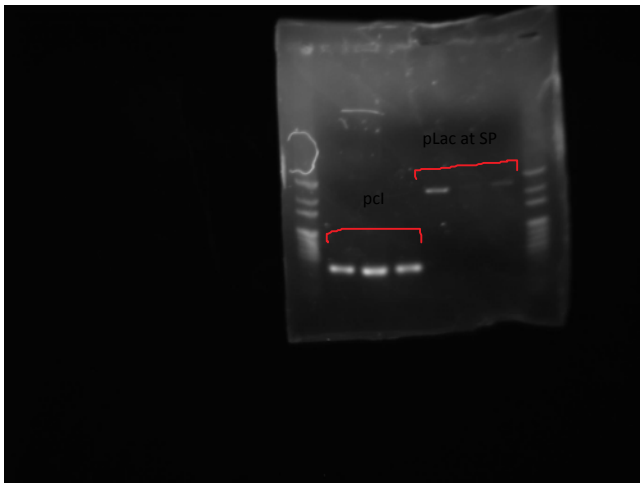
cI should be around 930+2070=3000
TetR Gen should be around



LacI seems good 1308+314=1622

LacI at SP should be 1308 + C resistance then we get around 1622 for PCR

pTet should be 54 + C resistance= 368 bp for PCR



pcI should be97+ C resistance which is 411 bp for PCR.

Transform RBS if need be.

We need gels. Low.

25

Wednesday, April 23, 2014
4:06 PM

Gel purify digested parts.

The five tubes can be found in the -20 with the labeled UGP box.
These are the digestions that were done yesterday. Please refer to the first diagram on the previous day. This is figure 1.

We need to digest the purified plasmid for all the oscillator parts.
We digested all the promoters at SP and all the generators at XP.

29

Tuesday, April 29, 2014
3:10 PM

3 in 1 for ligated part B - Melody and Rachel
Purified plasmid RBS B0034 and TetR by itself. - Andy and Phillip

RBS 2 may have a lower concentration because there was around 1.5 ml left in the original liquid culture tube.
Everything else should be fine though.

Edward loaded the Autoclave with new pipet tips and centrifuge tubes.

30

Tuesday, April 29, 2014
3:18 PM

pLac is already read of SP soo…
Cut RBS at XP

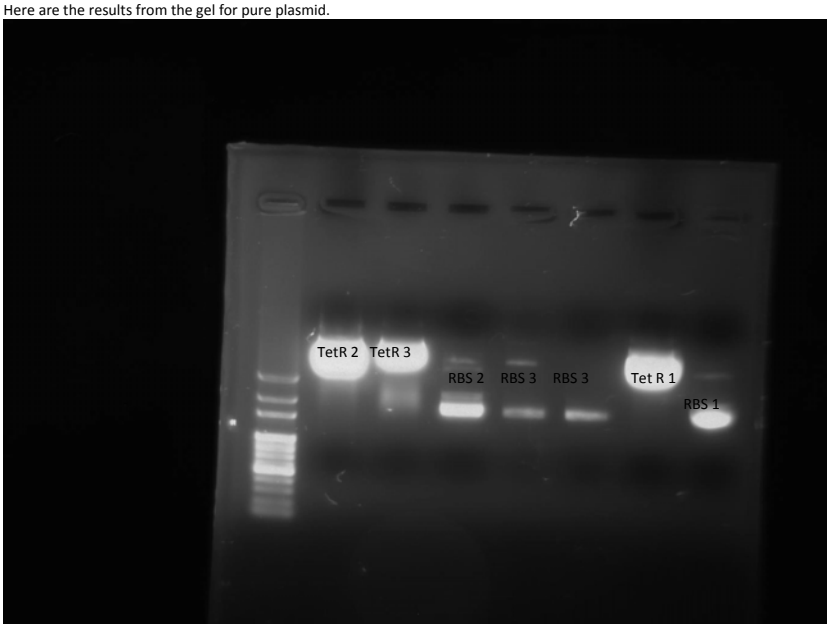
Cut TetR has to cut at XP.

Inventory:

- 18 small amp plates
- 11 big amp plates + 3 old big amp plates
- 16 small chlor plates
- 4 big chlor plates
- 3 tubes of Chlor
- A lot of tubes of Amp (8+)

Ran TetR and RBS-+

Here are the results from the gel for pure plasmid.



RBS was around 1.7k bp

The results of the gel on running the purified plasmid is that the attempted digestion of TetR may not be good. I recommend we create a liquid culture again for TetR and redo plasmid purification. The blurring of the gel results may be the result from having other DNA put into the plasmid purification process.

Julie & Lilee

Plasmid Purification for Osc 1-7

31

Wednesday, April 30, 2014
3:12 PM

Plasmid purification for TetR liquid culture.

Digest RBS at XP (two people, and compare results)

Digest TetR 1 at ES

Run gel for Osc B
Run gel to check if TermA at EX is alright.

Ask Jesse about sequencing purification.

Thursday, May 01, 2014
3:54 PM