# Jason Cham start 6/01/11

From AndersonLab wiki

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## Jcham 12:56, 01 June 2011 (PDT)

The two re-transformations that I did of pBca9525-bca1832 and pBca9525-bca1835 turned out fine. There were colonies that I picked and are now growing in the shaker.

I minipreppeed the cells that were growing yesterday: pBca9525-Bjc002 (a and b) and pBca9525-Bjc004 (a and c). I sent these 4 samples in for sequencing. Hopefully, they will sequenc well. I then transformed these 4 each into pca1601ca-bss-52. This is the reporter strain. I also transformed the negative control (pbca9525-bca1846) and the positive control (pbca9525-bca1835).

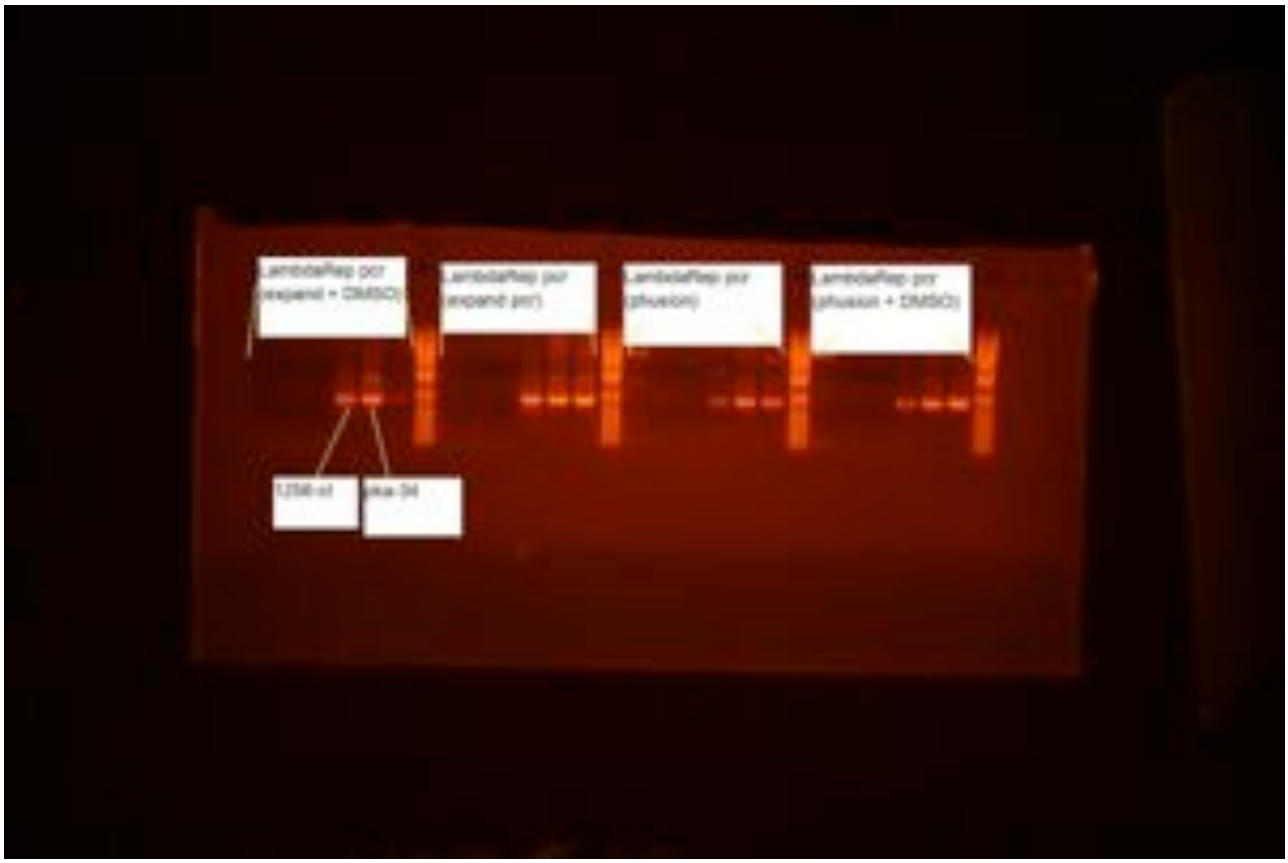
I set up the pcr for lambdaRep. 1. 1256-dc9 2. k9 3. pka-35 4. 1256-cI 5. pka-34 6. cI.1-dc33

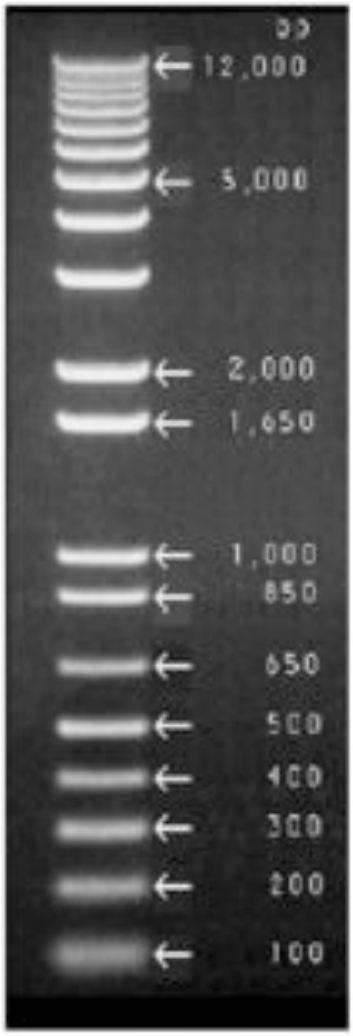
## Jcham 12:56, 02 June 2011 (PDT)

I took the innoculated pBca9525-bca1834 and pBca9525-bca183 out and miniprepped them.

The pcr's from yesterday turned out okay. I will use the bands from the lambdaRep pcr(expand+DMSO) in lanes 4 and 5. The backbones of those were 1256-cI and pka-34.

- lambdaRep_pcr_analytical_gel






1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

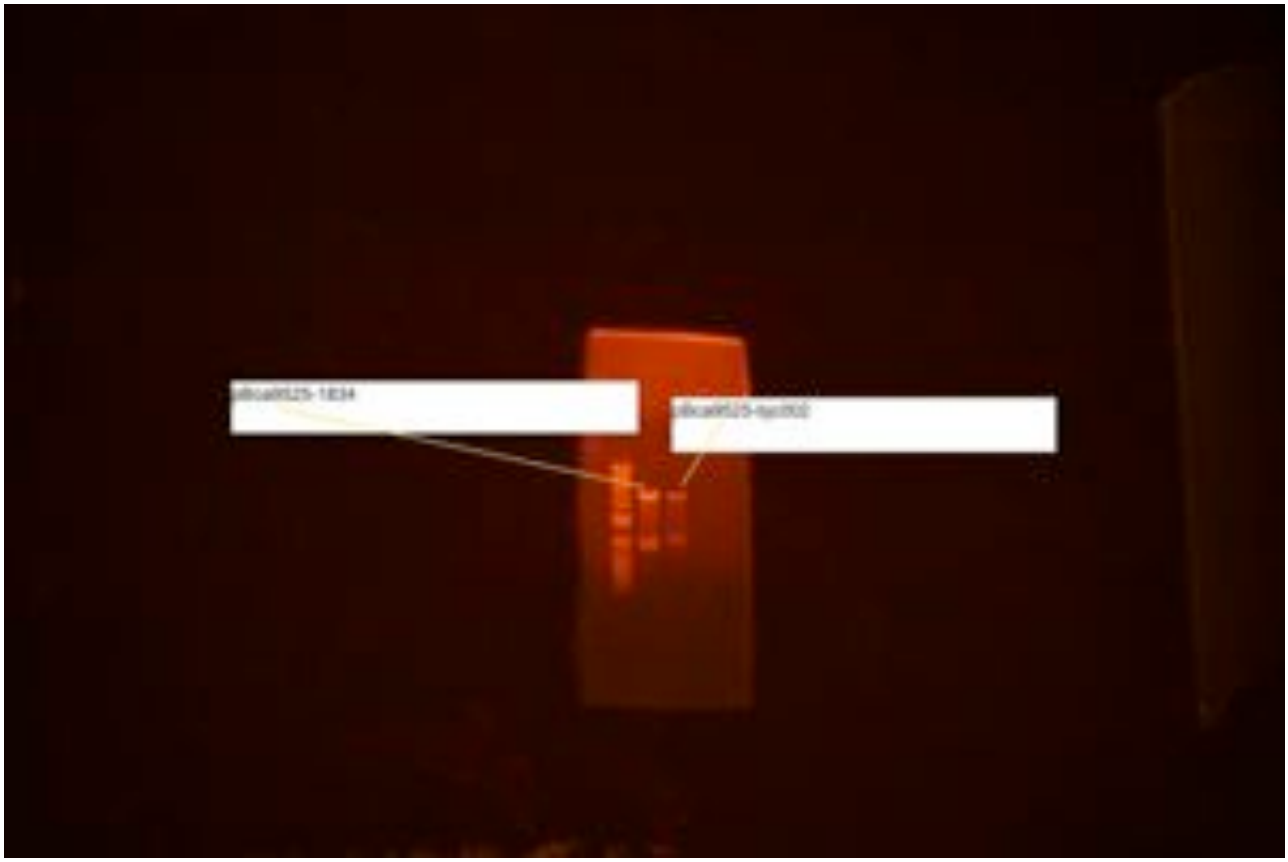
I zymo cleaned the pcr products and then digested with NheI and BamH1. I also digested the backbone pBca9525-bca1834 with NheI and BamH1. The digest came out fine for lambdaRep are about the correct size, but the backbone was not. I will have to redigest either pBca9525-bca1834 or one of my other constructs with the same backbone.

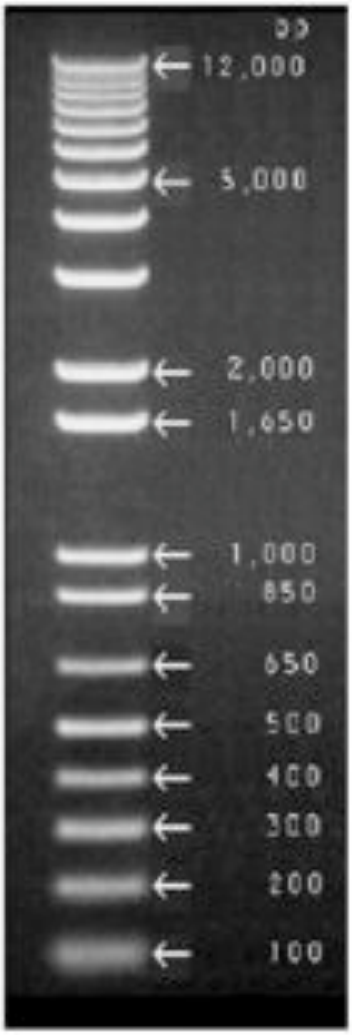
I cut out the gels of the digests and will gel purify, ligate, transform, plate tomorrow.

# Jcham 12:58, 03 June 2011 (PDT)

Today, I gel purified the gels that I cut out yesterday. I also digested the vector backbone pBca9525-bca1834 with NheI and BamH1. The gel picture is below. I took the large band. The drop out for pBca9525-1834 is rfp. I also cut pBca9525-bjc002. The drop out for pBca9525-bjc002 is bjc002. I gel purified, ligated, transformed, recovered, plated.

- backbone_digest






1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

## Jcham 10:53, 06 June 2011 (PDT)

The sequencing failed for pka-34 so I will continue with 1256-cI. I miniprepped it and then transformed it into the reporter strain pbca1601-ca-bss-52. I plated the positive and negative controls on amp and spec plates. I plated the 1256-cI on amp spec plates. I plated the extra experiment pBca9525-1839 on amp, spec, cl plates. I also plated extra bss52 cells to make extra comp cells for the reporter strain.

## Jcham 11:26, 07 June 2011 (PDT)

P_rffGH.rbs.ToxR-Nhe1.{<gsgsgs>}{LambdaRep} in 9525 are green with bss52 reporter cells I picked colonies today and innoculated into LB+CAS for starter colonies. I will do a tecan tomorrow.

# Jcham 12:26, 08 June 2011 (PDT)

I set up the tecan.

Row 1 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(2mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

Row 2 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(1mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

Row 3 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(.5mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

Row4 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(.25mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

Row 5 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(.125mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

Row 6 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(.06125mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

---

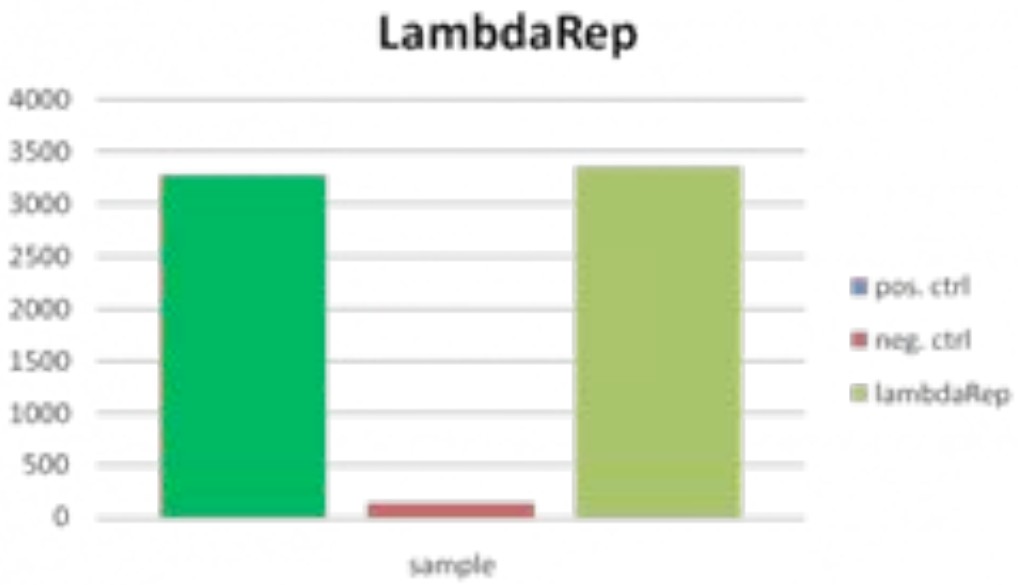
Row 7 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl w/ glutathione(.0306mM) +ctrl w/ gt -ctrl -ctrl 1839 1839 1839

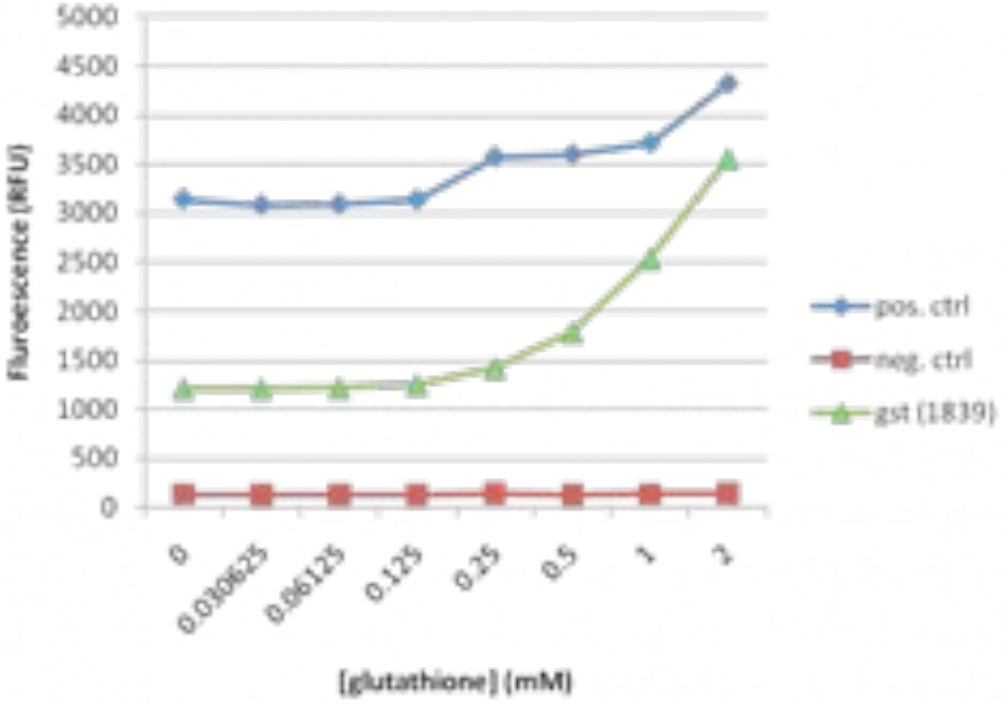
---

Row 8 +ctrl -ctrl lambdaRep1 lambdaRep2 +ctrl +ctrl -ctrl -ctrl 1839 1839 1839

# Jcham 11:26, 09 June 2011 (PDT)

I got the results back for the tecan. LambdaRep definitely had activity. It dimerized and the fluorescence was comparable or even greater than the +ctrl. The gst also showed that it had activity when glutathione was present. The +ctrl did slightly increase in fluorescence too.




# Jcham 11:30, 10 June 2011 (PDT)

prepared and made presentation today on progress.

# Jcham 11:30, 13-15 June 2011 (PDT)

I innoculated pBca9525-1839. I started to design oligos for library making. The construction file is below.

```
LAMBDA REP INTEFACE LIBRARY CONSTRUCTION FILE
jkc011_F 3' KpnI Split lambdarep forward- ccaaaGGTACCAAGTTAGCGTTGAAGAATTTAG
jkc012_R 3' SpeI split lambda rep reverse- cagttACTAGTGGCAGCAGCCACTGGTAACAG

jkc013_F 5' SpeI split lambdarep forward- cagttACTAGTCCAGTGGCGATAAGTCGTGTC
jkc014_R 5' KpnI split lambdarep reverse- cagttGGTACCTTTGAGAATTTTTGCAAGCA

ca1775  Forward KpnI for Kan+R6K from pth7028   ccatgGGTACCGCCTCCTCgctttcgctaaggatgatttc
ca1776  Forward KpnI for R6K from pth7028       ccatgGGTACCGCCTCCTCGCAGTTCAACCTGTTGATAG
ca1777  Reverse M13/  SpeI for Kan+R6K or R6K from pth7028      cagttATactagtGCCTCCTCcattgcagcactgg

------
PCR jkc011_F/jkc012_R on pBca9525-jkc0005     (2378 bp KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                (670bp, KpnI/SpeI)
Ligate, product is pBcajkc0006 (SpecR, R6k ori)

PCR jkc013_F/jkc014_R on pBca9525-jkc0005     (1923 bp KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                (2721 bp KpnI/SpeI)
Ligate, transform pir, product is pBcajkc007 (KanR,R6K ori)

Lambda interface library:
A59, Y60, E83, I84, M87, Y88, A63
```

Started on library construction for GST pick colonies to redo the tecan experiment.

# Jcham 11:17, 16 June 2011 (PDT)

```
GST INTERFACE LIBRARY CONSTRUCTION FILE
jkc015_F 3' KpnI Split GST forward- ccaaaGGTACCatgacggtactttgctgacg
jkc012_R 3' SpeI split GST reverse- cagttACTAGTGGCAGCAGCCACTGGTAACAG

jkc013_F 5' SpeI split GST forward- cagttACTAGTCCAGTGGCGATAAGTCGTGTC
jkc016_R 5' KpnI split GST reverse- cagttGGTACCatccagcagcaatgcagg

ca1775  Forward KpnI for Kan+R6K from pth7028   ccatgGGTACCGCCTCCTCgctttcgctaaggatgatttc
ca1776  Forward KpnI for R6K from pth7028       ccatgGGTACCGCCTCCTCGCAGTTCAACCTGTTGATAG
ca1777  Reverse M13/  SpeI for Kan+R6K or R6K from pth7028      cagttATactagtGCCTCCTCcattgcagcactgg

------
PCR jkc015_F/jkc012_R on pBca9525-1839     (2276 bp KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                (670bp, KpnI/SpeI)
Ligate, product is pBcajkc008 (SpecR, R6k ori)

PCR jkc013_F/jkc016_R on pBca9525-1839     (1896 bp KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                (2721 bp KpnI/SpeI)
Ligate, transform pir, product is pBcajkc009 (KanR,R6K ori)

GST interface library:
ALA9, CYS10, LEU32, LYS35, GLY50, GLN51, GLY66, ASN99, HIS106, LYS107, PHE113, TYR135
```

# Jcham 12:21, 20 June 2011 (PDT)

Set up pcr reactions for 3' and 5' split for LambdaRep interface library.

1. 3' split xpand +/- DMSO
2. 5' split xpand +/- DMSO
3. 3' split phusion +/- DMSO
4. 5' split phusion +/- DMSO

Set up pcr reactions for stuffer split

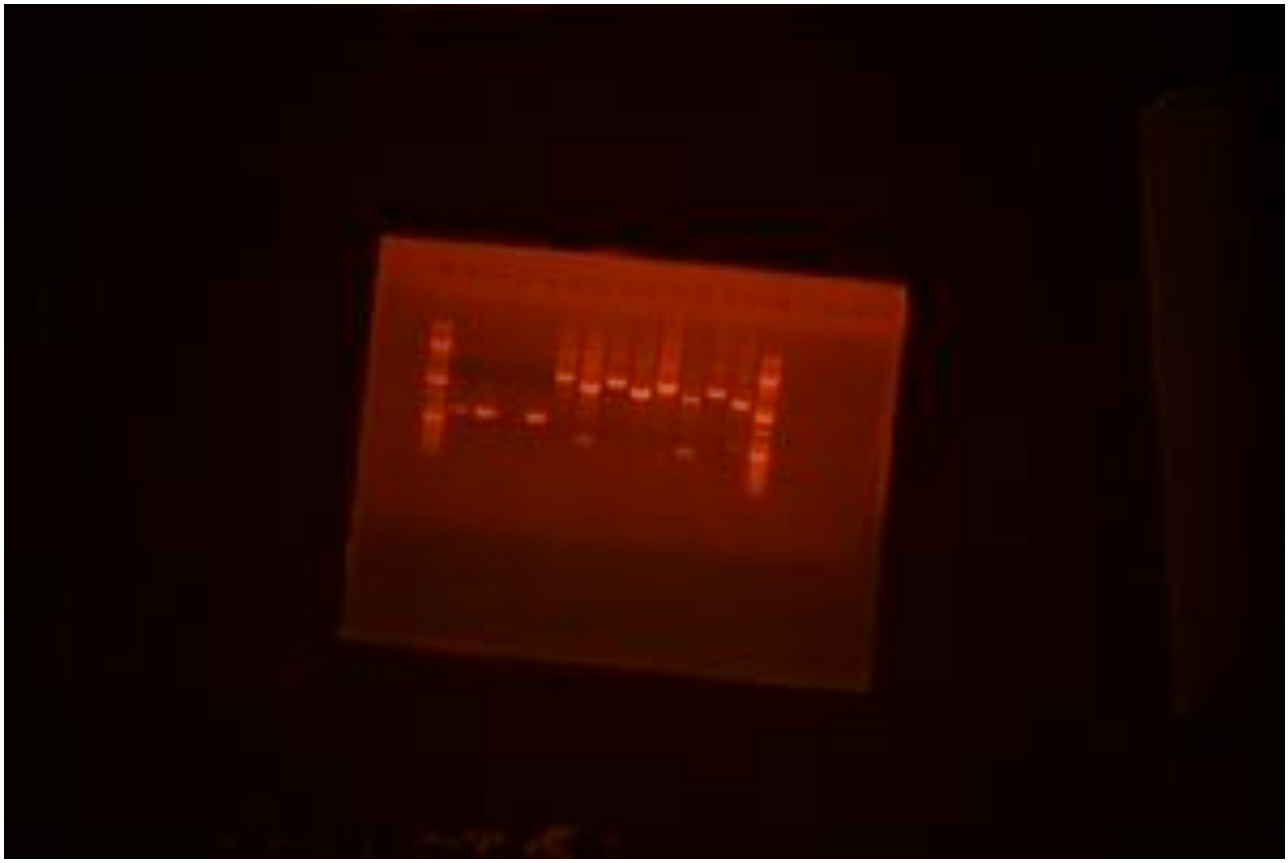
1. stuffer+kan xpand +/- DMSO
2. stuffer r6k xpand +/- DMSO
3. stuffer+kan phusion +/- DMSO
4. stuffer r6k phusion +/- DMSO

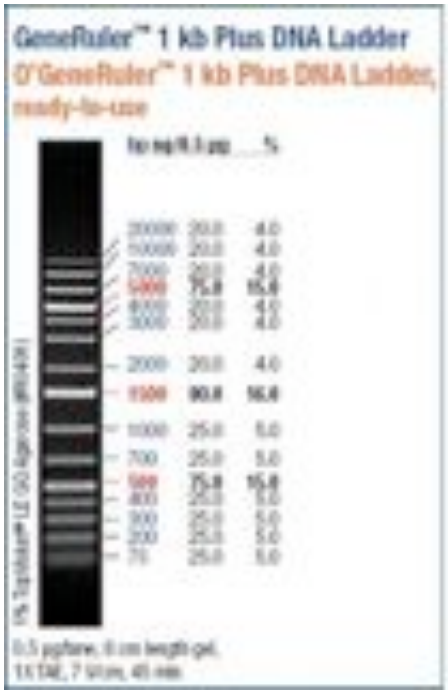
```
LAMBDA REP CORE LIBRARY CONSTRUCTION FILE (DBS or NBS)
jkc017_F 3' KpnI Split lambdaReap core forward- ccaaaGGTACCTTGGCTTATCCCAGGAATCT
jkc012_R 3' SpeI split lambdaRep core reverse- cagttACTAGTGGCAGCAGCCACTGGTAACAG

jkc013_F 5' SpeI split LambdaRep core forward- cagttACTAGTCCAGTGGCGATAAGTCGTGTC
jkc018_R 5' KpnI split lambdaRep core reverse- cagttGGTACCAAGTTCATTTTTCTTTTTTTC

ca1775  Forward KpnI for Kan+R6K from pth7028   ccatgGGTACCGCCTCCTCgctttcgctaaggatgatttc
ca1776  Forward KpnI for R6K from pth7028       ccatgGGTACCGCCTCCTCGCAGTTCAACCTGTTGATAG
ca1777  Reverse M13/  SpeI for Kan+R6K or R6K from pth7028      cagttATactagtGCCTCCTCcattgcagcactgg

------
PCR jkc017_F/jkc012_R on pBca9525-jkc005     (2501 bp KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                (670bp, KpnI/SpeI)
Ligate, product is pBcajkc010 (SpecR, R6k ori)

PCR jkc013_F/jkc018_R on pBca9525-jkc005     (1800 bp KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                (2721 bp KpnI/SpeI)
Ligate, transform pir, product is pBcajkc011 (KanR,R6K ori)

LambdaRep core library:
DBS or NBS- LEU18,TYR22,VAL36,PHE51,LEU65,LEU69  (and as a 7th, MET40)
```

```
LAMBDA REP CORE LIBRARY CONSTRUCTION FILE (DKG)
jkc019_F 3' KpnI Split lambdaReap core forward- ccaaaGGTACCAATGCATTAAATGCTTATAAC
jkc012_R 3' SpeI split lambdaRep core reverse- cagttACTAGTGGCAGCAGCCACTGGTAACAG

jkc013_F 5' SpeI split LambdaRep core forward- cagttACTAGTCCAGTGGCGATAAGTCGTGTC
jkc020_R 5' KpnI split lambdaRep core reverse- cagttGGTACCTTGATGCCATTAAATAAAGCAC

ca1775  Forward KpnI for Kan+R6K from pth7028   ccatgGGTACCGCCTCCTCgctttcgctaaggatgatttc
ca1776  Forward KpnI for R6K from pth7028       ccatgGGTACCGCCTCCTCGCAGTTCAACCTGTTGATAG
ca1777  Reverse M13/  SpeI for Kan+R6K or R6K from pth7028      cagttATactagtGCCTCCTCcattgcagcactgg

------
PCR jkc019_F/jkc012_R on pBca9525-jkc005     (2424 bp KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                (670bp, KpnI/SpeI)
Ligate, product is pBcajkc010 (SpecR, R6k ori)

PCR jkc013_F/jkc018_R on pBca9525-jkc005     (1877 bp KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                (2721 bp KpnI/SpeI)
Ligate, transform pir, product is pBcajkc011 (KanR,R6K ori)

LambdaRep core library:
DKG- LEU18,TYR22,VAL36,MET40,LEU50,PHE51,LEU57,LEU65,LEU69,VAL71,VAL73,PHE76
```

# Jcham 12:21, 21 June 2011 (PDT)

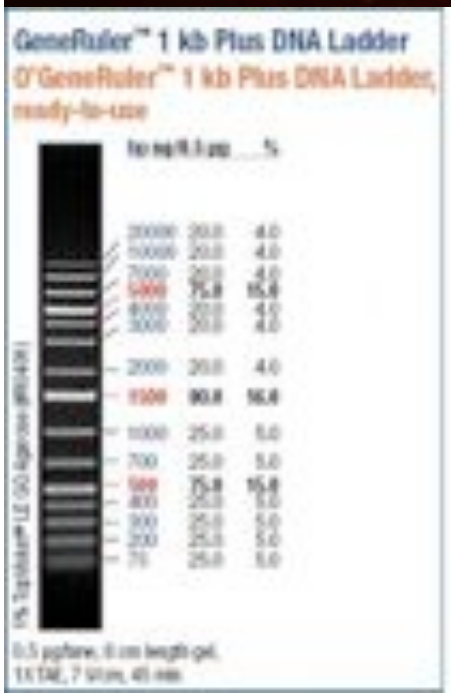
- pcr lambdaRep interface library






```
lane 1: 1kb+ ladder
lane 2: expand stuffer B
lane 3: expand+DMSO stuffer B
lane 4: phusion stuffer B
lane 5: phusion+DMSO stuffer B
lane 6: expand 3'
lane 7: expand 5'
lane 8: expand + DMSO 3'
lane 9: expand+DMSO 5'
lane 10: phusion 3'
lane 11: phusion 5'
lane 12: phusion + DMSO 3'
lane 13: phusion + DMSO 5'
lane 14: 1kb+ ladder
```

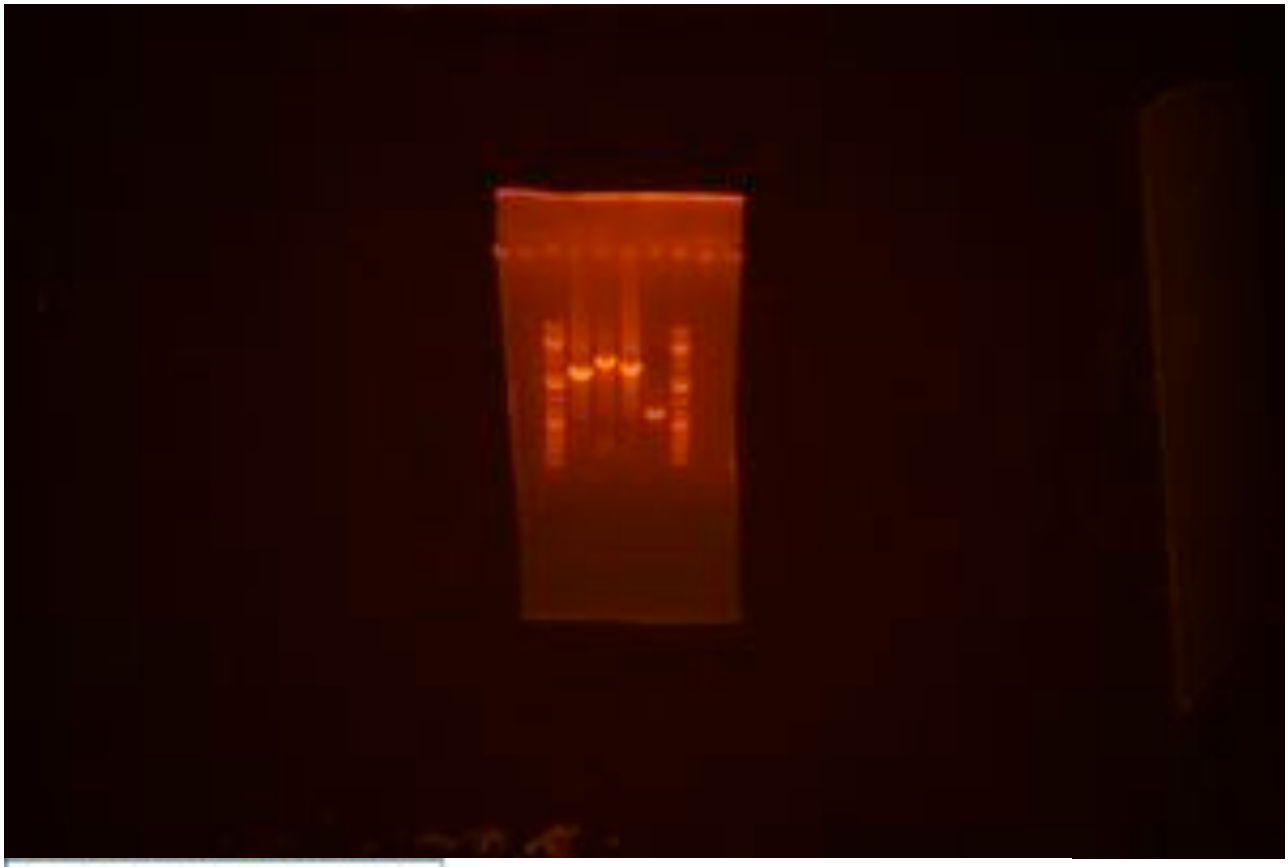
- pcr lambdaRep interface library

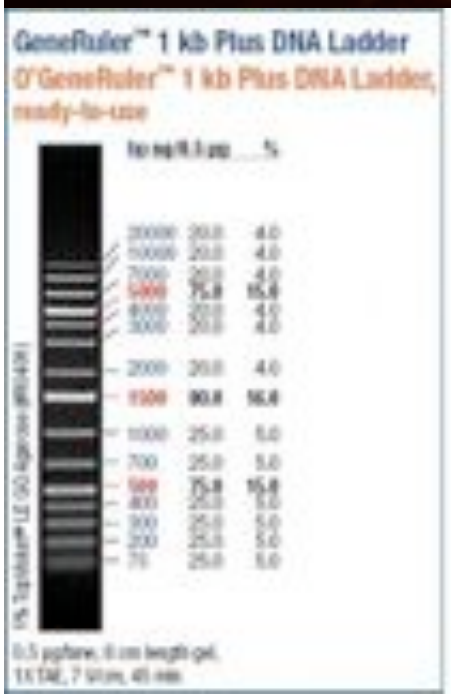



```
lane 1: expand stuffer A
lane 2: expand+DMSO stuffer A
lane 3: phusion stuffer A
lane 4: phusion+DMSO stuffer A
```

- Restrict, run gel, gel purify, Ligate, product is pBcajkc0006 ( 3' SpecR, R6k ori)
- Restrict, run gel, gel purify, Ligate, transform pir, product is pBcajkc007 (5' KanR,R6K ori)

- pcr lambdaRep interface library






```
lane 1: 5' lambdaRep kpnI/speI
lane 2: 3' lambdaRep kpnI/spe
lane 3: stuffer A kpnI/speI
lane 4: stuffer B KpnI/SpeI
```

- pick and grow pth7028

# Jcham 12:45, 22 June 2011 (PDT)

- miniprepped pth7028
- format the library creation page
- I redesigned the oligos for the lambdarep core library because the original construct did not split at the correct location.

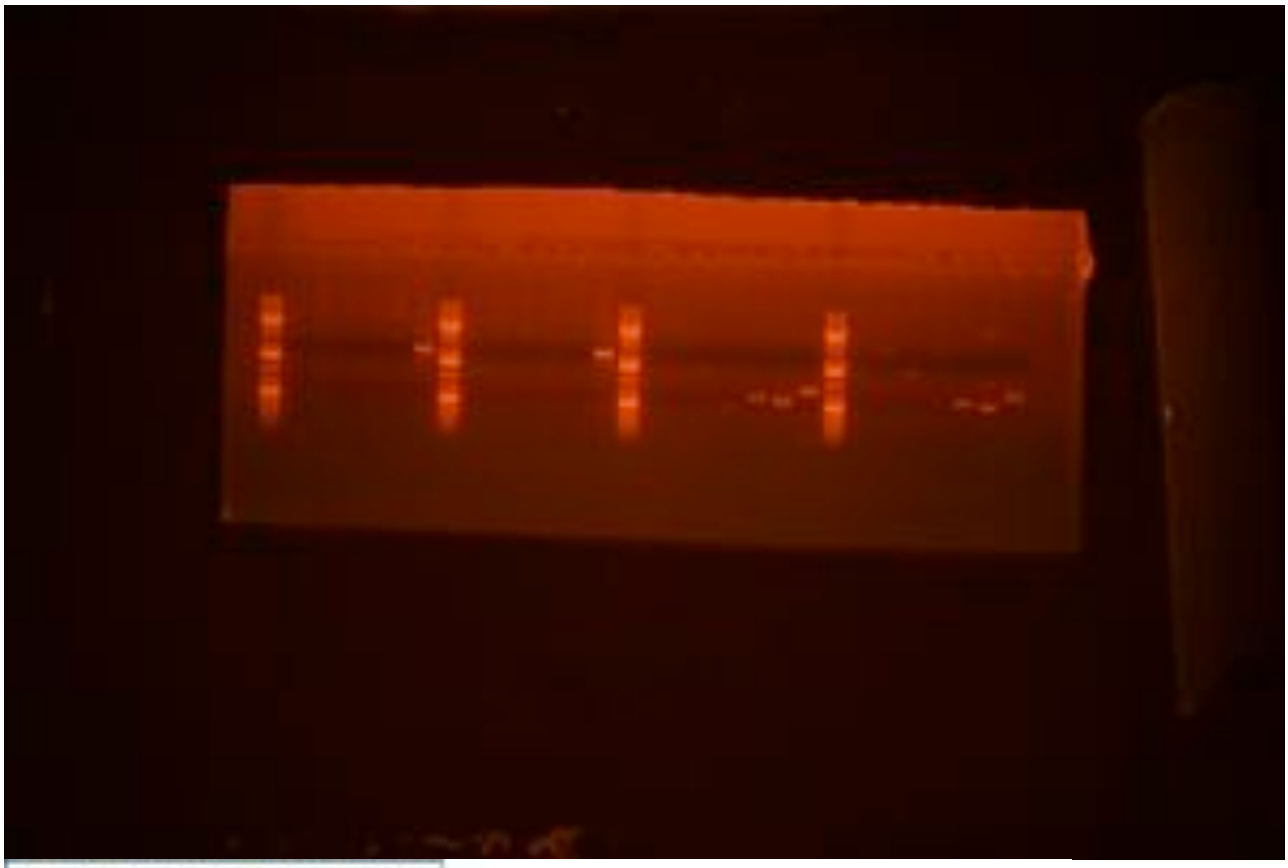
```
LAMBDA REP CORE LIBRARY CONSTRUCTION FILE (DBS or NBS)
jkc021_F 3' KpnI Split lambdaReap core forward- ccaaaGGTACCTTGGTGCTTTATTTAATGGC
jkc012_R 3' SpeI split lambdaRep core reverse- cagttACTAGTGGCAGCAGCCACTGGTAACAG

jkc013_F 5' SpeI split LambdaRep core forward- cagttACTAGTCCAGTGGCGATAAGTCGTGTC
jkc022_R 5' KpnI split lambdaRep core reverse- cagttGGTACCAACGCCTGACTGCCCCATCC

ca1775  Forward KpnI for Kan+R6K from pth7028   ccatgGGTACCGCCTCCTCgctttcgctaaggatgatttc
ca1776  Forward KpnI for R6K from pth7028       ccatgGGTACCGCCTCCTCGCAGTTCAACCTGTTGATAG
ca1777  Reverse M13/  SpeI for Kan+R6K or R6K from pth7028      cagttATactagtGCCTCCTCcattgcagcactgg

------
PCR jkc017_F/jkc012_R on pBca9525-jkc005     (2447 bp KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                (670bp, KpnI/SpeI)
Ligate, product is pBcajkc010 (SpecR, R6k ori)

PCR jkc013_F/jkc018_R on pBca9525-jkc005     (1854 bp KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                (2721 bp KpnI/SpeI)
Ligate, transform pir, product is pBcajkc011 (KanR,R6K ori)

LambdaRep core library:
DBS or NBS- LEU18,TYR22,VAL36,PHE51,LEU65,LEU69  (and as a 7th, MET40)
```

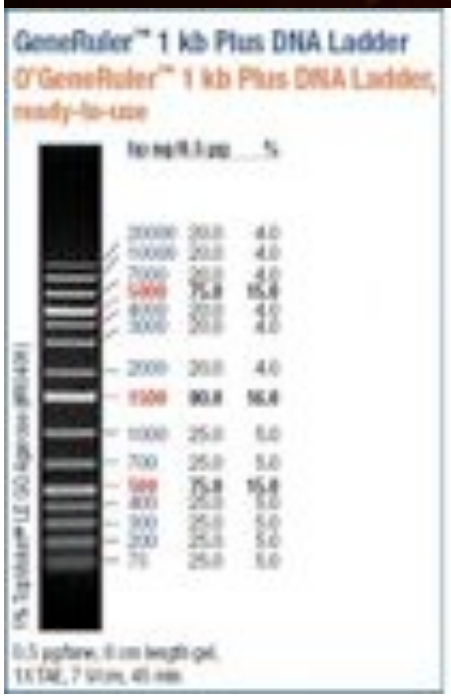
# Jcham 03:30, 23 June 2011 (PDT)

- set up pcr reactions for splitting GST, lambdaRep core 2, mukF core 1.
- set up pcr reactions for searching for new libraries: fabB, gyrA, hisS, mqsA, orn, tadA, yeaZ
- miniprep and sequence bjc005-jc1-L, bjc005-jc-R

# Jcham 11:39, 24 June 2011 (PDT)

- run analytical gel of pcr reactions from yesterday




GeneRuler™ 1 kb Plus DNA Ladder
O'GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use
20000 20.0 4.0
10000 20.0 4.0
7000 20.0 4.0
6000 75.8 15.4
5000 20.0 4.0
4000 20.0 4.0
3000 20.0 4.0
2000 20.0 4.0
1500 80.8 16.4
1000 25.0 5.0
700 25.0 5.0
500 75.8 15.4
400 20.0 5.0
300 25.0 5.0
200 25.0 5.0
75 25.0 5.0

```
lane 1: ladder
lane 2: gst3'
lane 3: lambdarep core 2 3'
lane 4: mukF 3'
lane 5: gst 5'
lane 6: lambdarep core 2 5'
lane 7: mukF 5'
lane 8: ladder
lane 9-15: lane 2-7 + DMSO
lane 16: fabB
lane 17: gyrA
lane 18: hisS
lane 19: mqsA
lane 20: orn
lane 21: TadA
lane 22: yeaZ
lane 23: ladder
lane 24-31: lane 16-23 + DMSO
```

- zymo clean gyrA, hisS, mqsA, orn, TadA, yeaZ, mukF 5'
- gel purify GST3' lambdaRep3' MukF3' Gst5' lambdarep5' fabB

- digest, gel purify

---

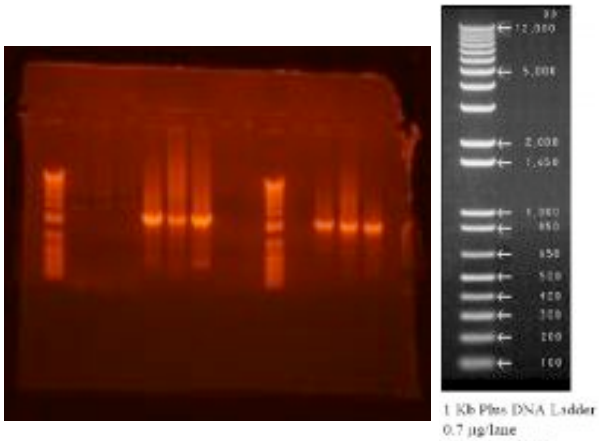
- set up new pcr using expand +/- dmso for splitting gst, lambdarep, mukF

```
Plan
1. check results of pcr (gst3' lambdarep 3' mukf3' gst5' lambdarep5' mukf5' +/- DMSO)
2. zymo clean gyrA, hisS, mqsA, orn, tadA, yeaZ, mukf5'
3. ligate with 1834, recover, transform
4. grow, miniprep
```

# Jcham 02:06, 27 June 2011 (PDT)

- analytical gel for (gst3' lambdarep 3' mukf3' gst5' lambdarep5' mukf5' +/- DMSO)

- split pcr

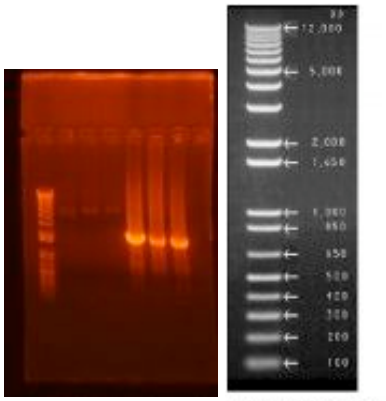


1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1:gst3'
lane 2:lambdarep 3'
lane 3:mukf3'
lane 4:gst5'
lane 5:lambdarep5'
lane 6:mukf5'
```

- zymo clean up first 6 (gst3' lambdarep 3' mukf3' gst5' lambdarep5' mukf5')
- digest with KpnI/SpeI

- digest of splits



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1:gst3' KpnI/SpeI
lane 2:lambdarep 3' KpnI/SpeI
lane 3:mukf3' KpnI/SpeI
lane 4:gst5' KpnI/SpeI
lane 5:lambdarep5' KpnI/SpeI
lane 6:mukf5'KpnI/SpeI
```

- run on gel and gel purify (the gel was slightly slanted, so the bands for the first 3 were higher than normal)
- ligate with stuffer A/B
- recover
- transform in jtk155
- plate on respective Kan or Spec

- gel purify digest of gyrA, hisS, mqsA, orn, tadA, yeaZ, mukf5'

I eluted with 50 uL. It's probably too little, so I set up a new pcr reaction

- set up new pcr for fabB, gyrA, hisS, mqsA, orn, tadA, yeaZ

```
   xpand +/- dmso and phusion +/- dmso.
```

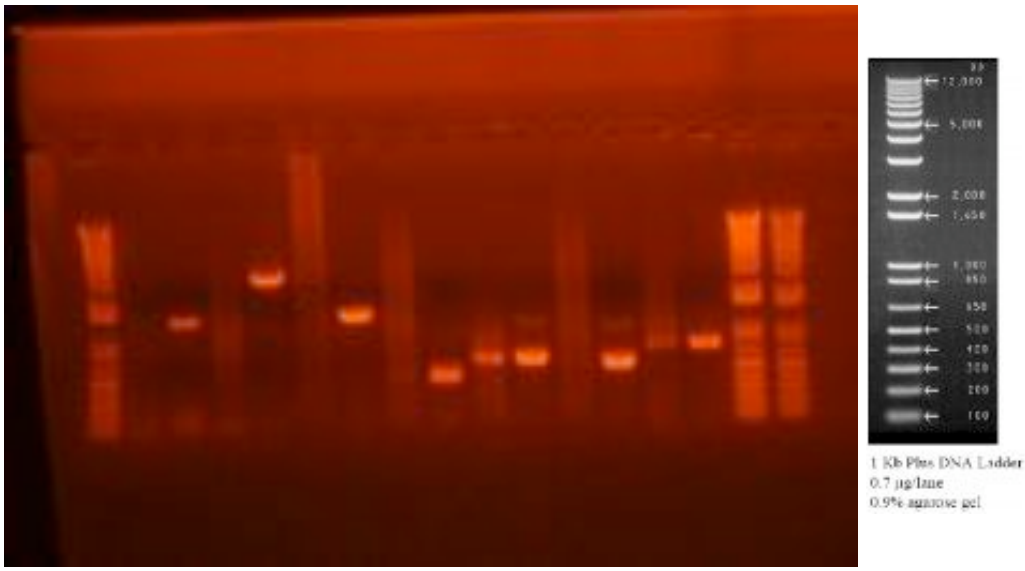
Nikit had pre-made stocks of pcr mastermix. I forgot to put in the polymerase. I put it in after half an hour and restarted. Hopefully, I still get products.

- set up splitting pcr for all five libraries in case the transformations failed and I get no colonies.

```
Plan for tomorrow
1. run analytical gel on pcr products for splitting (5) libraries and new library possibilities (fa
2. zymo whatever is good
3. digest splits and stuffers, run on gel, purify, ligate, transform into jtk155
4. digest new library possibilities, ligate, transform into jtk155
```

# Jcham 05:36, 28 June 2011 (PDT)

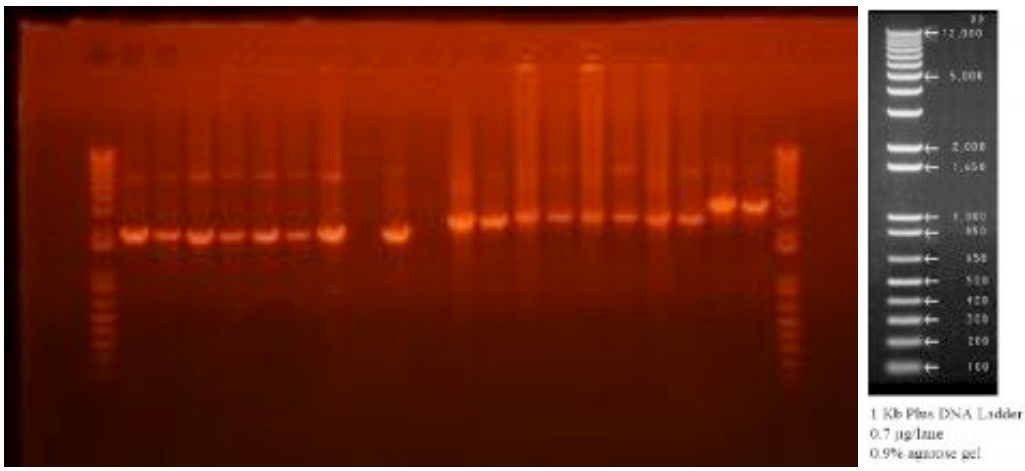
- run analytical gel on pcr products for splitting libraries and new library possibilities.





1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1-2: fabB xpand/phusion
lane 3-4: gyrA
lane 5-6: hisS
lane 7-8: mqsA
lane 9-10: orn
lane 11-12: tadA
lane 13-14: yeaZ
```





1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1-2: GST 5'
lane 3-4: LambdaRep 5'
lane 5-6: LambdaRep 2 5'
lane 7-8: LambdaRep interface 5'
lane 9-10: MukF 5'
lane 11-12: GST 3'
lane 13-14: LambdaRep 3'
lane 15-16: LambdaRep 2 3'
lane 17-18: LambdaRep interface 3'
lane 19-20: MukF 3'
```

- zymo clean up new library possibilities (fabB, gyrA, hisS, mqsA, orn, tadA, yeaZ)
- digest with nhe1/bamH1
- run on gel, gel purify,
- ligate with 1834 nhe1/bamH1
- transform, recover, plate on spec

- zymo clean splitting libraries (all 5)

# Jcham 06:53, 29 June 2011 (PDT)

- pick colonies for 7 new possible libraries. Hopefully sequencing will come out ok.
- sequencing for the bjc005-jc1 L and R failed.
- miniprep'd the GST L/R, LambdaRep2 L/R, MukF L/R. Sent for sequencing

digest splitting library pcr again

```
Plan
1. Miniprep 7 new libraries
2. check sequencing. If good, done. If bad, restart pcr. Also incorporate the pcr Xin Xin set up to
```

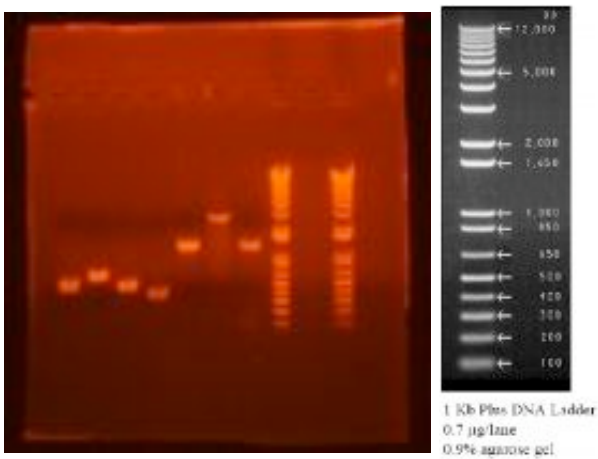
# Jcham 04:36, 30 June 2011 (PDT)

restart splitting pcr from beginning again.

phusion +/- DMSO expand +/- DMSO

---

Testing Seven New Possible Libraries FabB, GyrA, HisS, mqsA, tadA, yeaZ, Orn digest, gel purify, ligate, transform into JTK155.




1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: orn
lane 2: yeaZ
lane 3: tadA
lane 4: mqsA
lane 5: hisS
lane 6: gyrA
lane 7: fabB
```

```
Plan for tomorrow
1. pick colonies of new libraries.
2. run analytical gel on the splitting pcrs
3. zymo clean pcrs
4. digest splits and stuffer and run gel
5. ligate splits with stuffers
6. transform into pir jtk155 cells

Plan for Saturday
1. pick colonies of splits
2. miniprep the new libraries
3. transform new libraries into bss52

Plan for Sunday
1. miniprep splits
```