Miriam igem start

From AndersonLab wiki

May 31 2011

- digested 1778 and 1884 with 50 ul DNA (from my midi prep), 50ul NEB2, 5ul BseRI, 395ul H20

1. digest over night

- made a mega gel for the running out of gel
- streaked out DH10B from Josh's parts.

June 1 2011

- Run out digest on mega gel for 2-3 hours

- cut out

1. 1778- 1711 band
2. 1784-3169 band

- electroporation

1. put gels in bags
2. got 740ul of liquid
3. (1/10) 3 molar sodium acetate (74ul)
4. 1 volume isopropanol or 2-propanol (740ul)
5. put in freezer
6. spin for 5 mins
7. look for pellet
8. pour 1 ul 70% etoh spin 2 mins after each (x2)

- ligation over night

1. 600 total ul
2. Add 60uL T4 DNA Ligase Buffer, 15uL T4 DNA Ligase & 525uL DNA fragments 1778 & 1784
3. put 100ul into 6 PCR strips
4. overnight instant incubate

June 2 2011

- zymo clean up ligation elute to get 48ul of DNA

1. Separate 6 PCR Tubes to 4 Eppendorf Tubes (150uL volume in each)
2. Add 3x volume ADB Buffer- 450uL~500uL
3. Heat several minutes @ 55 degrees C

4. Spin down & discard flow through- 45 sec @ 13.4k rpm
5. Spin 90sec to dry
6. Elute all 4 columns into 1 Eppendorf w/ 12uL ddH2O each- Total 48uL DNA

- Competent cell prep

1. Big flask fill with 1L 2YT (save some 2YT)
2. 5 ml of DH10B culture
3. 1.5 hrs, the first OD
4. blank with 1 ml 2YT
5. every 30 min until at .1
6. then start timing every 10 mins
7. put 10% glycerol on ice
8. clean out rotar, start cooling 5 degrees 300 rpm
9. get bottles used for spinning down and cool
10. once at .5, pour cells into bottles, balance them
11. spin 4 mins at 7000 rpm
12. pour out supernatant
13. combine bottles into 2 bottles: pour 10% glycerol 1/4 full on 2 use sterological pipet to dislodge pellet, then combine
14. pour 10% glycerol into the 2 bottles to fill to the top
15. put blue lid cell collection tubes on ice
16. pour SOB into a large flask
17. spin again 4 mins at 7000rpm
18. pour out supernatant
19. make sure to get rid of all the glycerol
20. add a little glycerol
21. spin 4 min at 7000 rpm
22. get blue topped cuvets, put 6 on ice
23. 48 ul of DNA, 6 cuvets, 7ul of DNA in each
24. dump supernatant
25. get rid of the rest of the glycerol with a pipette
26. resuspend with a pipette
27. pour into blue topped tubes

- Transform
- Electroporation

1. Place electroporation cuvettes, eppendorfs, 15mL Falcon Tube, & DNA on Ice
2. Remove supernatant & add to Falcon Tube
3. Add 320uL cells to Eppendorfs & 4uL DNA- Electroporate (DNA volume varied from 3-7uL)
4. Time Constant should >4, ~2.2-3.4=High Salt Content
5. Rescue w/ 1mL SOB Media & inoculate 1L SOB in Flask- Shake in Warm Room for 1hr. (2:30-3:30PM)
6. Negative Control- Plate competent cells on Spec & incubate 37 degrees C overnight

- Titer

1. Incubate 2 Spec 2YT plates
2. Add 1mL 1000X Spec to flask & mix
3. Cell Density ~1e5-1e9- Assume titer as 1e8 for 100cfu

4. Take 10uL + 990uL 2YT in Eppendorf & Plate 100uL (100cfu=1e8 Transformants)
5. Also, plate 100uL directly (100cfu=1e6 Transformants)
6. Plated serial dilution of 10uL culture in 90uL LB+Spec in 8 PCR Tubes- Pipetted 3 rows on Spec plate
7. Shake flask in Warm Room overnight

June 3 2011

- Transformation Results

1. Only 98 colonies on plate w/ 100uL direct from flask
2. No colonies from 100uL 1/100 dilution titer or 5uL serial dilution titer
3. Discarded electrocompetent overnight cell culture (large flask)

- Ligation Parallel Test

1. Compare ligation DNA w/ control pUC18 using chemically competent cells
2. Find #transformants/ug DNA
3. Add 1uL pUC18 DNA+30uL KCM to tube 1 & 0.5uL library ligation+4.5uL Box H2O
4. Incubate on Ice for 1min.- Add 50uL chemically competent MC1061
5. Incubate on Ice for 10min.- Heat shock @ 42 degrees C for 90sec
6. Incubate on Ice for 1min.- Rescue w/ 200uL 2YT
7. Shake @ 37 degrees C for 1hr.- Plate 50uL pUC18 on AMP & Ligation on Spec
8. Incubate @ 37 degrees C overnight
9. Expected- 1E6=20cfu

June 6 2011

- ran a 15ml scale one pot reaction (Digestion and ligation) used gold program on PCR machine

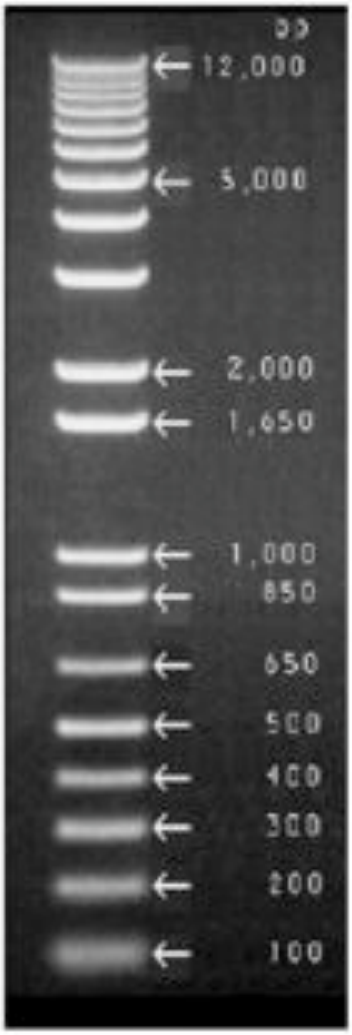
1. .3 ul BseRI, .3 ul ligase, 1.5ul T4 DNA ligase buffer, 1.1ul 1778, 1.1 ul 1784, 11ul h20

- transformed

1. dilute 1ul of mix into 10 of h2o
2. 1ul of dilution into BSS52 cells
3. recover and plate on spec

- test on a gel

1. run other 10 ul of dilute and 5ul of straight up on a gel
    - 1785 one pot



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

- Parallel Ligation Test w/ pUC18 & One Pot Reaction

1. 1ul of .1ng/ul of puc into Bss52, rescue with 200ul of 2YT, plate 50 ul on spec

June 7 2011

- checked plates- 1785 plate has 32 colonies ~8 are green
- started large scale ligation (40x of above ligation)
- plated on bss52 (does not work for puc, redoing)

June 8 2011

- competent cell prep... but it leaked so we planned on working on it tomorrow
- transformed into bss52 cells

June 9 2011

- competent cell prep
- transformation
- time constants are low (2-3)
- went through with transformation as planned

June 10 2011

- Mini prepped to get 300ul of the competent cell prep

- plated DH10B
- Presentation for lab meeting

June 12 2011

- prepped all the materials for competent cell prep
- picked a colony of DH10B

June 13 2011

- Competent cell prep to test glycerol cleanness

1. one with glycerol and straight box water
2. one rinsed with box water twice then box water and glocerol
3. one rinsed with 18.2 milliQ h20 and then 18.2 h20 with glycerol
4. only filling bottles half way with glycerol, testing 4 bottles NOT consolidating
5. 320ul zap to see which works the best
6. with the one that works the best, do a 50ul zap with 1ul puc, rescue in 10ml
7. 10ul + 990ul dilution with 2YT, plate 100ul
8. serial dilution

- So here are my results:

1. Regular glycerol (no rinse): TC=5.2
2. Regular glycerol (rinse): TC=5.6
3. 18.2 milliQ Glycerol with rinse: Tc=5.4
4. 1ul of puc into 50ul of the regular glycerol with rinse and got a TC=5.6

June 14 2011

- 5.7 E9 cfu/ug of .1ng/ul wit h50ul electrocompetend cells in .1mm gap cuevett
- comp cells are not the problem

June 15 2011

- looking for new options of dimers in yeast

1. not enzyme
2. no metabolism
3. N-term on the same side

June 16 2011

- looking at proteins more
- picked a colony of DH10B for comp cell prep
- washed dishes, preparing for com cell prep

June 19 2011

- comp cell prep
- 7ul DNA, 320 ul cells TC: 4.8, 4.6, 4.4, 4.6, 4.6, 4.4
- 10-12ul DNA, 500ul cells TC: 4.4
- incubate for 1 hour
- titer and serial dilution
- got acetylsalicylic acid, estradiol, caffeine, ascorbic acid 2-phosphate for terry

June 20

- comp cell prep transformation worked well!
- 3.178 x 10^9 diversity
- midi preping 256.1 ml to get 500ul
- transform 1:200ul dilution with water, 1ul heat shock into 50ul jtk155 cells (30ul kcm)

1. plate 50ul
2. pick 4 colonies
3. sequence with primer ca1786

July 5

- comp cell prep of MSD002 1788 d4-3 cells into mukF library test (in notebook)
- 8ul of DNA, 1920ul of cells
- TCs: 4.8, 5.0, 5.0, 5.0, 4.8, 4.8
- titering: 10ul of cells with 990ul h20, plating 100ul of that

1. 1ul DNA= 22864 colonies
2. 1ml= 2.3 10^10 cfu

- separating the 2 beakers into 1 with spec and 1 with spec/cam

July 6

- mini prep 2ml from each
- receed 2ml of each into 2YT broth with cam
- spin down 250ml of each and store in the -80
- titer again

1. 1ul to 1000ul 2YT
2. grow on spec trim

July 7

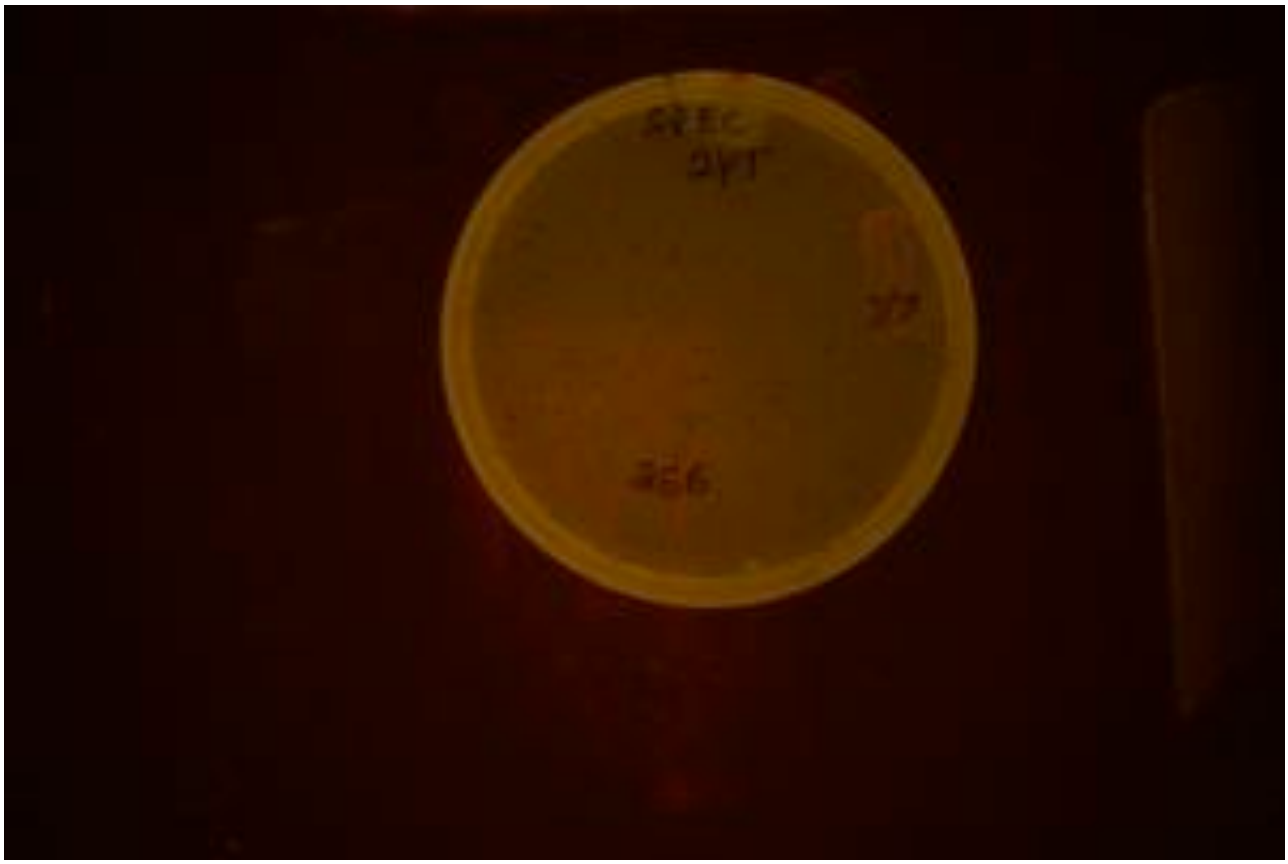
- mini preped 2ml from each 2YT sample
- plated 10^-6 and 10^-8 and serial dilution of all 4
- transformed 1ul:100ul h20 into bss52 cells
- plate on spec/ac

- looked for which samples to use
- midi prepped the spec 2YT sample
- results:

- transformations:

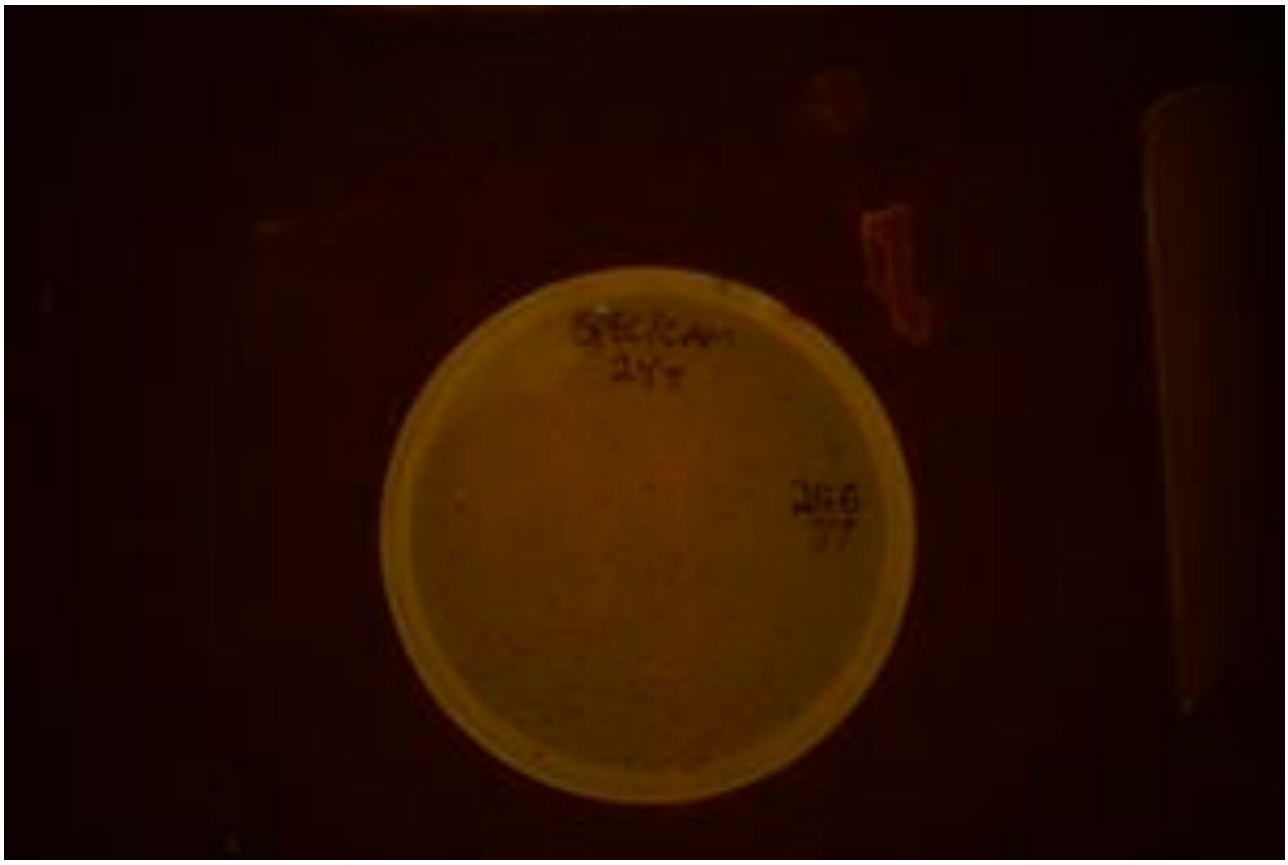
1. spec SOB: G:143, regular: 146

1. spec 2YT: G:7, regular: 256



1. spec/cam SOb: G:15, regular: 173

1. spec/cam 2YT: G:9, regular: 256

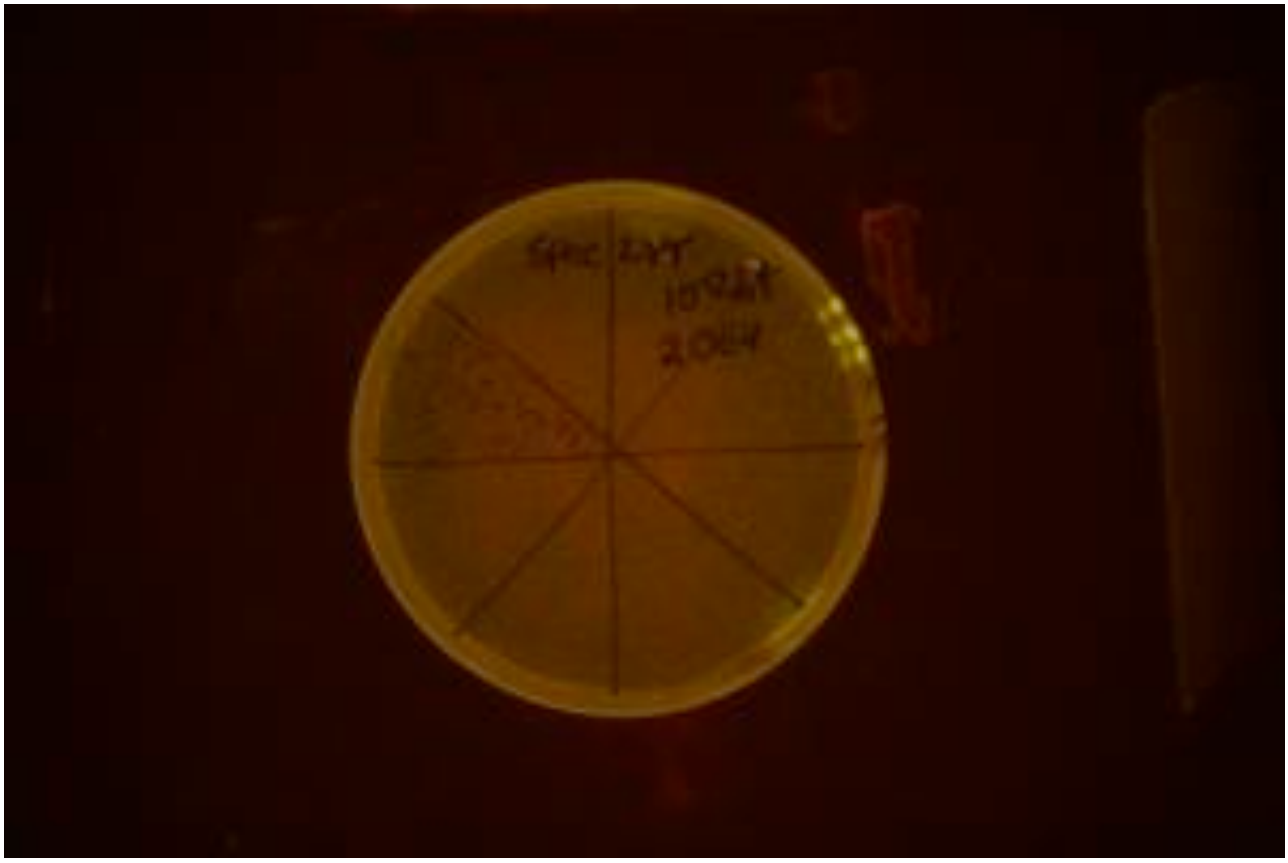


- titering plates

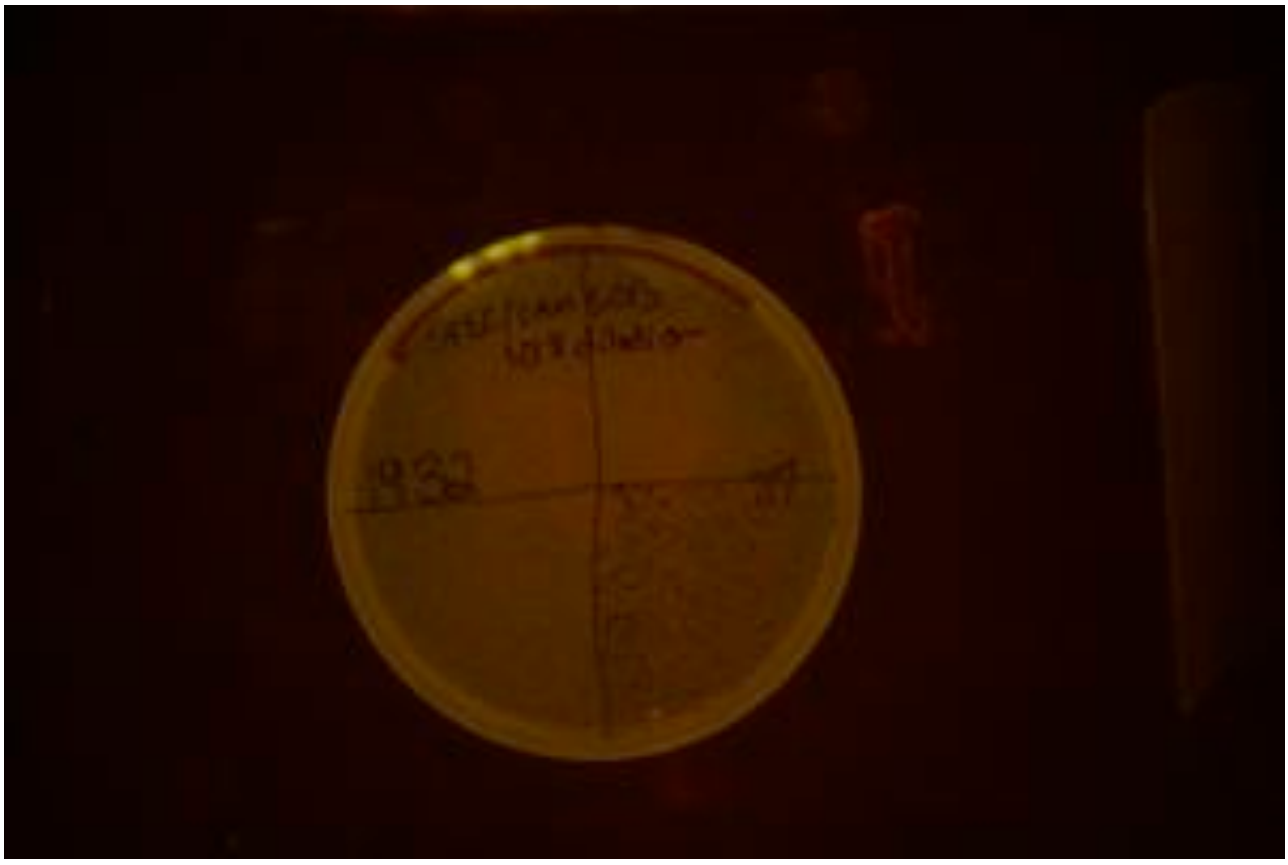
1. spec SOB: 6.3x10^9

1. spec 2YT: 2x10^11 (jiffy, may have messed up)

1. spec 2YT: 2x10^11 (iffy, may have messed up)



1. spec/cam SOB:1.9x10^11



1. spec/cam 2YT:3.6X10^9

July 11

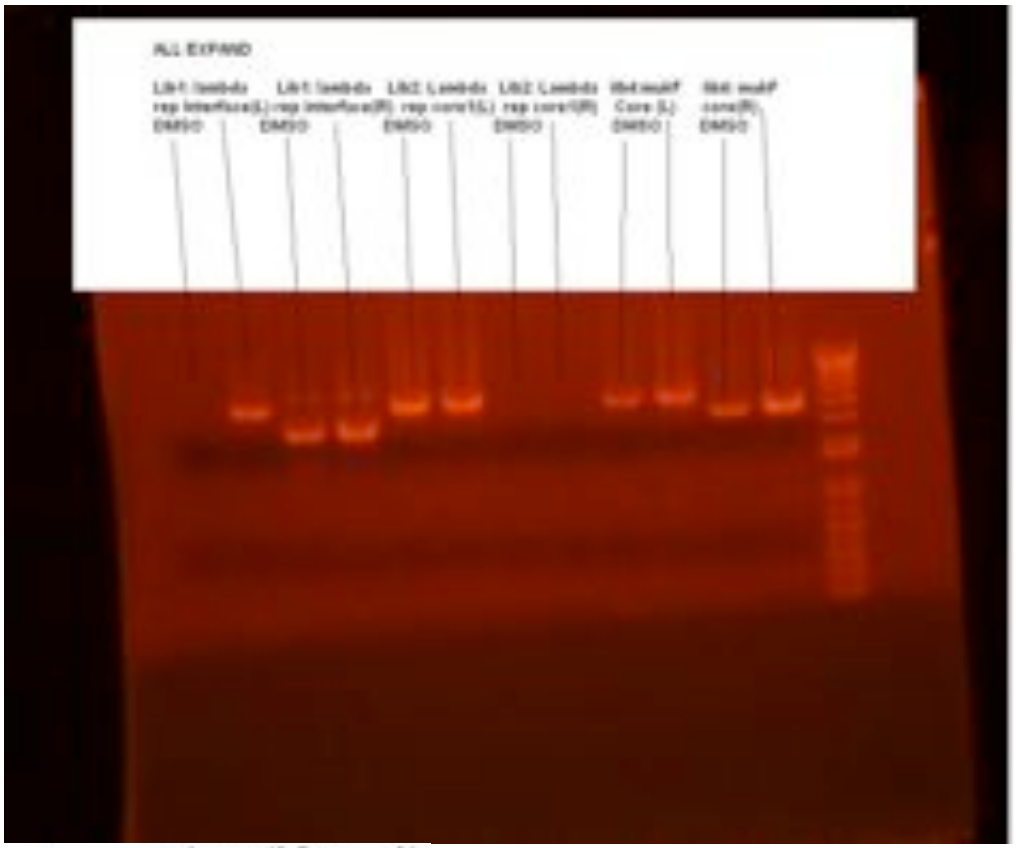
- set up EIPCR of jc1 jc2 and np1 libraries
- streaked out jtk155 cells for comp cell prep on wednesday
- HELPED EVERYONE AS USUAL

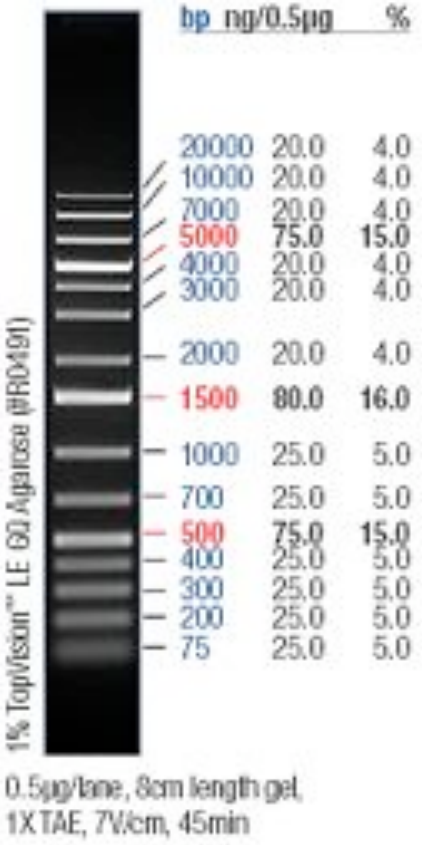
July 12

- mapped EIPCR

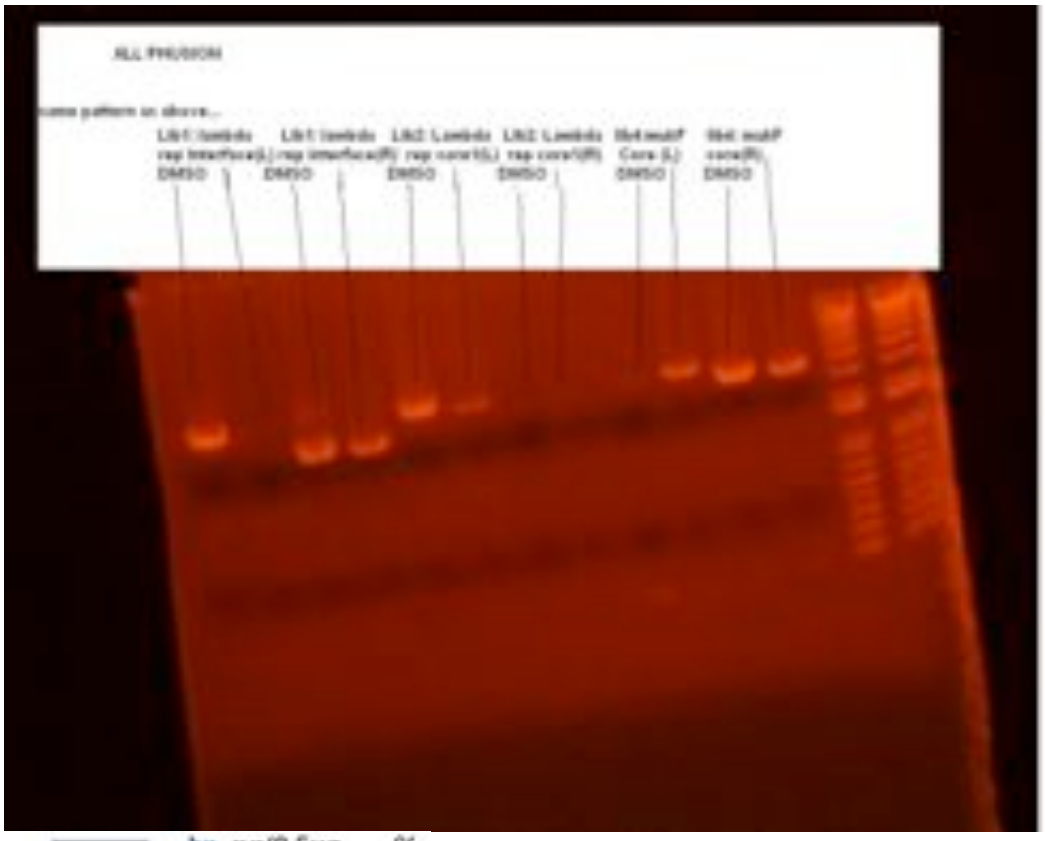
results:

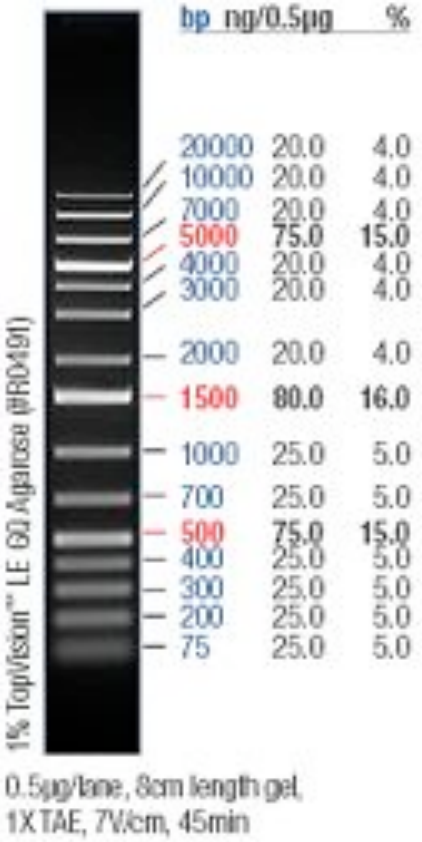
- 20110712 lib 1, 2 and 3 after EIPCR with all con ditions(DMSO, Phusion, Expand)





- 20110712 lib 1, 2 and 3 after EIPCR with all con ditions(DMSO, Phusion)





- washed dishes
- gold rxn on all besides jc2R (lambda rep core 1 R)

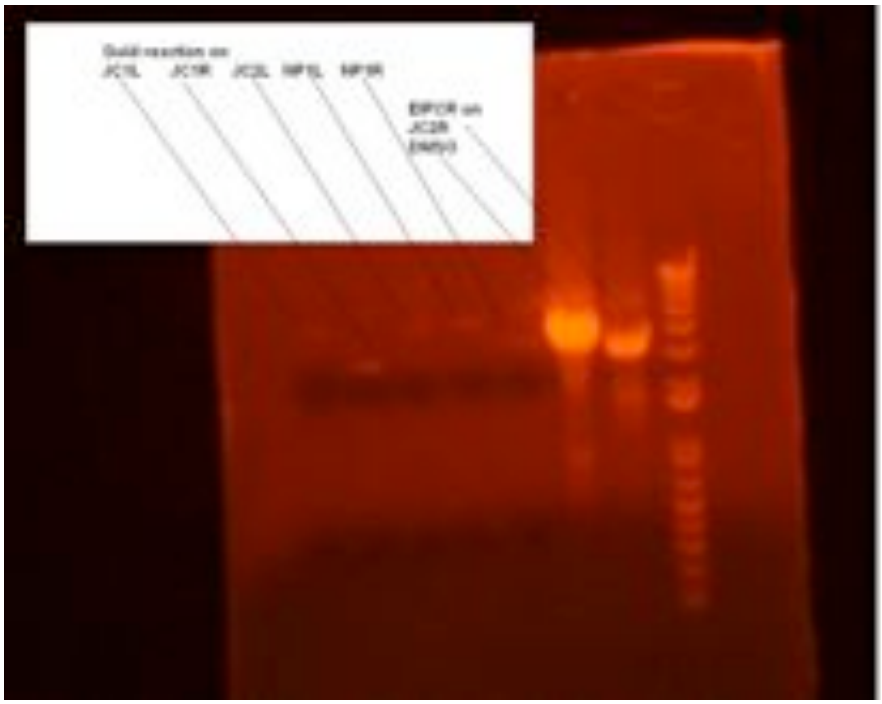
1. Expand DMSO for all but jcR1 which was expand with no DMSO

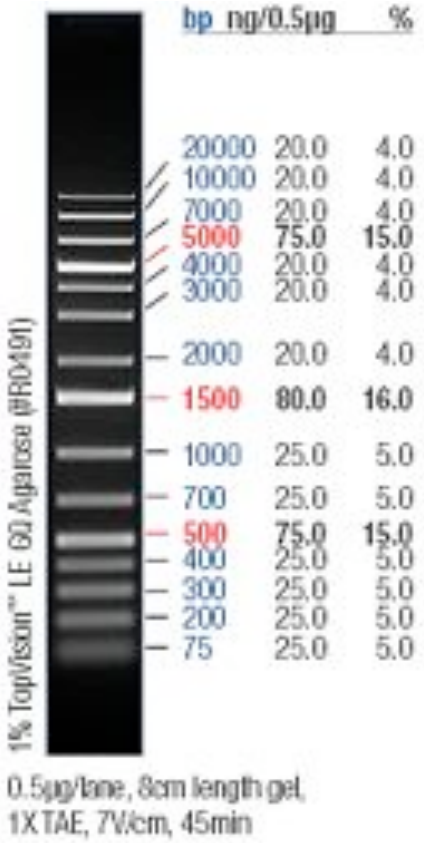
- redoing EIPCR rxn on jcR2
- asked nikit to reorder oligos with more GC content
- picked a colony of the jtk155 plate

July 13

- made LB
- set up comp cell prep
- ran gold rxn on a gel
- ran EIPCR of JC2R, it worked!

1. gel purify the expand without DMSO
2. set up gold reaction

- zymo clean up of gold rxn eluted with 8
- 



July 14

- getting ready for 2:45 am comp cell prep for gabe
- EIPCR for JC3R/L and NP2R/L
- zymo clean up of gold reaction eluted with 8ul

July 16

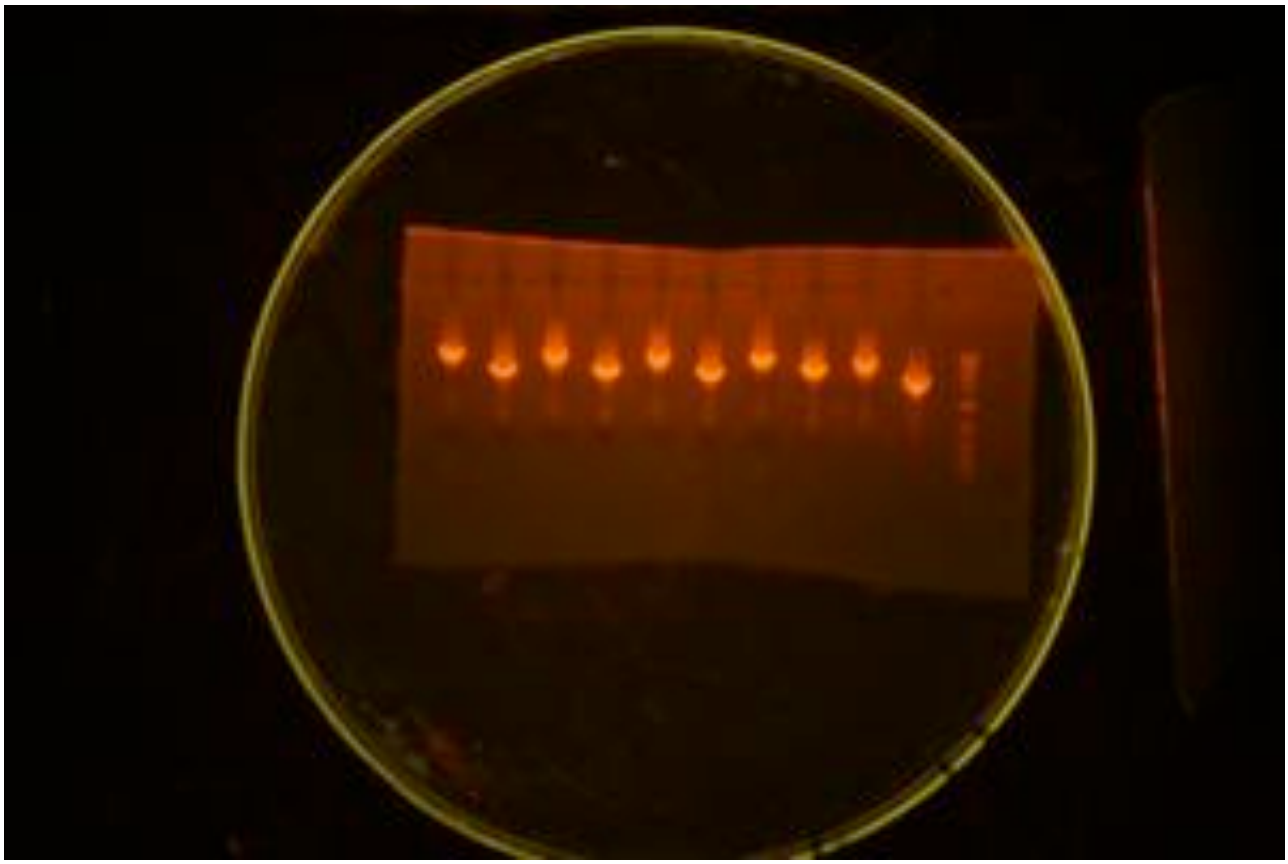
- comp cell prep at 6 am!!
- it has been about 3.5 hours since gabe put them in and the OD is still about .07 :(
- comp cell prep...TCs below
- NP1R- 4.8 1 ml rescue 7ul DNA
- JC1R- 4.6-4ul in 320 of cells,4.2-4ul in 100ul of cells, 2ml rescue
- JC3L- 4.4 100ul cells, 1ml rescue, 3.5 DNA
- JC2L- 4.6 100ul cells, 1ml rescue, 3.5 DNA
- JC2R- 4.6 100ul cells, 1ml rescue, 3.5 DNA
- NP2R- 4.6 100ul cells, 1ml rescue, 3.5 DNA
- JC3R- 4.2 100ul cells, 1ml rescue, 3.5 DNA
- JC1L(1)- 3.2 100ul cells, 1ml rescue, 3.5 DNA
- JC1L(2)- 3.4 100ul cells, 1ml rescue, 3.5 DNA
- NP2L- 4.4 100ul cells, 1ml rescue, 3.5 DNA
- NP1L- 4.4 100ul cells, 1ml rescue, 3.5 DNA
- for titer plate:

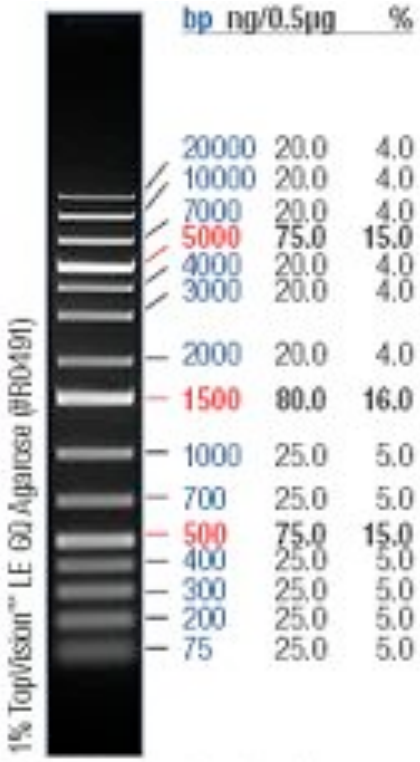
1. plated 10ul of 10^-6 and 10^-8
2. plated serial dilution

July 18

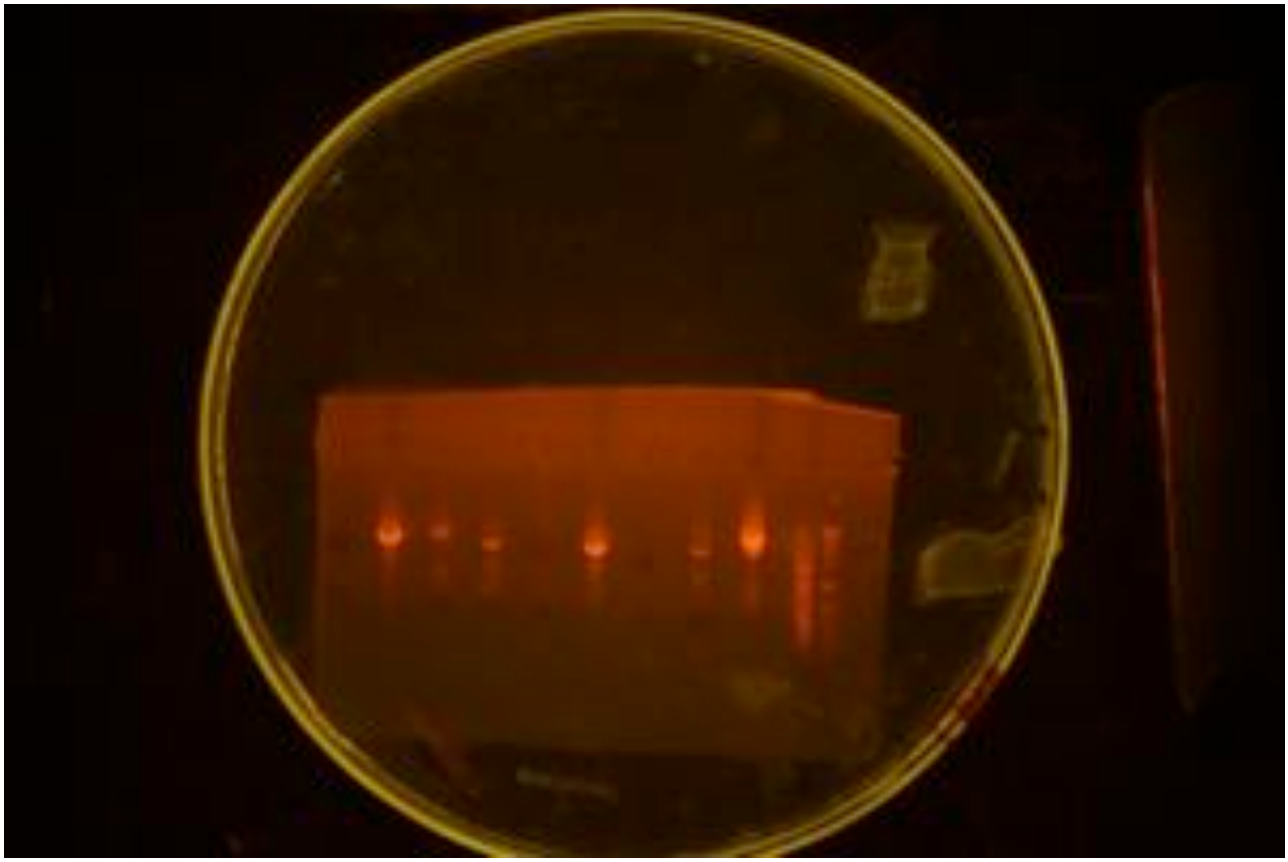
- Everything was contaminated because the 2YT I used to rescue was contaminated..
- started over at the EIPCR step.
- gel purify

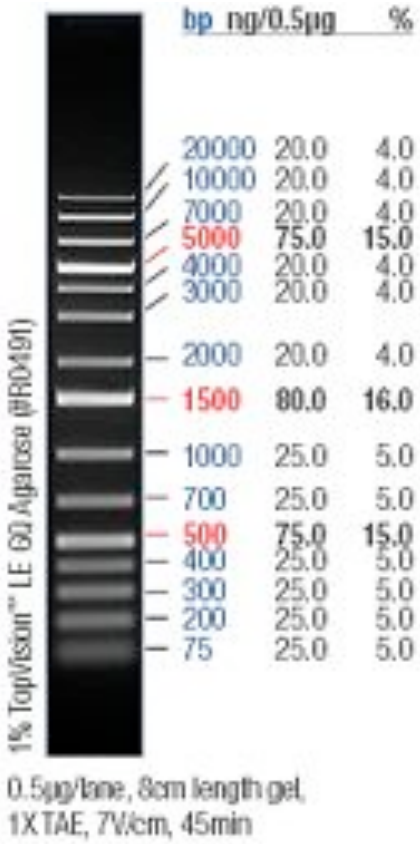
1. order is JC1L, JC1R, JC2L, JC2R, JC3L, JC3R, NP1L, NP1R, NP2L, NP2R
2. bottom is DMSO

- 



0.5µg/lane, 8cm length gel,
1XTAE, 7V/cm, 45min





- Doing all 5 libraries together now
- set up gold reaction, will do another comp cell prep tomorrow

July 18

- zymo clean up of gold rxn
- cell prep
- transformed in 3.5ul DNA into 100ul of cells
- Time constants:

1. JC1L-5.0
2. JC1R-5.0
3. JC2L-4.8
4. JC2R-4.8
5. JC3L-4.8
6. JC3R-4.8
7. NP1L-4.8
8. NP1R-4.8
9. NP2L-5.0
10. NP2R-4.8

- because the jc1 library is so large, inoculated in 100ul of 2YT
- Titer plates of 10^-6, 10^-8 and serial dilution
- made electro comp cells of EC100D PIR 16

July 19

- Comp cells are not resistant to trim :(and there are a few tiny tiny colonies on spec

diversity:

1. JC1L- 9 x 10E8
2. JC1R- 3.2 x 10E9
3. JC2L- 2 x 10E8
4. JC2R- 1.1 x 10E10
5. JC3L-1 x 10E9
6. JC3R- 2.1 x 10E10
7. NP1L- 2 x 10E8
8. NP1R- 4.9 x 10E9
9. NP2L- 3 x 10E9
10. NP2R- 9.9 x 10E9

- from serial dilution, picked 1 or 2 of the most concetrated clumps and inoculated into 3 ml... will mini prep tomorrow
- picked a single colony from each lib for mini and sequencing
- mini prepped 4 ml of the jc1L and JC1R library
- ALL FAILED BAD MATH WRONG TITERING, DIDNT SAVE ALL OF THE CELLS.
- COMP CELLS BROKEN (i put tss instead of glycerol in them)
- going to do a comp cell prep tomorrow.
- prepping now.

July 20

- Comp cell prep on 10 libraries
- Titering protochol:

1. 1ul of 1 ml of rescue into 1ml of 2YT.
2. plate 10ul to get 10 colonies (1E6)
3. 100cfu= 1E7
4. 10cfu= 1E6

- receed JC1L and JC1R into 500ml of 2YT grow over night for midi prep in the future
- receeded NP1L and NP1R into 500ml of 2YT by accident. going to do a mini prep on it
- Poured 600ul of DNA juice onto a large plate hoping for a lawn

July 21

- Counted the titering plates

1. JC1L- 5E5
2. JC1R- 7.2E6
3. JC2L- 1.4E6
4. JC2R- 8.24E7
5. JC3L- 6.2E6
6. JC3R- 1.056E8
7. NP1L- 4.9E6
8. NP1R- 5.04E7

8. NP1R- 5.04E7
9. NP2L- 1.7E6
10. NP2R- 9.76E7

- picked a colony from each for sequencing
- scraped plates and mini prepped each library besides JC1R and JC1L
- Spun down 250ml of JC1R and JC1L for midi prep at a later time
- Diluted the DNA from mini preps 1:20 dilution for EIPCR

July 22

- mini prepped the picked colonies to send for sequencing
- dishes
- waiting for polymerase to come in to set up EIPCR

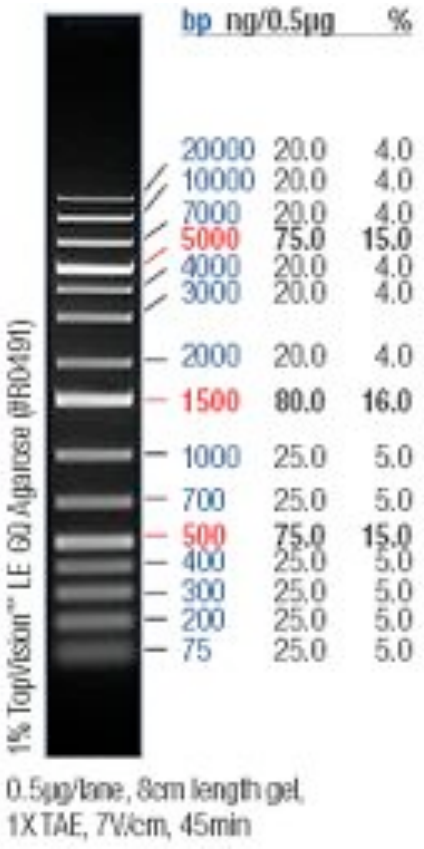
July 25

- helped nikit with a comp cell prep

July 26

- set up EIPCRs for j2, j3, n1,n2, x1, x2
- NP2R and X2L failed so setting up an EIPCR for those
- gel purify
- set up gold reaction
- help spencer with cell prep
- order is JC2L, JC2R, JC3L, JC3R, NP1L, NP1R, NP2L, NP2R, XL1L, XL1R, XL2L, XL2R
-

600px



July 27

- Transformations 3.5 DNA 100ul of cells (purple work better)
- TCs:

1. JC2L: 5.2
2. JC2R: 5.0
3. JC3L: 4.6
4. JC3R: 5.0
5. NP1L: 5.2
6. NP1R: 5.0
7. NP2L: 5.0
8. X1L: 4.6
9. X1R: 4.6
10. X2R: 4.4

- Receeding JC2L, JC2R, NP1L, NP1R and JC3R into 100ml of 2YT (they are done with mutagenesis)
- titering: 1ul into 1ml of 2YT, plate 100ul
- plate the rest on big plates, 600ul-1ml

July 28

- transformations failed
- started over at 2 steps

1. EIPCR- NP2L, NP2R, X2L all did not work. set up a gold reaction

2. after last EIPCR(from last transformation) NP2R, X2L did not work, set up a gold reaction, transforming with the same procedure as yesterday

July 29

- transformation failed again. Going to start over from EIPCR step
- TC:

1. JC2L: 5.2
2. JC2R: 5.0
3. JC3L: 5.0
4. JC3R: 5.4
5. NP1L: 5.2
6. NP1R: 5.0
7. X1L: 4.6
8. X1R: 4.6
9. X2R: 4.6
10. puc18: 4.8 (100ul of 10/990 dilution)

- all others got the usual dilution

July 30

- transformation worked! (except for NP1L.. designing primers again)

1. JC2L: 2E5
2. JC2R: 2.2E6
3. JC3L: 8E5
4. JC3R: 7.9E6
5. NP1L: 0
6. NP1R: 9E5
7. X1L: 4E5
8. X1R: 8.6E6
9. X2R: 1E5
10. puc18:48 (200cfu= 1E9)

- mini prepped J3L, X2R, X1R,X1L, N2L, N2R
- midi prepped JC1L, JC2L, NP1R, JC3R, JC1R, JC2R
- picked a colony from all but NP1L for sequencing

July 31

- streaked out JTK 155

August 1

- set up EIPCR for the J3L, N1L, N2L, N2R, X1L, X1R, X2L, X2R

- set up EIPCR for the j3L, n1L, n2L, n2R, x1L, x1R, x2L, x2R
- set up one pot reaction for jc1 and jc2 (600ul scale0

August 2

- 2 comp cell preps
- clean up of the one pot reaction on the 2 libraries (eluted with 8ul each=48ul total)
- transform into cells
- titer the usual way!

- n1L, j3L recovered in 250 mL of 2yt
- titer 25 uL of n1L and j3L should give 100 cfu = 1e6

- jc1 and jc2 recovered in 1L of homemade SOB
- titer 10 uL into 990 uL of 2yt.plate 50 uL. 200 cfu = 1e9

- x1L, x1R, x2R recovered in 1 mL of 2yt
- titer 1 uL into 1000 uL of 2yt. plate 10 uL. 100 cfu = 1e6
- plate on big plates

```
Recover in 250ml--> titer 25ul (100cfu=1E6)
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```
Recover in 1L--> titer 10ul into 990ul of 2YT, plate 50ul (200cfu=1E9)
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```
Recover in 1ml--> titer 1ul into 1000ul 2YT, plate 10ul (100cfu=1E6)
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August 3

- Miriam

1. electroporate np2L(2/3), np2R(2/4), x2L(1/3), x2R($), Jc3L($)
2. streak out DH10B

- Jason

1. mini prep x1L, x1R from the scrape ===> sequence
2. pick colony and miniprep ALL x1L x1R ==> sequence
3. zymo gold np2L(2/3) np2R(2/4) x2L(1/3)
4. set up an EIPCR for X1R(3/3$) and X1L(3/4)
5. set up a gold reaction for X1R(3/3$) and X1L (3/4)
6. make primers for NP1L

August 4

- miriam

1. midi JC3L, X2R (NOPE MUST REDO)
2. one pot JC3 (NOPE)

3. electroporate X1L(3/4) and X1R(3/3$)
4. comp cell prep jc1, jc2 DONT FORGET VOLTAGE AND ANTIBIOTICS

- count from yesterday:

1. N2R: 6E7
2. X2L: 2E5
3. N2L: 8E5
4. X2R and J3L no colonies, must redo starting from EIPCR

- jason

1. zymo JC1 and JC2
2. mini prep picks from the colony of x1L x1R for sequencing
3. mini prep from a scrape np2l(2/3), np2R(2/4), x2L(1/3)
4. zymo clean up of X1L and X1R
5. EIPCR and gold of np2l(3/3$), np2R(3/4), x2L (2/3), J3L (3/3$), X2R (3/3$)

August 5

- miriam

1. transformation results: Jc1 Jc1= 0 colonies :(X1R=5.7E7 X1L=2.8E5
2. midi prep X1R
3. electroporate np2R(3/4), J3L (3/3$)
4. one pot reaction of jc1 and jc2
5. transform 1ul of gold rxn into bss52 plate
6. run 1ul on a gel

- Jason

1. mini prep from a scrape x1L(2/3) mini prep (sequence)
2. pick a colony of X1L(2/3) for sequencing
3. set up EIPCR and Gold of X1L (3/3$)
4. zymo clean up of np2l(3/3$), np2R(3/4), x2L (2/3)

August 6

- miriam

1. results: N2R lots and lots of colonies, J3L=3.2E5 cutting it close but continuing
2. electroporate X1L and N2L (3/3$)
3. spin down j3l
4. test transformation of Jc1 and Jc2 (1ul into 50 ul of cells rescue in 200 ul plate 50 ul on one plate and 150 on another)
5. run on a gel 1ul of jc1 and jc2-DNA there looked good

- Jason

1. mini prep colony of X1L (2/3)
2. pick a colony for sequencing of np2L, np2R, X2L

2. pick a colony for sequencing of np2L np2R X2L

3. scrape and mini prep np2R(3/4), x2L (2/3)
4. EIPCR and gold np2R (4/4$), X2L (3/3$)

August 7

- miriam

1. midi prep X1L, X2L, NP2R
2. set up one pot for X1 and j3
3. electroporate N1L and X1L again
4. picked a colony of DH10B

- Jason

1.

August 8

- miriam

1. comp cell prep for J1, J2, J3, X1, puc (DH10B) (DONT FORGET VOLTAGE)
2. streak out DH10B
3. electroporate

August 9

- midi prep jc1, jc2, jc3 give to nikit
- streak out DH10B

August 10

- midi prep np2l
- set up one pot reaction for NP2
- pick 10ml of DH10B for comp cell prep tomorrow

August 11

- comp cell prep of NP2
- Clean up NP2
- Electroporate X1L

August 24

- comp cell prep for jc1 jc2 jc3
- cells= 164-J 2993 008
- bad time constants (2.8-3.6)
- 3.5ul of dna into 400ul cells
- rescued in 1L of SOB
- titered

August 25

- spoke to chris and Gabe about how we will be proceeding with the positive selection
- first day of school confusion... Gabe helped me out :)

August 26

- transferred the plated libraries from the first round of selection onto new plates (10^6 dilution)
- did a test of the transformation straight up (o, 10, 20 ug of tet see gabe for pictures) LOOKED GOOD

August 27

- scrape and mini prep the second round of transformations
- transform 1/10th of a ul into 75ul of bss52 cells
- recover in 200ul of 2YT
- split between 2 plates (plus and minus chemicals)

August 29

- electroporated the mini preps into D4-3 (100ul cells, 3.5ul DNA)
- recovered in 250ml 2YT cam/trim
- titered 2.5ul cells into 250ul 2YT, plated 12.5 ul
- added spec to over night culture
- did transformation for spencer

August 30

- mini prepped 2ml of the overnight culture
- transformed 1/100ul into 75ul of cells+KCM
- receded 500ul of overnight culture into 500ml of 2YT with trim, cam, spec
- plates from last night: JC1= 1.6E9, JC2=2.2E8, JC3= 2.76E9

August 31

- Comp cell prep to make -80 stocks of 164J 2993

September 1

- mini prepped 4ml of the over night culture
- transformed 1/100 ul of the mini prep into 75ul of cells and KCM
- plated 50ul
- receded 1ml into 1L 2YT with Cam, trim, spec for last step of negative selection

selection protocol: Miriam 6 September 2011 ToxR-Chimera/MSD/TetR selection Protocol

1. Comp cell prep (fresh for large libraries) off MSD002-D43 (includes pC+,exsA)

(NEGATIVE SELECTION)

1. electroporate 3.5ul of library into 100uL cells
2. recover in 250ml for 1 hr upstairs @ 37
3. dilution of 2.5ul into 250ul 2YT
4. then plate 12.5 ul of that dilution where ~200cfu à 1e9 diversity
5. add 1:1000 CAM, TRIM, & (library antibiotic)

Grow up, overnight.

1. Reseed 1ml à 250ml, same antibiotics
2. MP 4mL
3. 1:100 dilution of DNA
4. Transform 1:100 dilution, 1 uL into 75 ul cells w/ KCM
5. Cells = Bss52 (amp, cam) reporter cells with ffgfp
6. recover in 200ul 2yt for 1 hr
7. plate 50ul on CAS

Grow up, reseeded flask, overnight repeat 6-10 Grow up reseeded flask, overnight

1. MP/transform into Bss52 as before
2. electroporate MP (3.5ul) into 100ul 164j-002993-poo8 cells (POSITIVE

SELECTION)

1. recover in 250ul (2yT or SOB)
2. plate 2.5ul:250uL dilution, plate volume 12.5ul
3. plate on A,Tet,(lib antibiotics)

Grow up overnight

1. INDUCTION step, reseed 2ml:400ml, 2yt or SOB plus SELECTION

CHEMICALS (1:1000)

1. Plate 1:1e6 dilution of library onto A, Tet, +/- chemicals. Lib antibiotics to

test initial activity

1. [illegible]

1. wait 5 hrs

2. plate 1ml onto large selection plate (A,Tet, chemicals, library antibiotics)

Grow up, overnight

1. add 400ul 2yt to plates, scrape, replate 100ul

Grow up, overnight

1. scrape plate, miniprep 500ul
2. transform into Bss52 cells
3. plate +/- chemicals , CA, library antibiotics

Grow up, overnight

1. Pick colonies off +chemical plate into 384 well plates, check on/off ratios off both
2. plates from #22, and screen for induction on a per-colony basis

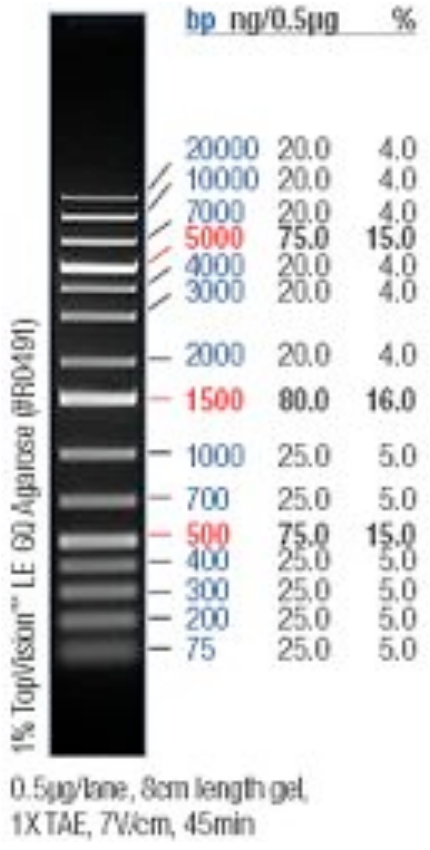
September 15

- I have been doing selections until now. sadly everything has been constituative. :(
- I will start working on libraries! My loves!
- I am going to finish the last EIPCR of X1L

September 16

- Ran gel of X1L EIPCR 4

600px



- set up gold

September 17

- zymo the gold (elute 8 ul)

September 18

- transform into Ec100Dpir 16 TC: 5.0
- plate 25ul on a kan plate

September 19

- 42 cfu= ~10^5 .... not 10^9
- thats ok! DKG is only 6 aas, and there are six spots.. so 6^6 ~ 10^5
- midi prepping

September 20

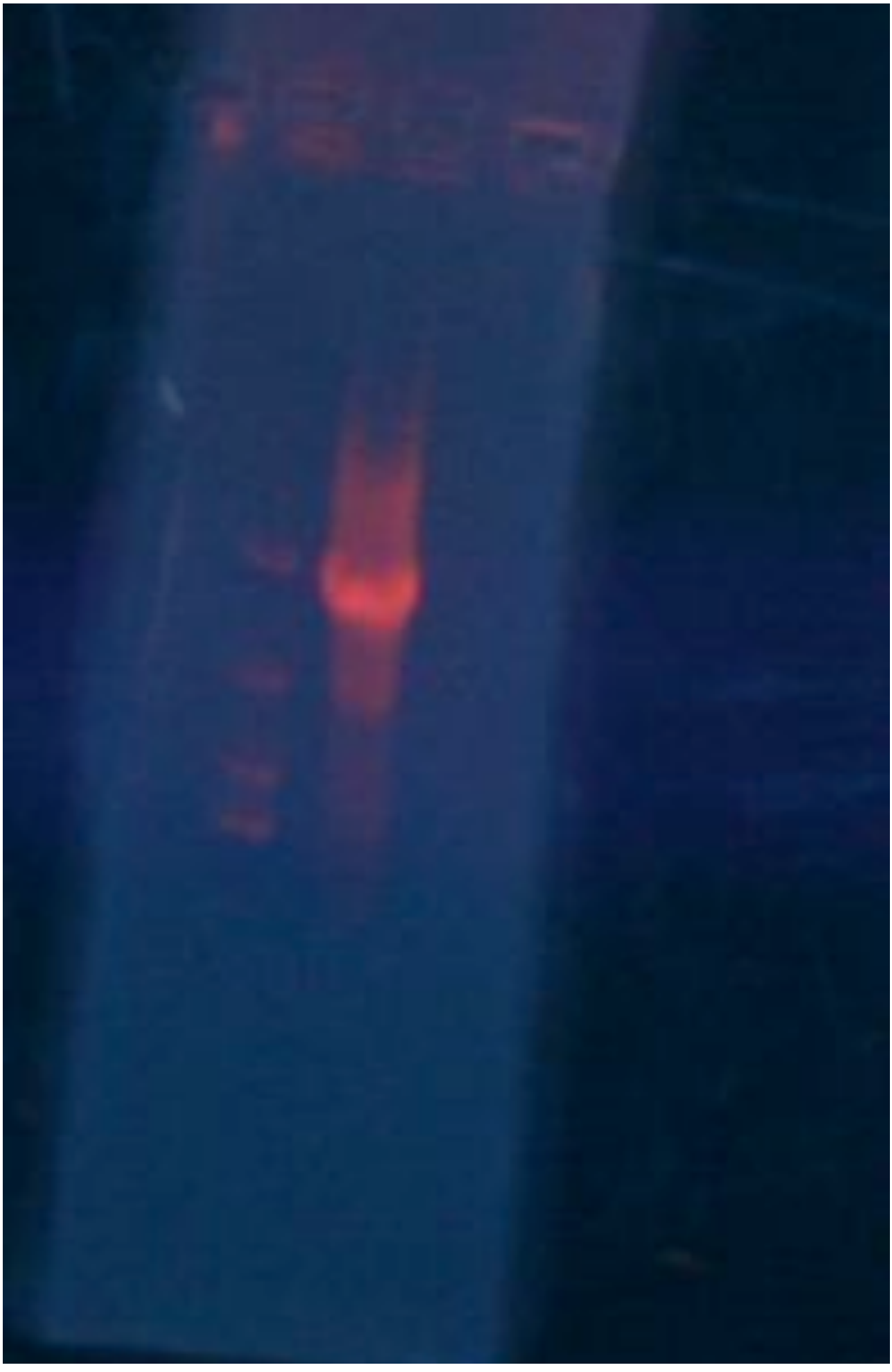
- sequencing failed. :(
- going to look into reodering the oligos

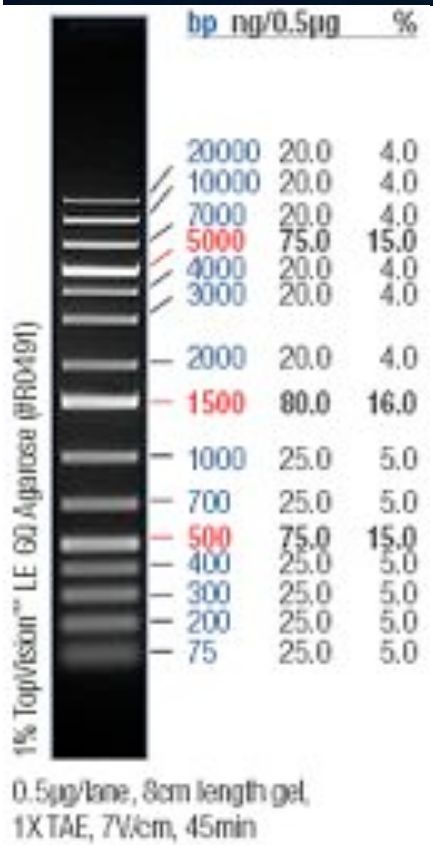
September 21

- got new oligos from EIPCR 3 (jkc32) forward and EIPCR R (jkc33)for X1L
- setting up EIPCR 3

September 22

- ran on gel





- gel purify
- gold reaction

september 23

- zymo clean up
- transform in EC100D pir 16
- recover in 1ml
- titer
- plate 600ul straight up on a plate