Nikit start 6/1/11

From AndersonLab wiki

Contents

Nikitpatel 20:39, 31 May 2011 (PDT)

Thought of the day: First day of iGEM! We're gonna win it all.... hopefully?

- TetA Reporter Project

  - Colony PCR 4 TetA RBS library hits (with ca998/G00101)
  - GP band at 1788 bps of three of them (1,2,4) and sent them for sequencing
  - Create starter cultures of all 32 cultures just in case sequencing is messy

- MSD 1.0/2.0 Characterization

  - Miniprep p1600-Bnp005 (4 cultures)
  - Miniprep p9145-Bss39 (2 cultures) - because stalk was running low
  - Sent both for sequencing

- ExsA/ExsD Threshold Gating

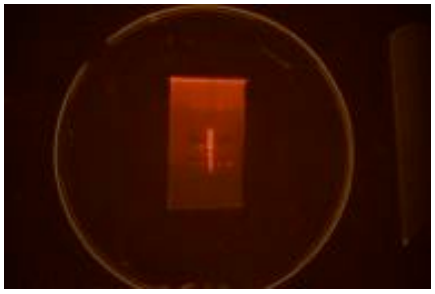
- Pick 16 colonies of Bss39N

Nikitpatel 16:04, 1 June 2011 (PDT)

Thought of the day: Cleaning day today! Everyone is claiming their territories!

- TetA Reporter Project

  - Miniprep 30/32 cultures
  - Silent mutation in Culture A1 but otherwise is a member of the library (sent miniprep for sequencing)
  - Culture B1 has many mutations in coding sequence (sent miniprep for sequencing)
  - Culture D1 sequencing failed



- ExsA/ExsD Threshold Gating Project

  - Miniprep 16 cultures
  - Eco/Bam Digest
  - Send samples 1,11,15 for sequencing

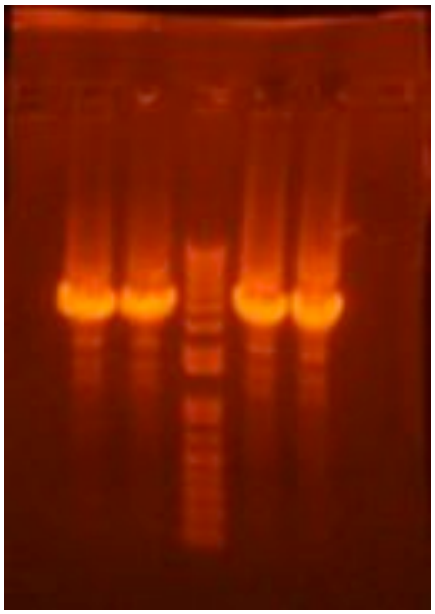
Nikitpatel 13:30, 2 June 2011 (PDT)

Thought of the day: We need to come up with an iGEM chant and/or cheer!

Construction of Pctx.TetA RBS Library:

```
EIPCR ca1841/ca1842 on pBgl00001-Bgl9108      (3365bp, HindIII)
Product is pBca1855 library (TriR, p15A)
--------
ca1841  EIPCR of pBgl00001-Bgl9108
CCATAaagcttcccgtcgtaAGATCcag
ca1856  New tetA library based on pPROBE-T1
ctgacaagcttAAGTATAAAGAAACTANNNGGNGAACGNtgtccaccaacttatcagtg
```

Lanes 1 and 2 are A1 PCRs. Lanes 3 and 4 are B1 PCRs.



- Sequencing Confirmation

  - TetA cultures A1 and B1 have good reads. A1 has 2 silent mutations and 1 mutation that converts Val to Gly. (Professor says this is okay.)
  - Bss39N Failed. Need to re-PCR with phusion just in case

- Research on ligand-dependent dimerizing GPCRs

Nikitpatel 12:06, 3 June 2011 (PDT)

Thought of the day: Wise words from Rebecca Black - It's Friday! Friday! Gotta get down on Friday!

- E/B Digest of TetA Library, Ligate
- Transform, plate on Trim

  - Titer (1E-3)
  - Plated rest on Trim

- Pour Plates:

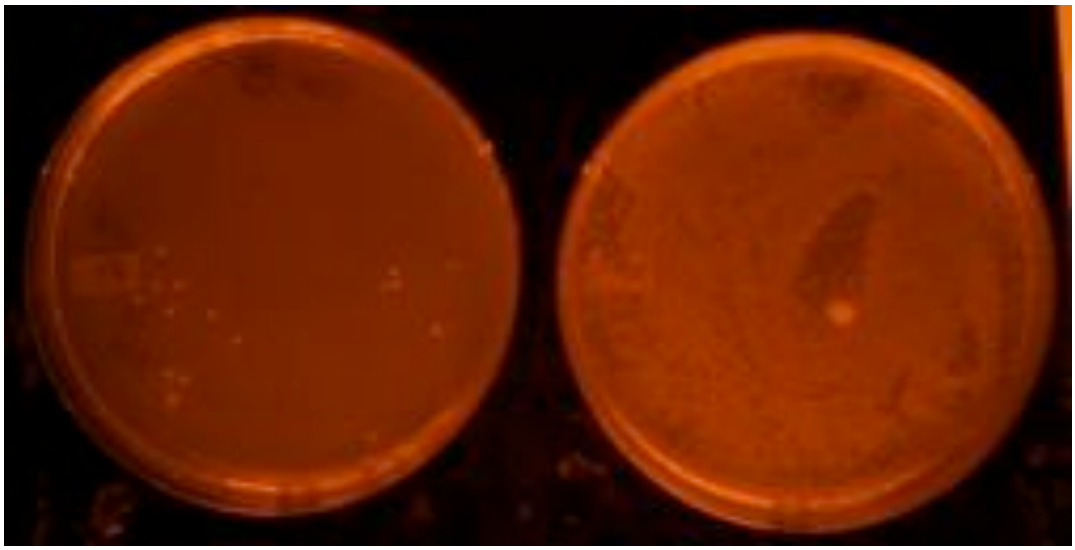
  - Trim
  - Trim/Tet (Apparently there is a special trick to dissolve Tet into LB/Agar. Ask for future use.)
  - Trim/Ni (2.3 mM Ni put into LB/Agar)

- Discussed GPCR Research with Gabe and Chris

  - Many would be too hard to engineer
  - Chemoking GPCRs are probably the only applicable and feasible one to even try

Nikitpatel 11:03, 4 June 2011 (PDT)

- Trim Titer plate on Left. (Plated 100 uL from 1mL of 1E-3 dilution... does that mean 1E-4 even though nothing was diluted?)
- Rest of transformants plated on right.



- Scrape TetA Library hits off of Trim (Right)

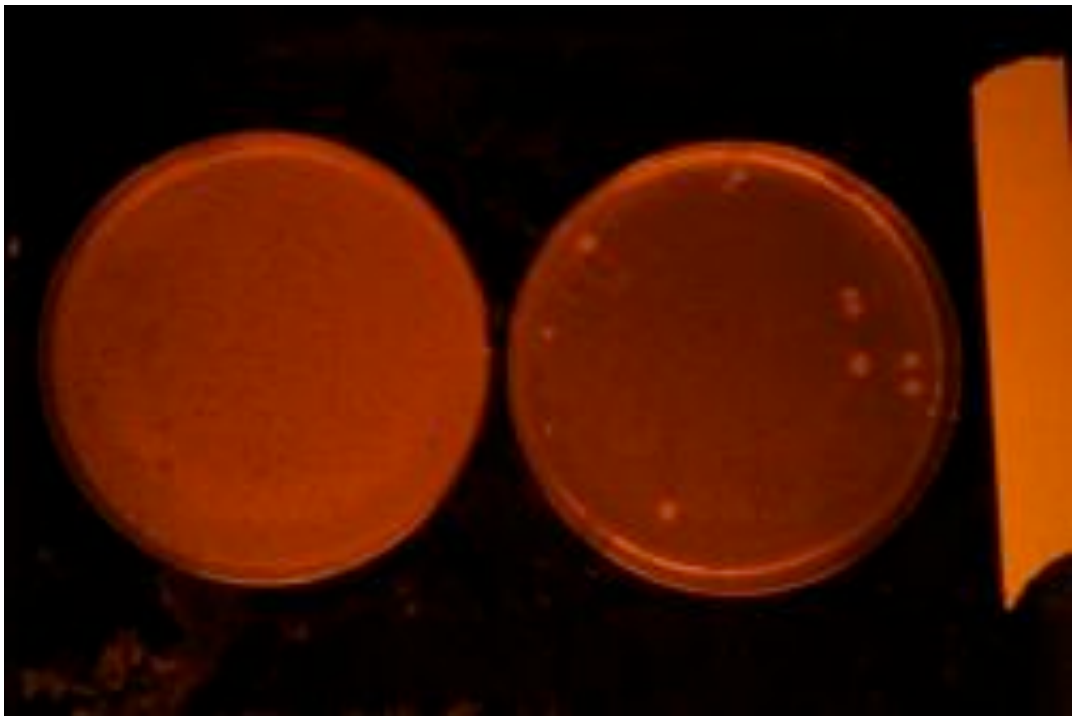
  - Miniprep 200uL of it for backup

- Make 1E-3 and 1E-6 dilution of the cells
- Plate 1E-6 and 1E-3 on Ni/Trim and Tet/Trim and Trim

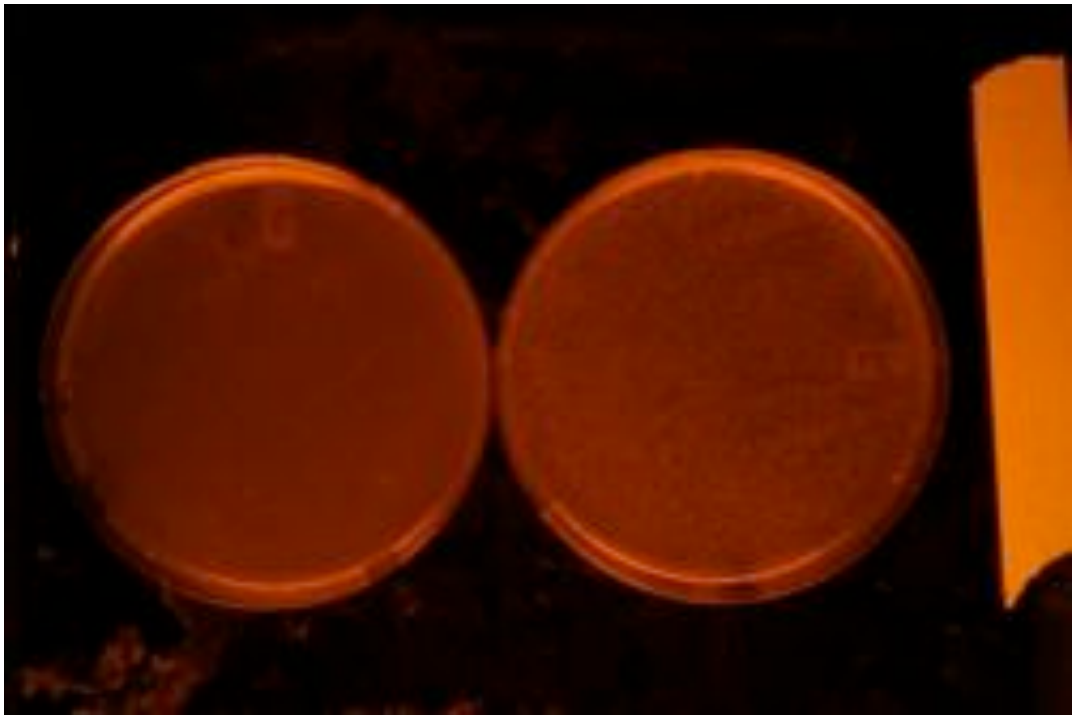
Nikitpatel 09:24, 6 June 2011 (PDT)

LOTS OF CONTAMINATION! Note for next time, big clumps of bacteria are usually contamination!!!
For all images below 1e-3 dilution on left. 1e-6 on right.

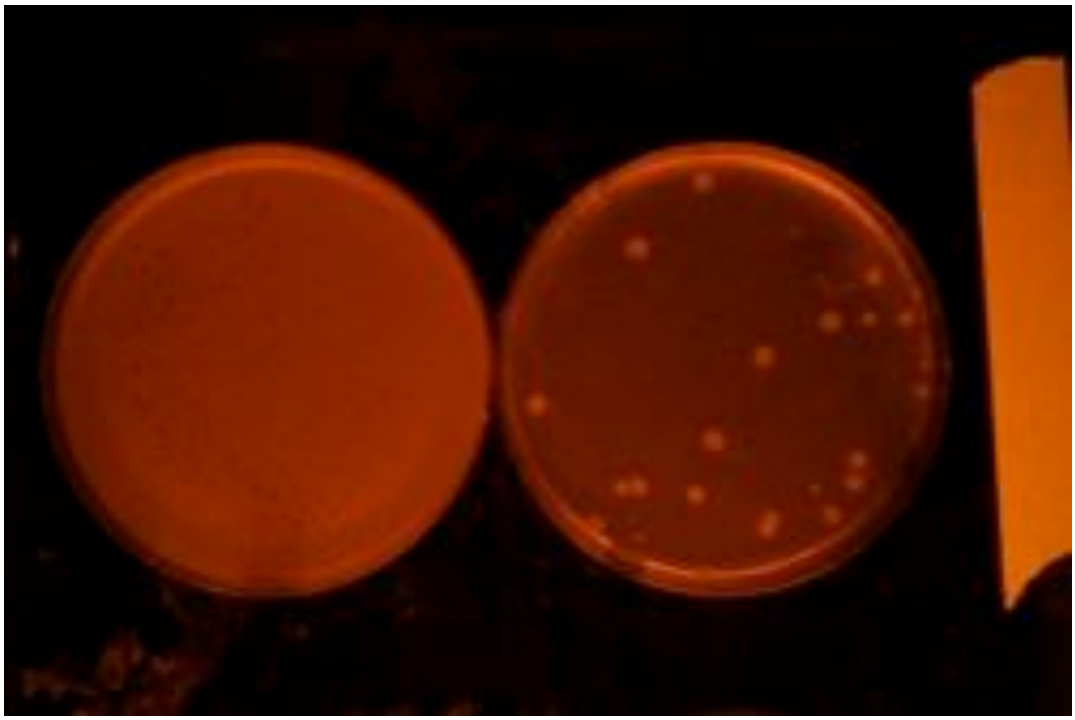
Trim Plate:



Tet/Trim Plate:



Ni/Trim Plate:



- Made Tet/Trim/Spec Plates
- Re-digested, ligated, transformed A1 PCR fragment. Plated on Trim.
- In parallel, I re-transformed the miniprep from the naive library taken from the scrape off of Trim.
- For both transformations, diluted 10uL of cells into 990uL 2YT. Took 100 uL from dilution and plated for 1E-3 Titer.

  - *Both transformations were in JTK EI strain*.

- Planned out group presentation outline.
- Homework: Research on topics for iGEM backup plan.

Nikitpatel 12:59, 7 June 2011 (PDT)

- The library I redid looks good and has around 200xE3 transformants.

  - Scraped the plate
  - Plated 1E-3 on Ni/Trim and 1E-6 on Tet/Trim, Ni/Trim, Trim

- The retransformed library has no contamination!

  - 1E-3 and rest plates on Trim and Ni/Trim both had lawns
  - 1E-3 Tet/Trim plate had around ~100 colonies on it
  - Scraped the rest Ni/Trim plate. Made Comp cells. Transformed in 2uL of Bca1832. Plate on Tet/Trim/Spec (1E-3 and rest).

- Pour Trim/Spec petri strips and plates for Spencer

- Research on Progesterone Receptor

- Discuss iGEM Group Meeting Presentation

Discuss iGEM Group Meeting Presentation

Nikitpatel 16:01, 8 June 2011 (PDT)

- Only 12 colonies found on positive selection

  - This is no GOOD!
  - Will redo the selections with more QC and titering to see what's going on.

- Redoing TetA selection process

  - Scrape library off of older Trim plate
  - Titer on Trim, Trim/Ni, Trim/Tet (10E-1 to 10-8)
  - Plate on Trim/Ni with (10E-1,3,5)

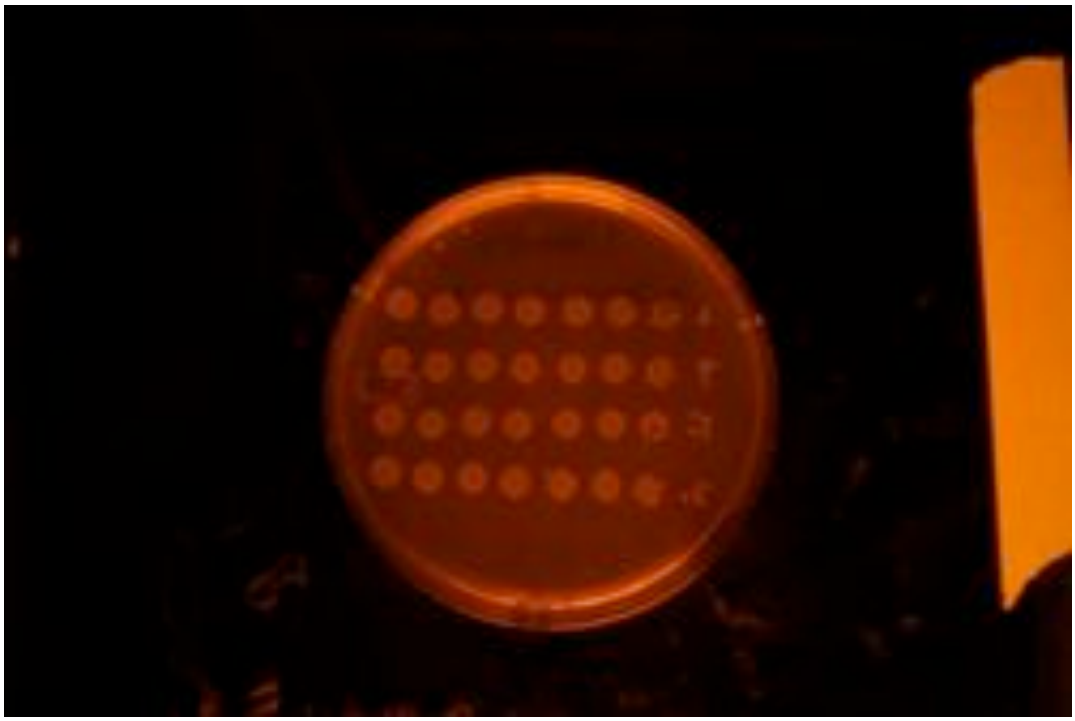
- iGEM Group presentation and backup plan meeting

- Do lab bench cleanup

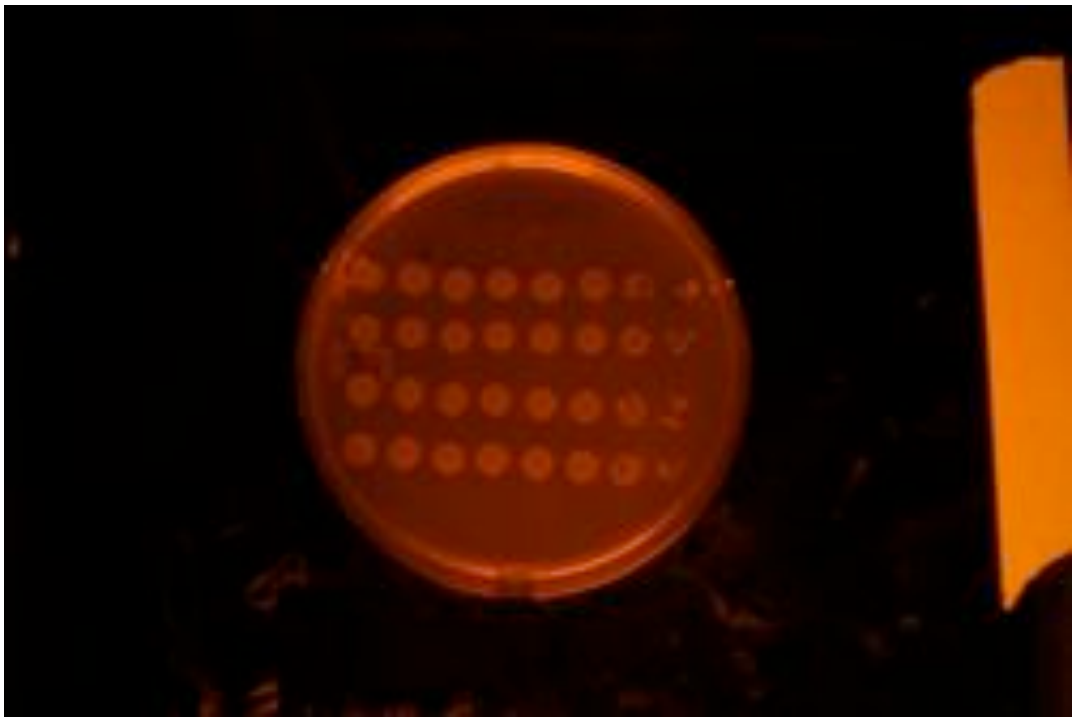
Nikitpatel 16:01, 9 June 2011 (PDT)

- Titer plates:

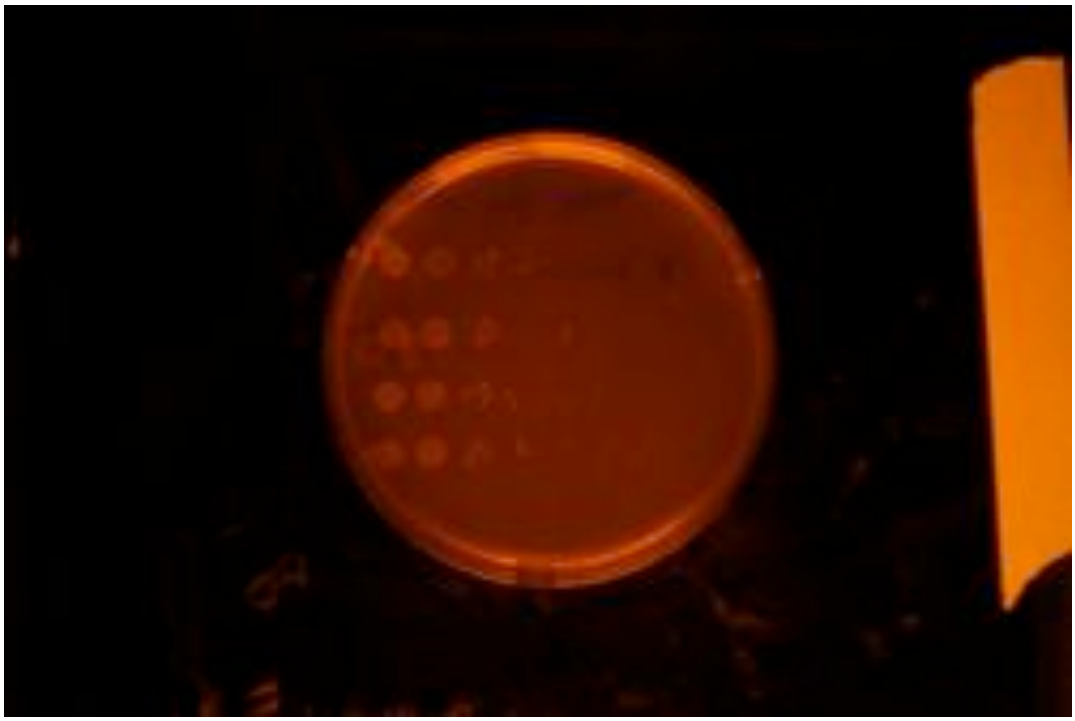
Trim: 6E8



Trim/Ni: 5E8



Trim/Tet: 2E4



- Got lawn on all 3 Trim/Ni plates

  - Scraped 10-5 lawn from Trim/Ni
  - Made competent cells
  - Transformed in Bca1832
  - Plated on Trim/Spec (Straight and 10E-3)

Nikitpatel 10:42, 10 June 2011 (PDT)

- Found 1000*E3 colonies on Tet/Spec plate (found from D3 plate)
- Scrape Tet/Spec plate

  - Plate on 3 dilutions of Trim/Tet/Spec (rest, D1, D3, D5) with titer on all 3 (Total 7 plates)

- Group presentation

Nikitpatel 10:42, 11 June 2011 (PDT)

- Trim/Tet/Spec plates

  - Found 500 on D1 (5000 colonies), 75 on D3, 88 on D5

Nikitpatel 09:51, 13 June 2011 (PDT)

- Tuning TetA

  - Scrape Trim/Tet/Spec Plates
  - Miniprepped
  - Diluted 1:100 to seperate the two plasmids
  - Transformed 2 uL in MC1061
  - Plate on Trim

Nikitpatel 12:31, 14 June 2011 (PDT)

- Tuning TetA

  - Pick 96 colonies into LB/Trim
  - Grow up for a 4 hours
  - Spot on Trim, Tet, Spec

- GST Experiment (Re-doing)

  - Split 96 well plate in half (one side without Glutathione and with Glutathione)
  - Lane 1: Positive Control
  - Lane 2: Negative Control
  - Lanes 3-4: ToxR-LamdaRep
  - Lanes 5-6: ToxR-GST
  - Glutathione is 2mM in row A and is halved all the way down to zero

- Checked Jason's Oligos for ToxR-LamdaRep

  - Needs to be redone

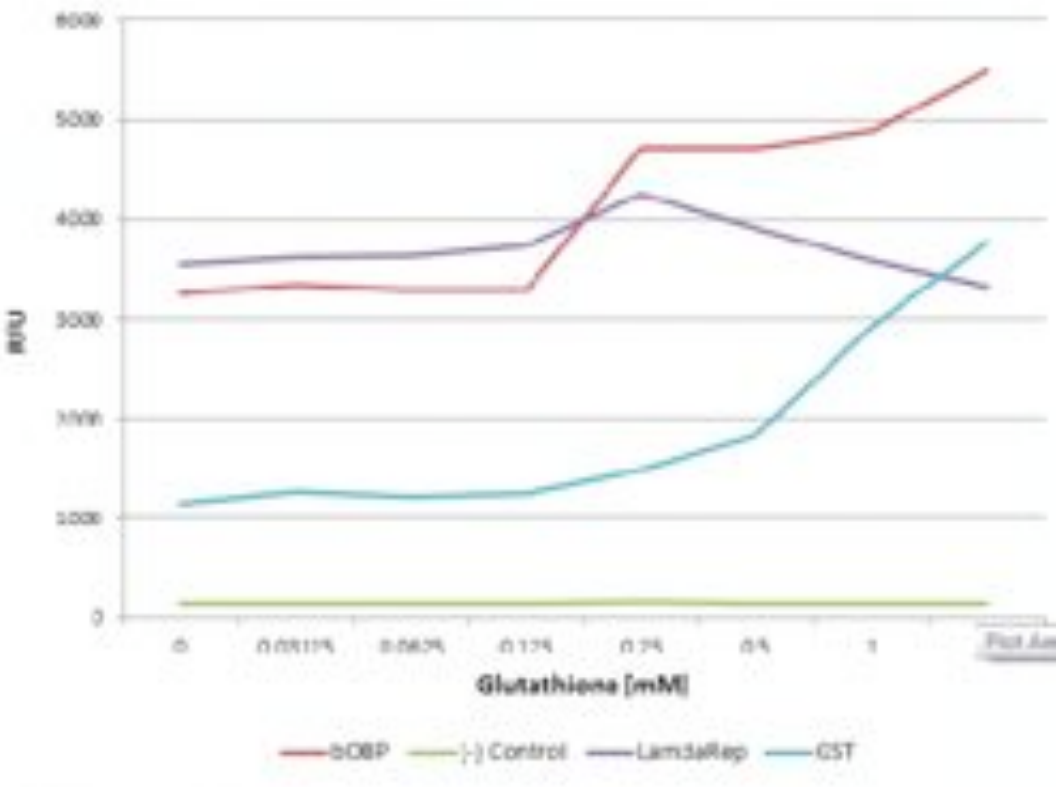
- Start another ToxR-MukF library with Xin Xin
- Helped Merriam quickly data-mine the yeast KO ORFs that have crystal structures

  - Lesson Learned: Tim is a beast with computers!

Nikitpatel 10:24, 15 June 2011 (PDT)

- GST Experiment (Re-doing)

  - Saved Tecan Read
  - Below are the results:



  - LamdaRep seems to be a better positive control
  - bOBP seems to respond to the Glutathione (as seen by Jason's previous run)

    - this is extremely fishy!
    - one hypothesis is that the glutathione stabalizes the protein just as its natural ligand (dihyrdomyrcenol) stabalizes bOBP
    - dihydromyrcenol is similar in size and shape to glutathione but has completely different polarity which makes this an interesting problem

  - We will redo this experiment, so we picked fresh colonies of bOBP, lamdaRep, (-) control, and GST fusions with ToxR. We will be sure to include the following on our next run:

    - Test a larger range of Glutathione (start from 8mM and then halve each time) to find the full range of GST induction

- Test dihydromyrcenol on bOBP and/or GST to see what its affect on the protein is

- Tuning TetA

  - Spots aren't the greatest
  - Got 14 colonies that do not show up on either Tet or Spec but do show up on Trim
  - Took grown cultures and performed competent cell prep, transformed in Bca1832, plated on Trim/Spec

- ToxR-MukF Library #2

  - Looked at crystal structure
  - Performed Alanine Scanning using Robetta Web tool

Nikitpatel 11:37, 16 June 2011 (PDT)

- Tuning TetA

  - Retransformed plates look good.
  - Since we are going to SB 5.0, I will grow them up and spot tomorrow

- GST Experiment

  - Started another TECAN run

Nikitpatel 11:18, 17 June 2011 (PDT)

- Tuning TetA

  - Picked colonies and will grow up for 4 hours
  - spot on Tet/Spec, Ni/Spec, Trim/Spec

- GST Experiment

  - Even more funky results

Nikitpatel 11:45, 20 June 2011 (PDT)

Splitting of ToxR-MukF Core Library #1

```
PCR nbp007F/jkc012R on pBca9525-Bca1832                          (2984 bp, KpnI/SpeI)
PCR ca1776/ca1777 on pth7028                                    (670 bp, KphI/SpeI)
Ligate, transform pir, product is 1832-np1-R                    (SpecR, R6K ori)


PCR jkc013F/nbp008R on pBca9525-Bca1832                         (1896 bp, KpnI/SpeI)
PCR ca1775/ca1777 on pth7028                                    (2721 bp, KphI/SpeI)
Ligate, transform pir, product is 1832-np1-L                    (KanR, R6K ori)

----

nbp007F 3' splitB Forward with KpnI on MukF                     ccaaaGGTACCATGCGTTTGAGC
jkc012R 3' SplitB Reverse with SpeI on ColE1                    cagttACTAGTGGCAGCAGCCAC

jkc013F 5' SplitA Forward with SpeI on ColE1                    cagttACTAGTCCAGTGGCGATA
nbp008R 5' SplitA Reverse with KpnI on MukF                     ccaaaGGTACCATCACTCACATG

ca1775  Forward KpnI for Kan+R6K from pth7028                   ccatgGGTACCGCCTCCTCgctt
ca1776  Forward KpnI for R6K from pth7028                       ccatgGGTACCGCCTCCTCGCAG
ca1777  Reverse M13/SpeI for Kan+R6K or R6K from pth7028        cagttATactagtGCCTCCTCca
```

- Ordered two new oligos for MukF core library
- TetA Tuning

  - After transforming in Bca1832, the 14 hits grew on both Tet AND on Ni. (negative selection is crappy, but positive is better)
  - Theory: Liquid culture with Ni is better at killing bacteria

- Testing BoBP Library

  - Transformed in Pctx-ExsA (1788 D4-3) into MSD 2.0
  - Plated on Trim/Chl

- TetA Race Experiment

  - Picked Pctx-TetA G2 colony into LB + Trim

Nikitpatel 11:19, 21 June 2011 (PDT)

- Give Stuffers to Jason

- Testing BOBP Library

  - Pick D4-3, culture in Trim/Chl
  - Made comp cels (along with -80 stocks)
  - Transformed in Lib 51 and 53 of BOBP
  - 1) Plate on Trim/Spec and Trim/Spec/Kan
  - 2) Plate immediately on Chl/Spec/Trim (Titer)

- Make Comp Cells of Pctx-Teta G2

  - Mix 50:50 GFP and Bca1832 (whats the backbone on GFP?)

- Trans into Comp Cells
- Plate Spec+Trim

- Design EIPCR Mutagenesis Primers and get it approved

EIPCR Mutagenesis Round 1 on 3' Split B

```
PCR nbp009F/nbp010R on 1832-np1-R                                (3648 bp, BsaI)
Ligate, transform pir, product is 1832-np1-R-M1                  (SpecR, R6K ori)
----
nbp009F Forward Mutagenesis #1 on MukF Core Lib - 3' SplitB      ccaaaGGTCTCCATGCGNBSGAG
nbp010R Reverse BsaI primer                                      ccaaaGGTCTCCGCATGGTACCG
```

EIPCR Mutagenesis Round 2 on 3' Split B

```
PCR nbp011F/nbp012R on 1832-np1-R-M1                             (3648 bp, BsaI)
Ligate, transform pir, product is 1832-np1-R-M2                  (SpecR, R6K ori)
----
nbp011F Forward Mutagenesis #2 on MukF Core Lib - 3' SplitB      ccaaaGGTCTCGGCGTGNBSGCC
nbp012R Reverse BsaI primer                                      ccaaaGGTCTCCACGCCGATGGT
```

EIPCR Mutagenesis Round 1 on 5' Split A

```
PCR nbp013F/nbp014R on 1832-np1-R                                (4611 bp, BsaI)
Ligate, transform pir, product is 1832-np1-R-M1                  (KanR, R6K ori)
----
nbp013F Forward Mutagenesis #1 on MukF Core Lib - 5' SplitA      ccaaaGGTCTCGACTCTCTNBSC
nbp014R Reverse BsaI primer                                      ccaaaGGTCTCAGAGTCGGTCTA
```

EIPCR Mutagenesis Round 2 on 5' Split A

```
PCR nbp015F/nbp016R on 1832-np1-R-M1                             (4611 bp, BsaI)
Ligate, transform pir, product is 1832-np1-R-M2                  (KanR, R6K ori)
----
nbp015F Forward Mutagenesis #2 on MukF Core Lib - 5' SplitA      ccaaaGGTCTCGGCGAGNBSGTG
nbp016R Reverse BsaI primer                                      ccaaaGGTCTCCTCGCCTTCACT
```

This week

- Wednesday

  - Scrape plate on Chl. + Titer
  - Scrape Chl, miniprep hold, ScrapeChl, induce, plate on Amp

- Scrape Cm, miniprep hold. ScrapeCm², induce, plate on Amp.

- Thursday

  - Done
  - Scrape Amp plate. Miniprep hold.

- Friday

Nikitpatel 10:34, 27 June 2011 (PDT)

- Yesterday Night:

  - Scrape Fast and Old 51/53 Library from negative selection
  - Inoculated into 3mL of T/A/S
  - Scraped GFP-MukF competition plate off of T/S and innoculated into T/S, T/S/0.5mM Ni, T/S/1mM Ni, T/S/10mM Ni, T/S/Tet

- Inoculated 51 and 53 scrapes into T/S and 1mM DHM

  - Grow for 6 hours. Plate on Spec/Amp.

- Plate GFP-MukF Competition Cultures on T/S

- Order EIPCR Oligos

Nikitpatel 17:58, 28 June 2011 (PDT)

- Made LB-Agar, poured plates with Miriam
- bOBP Library

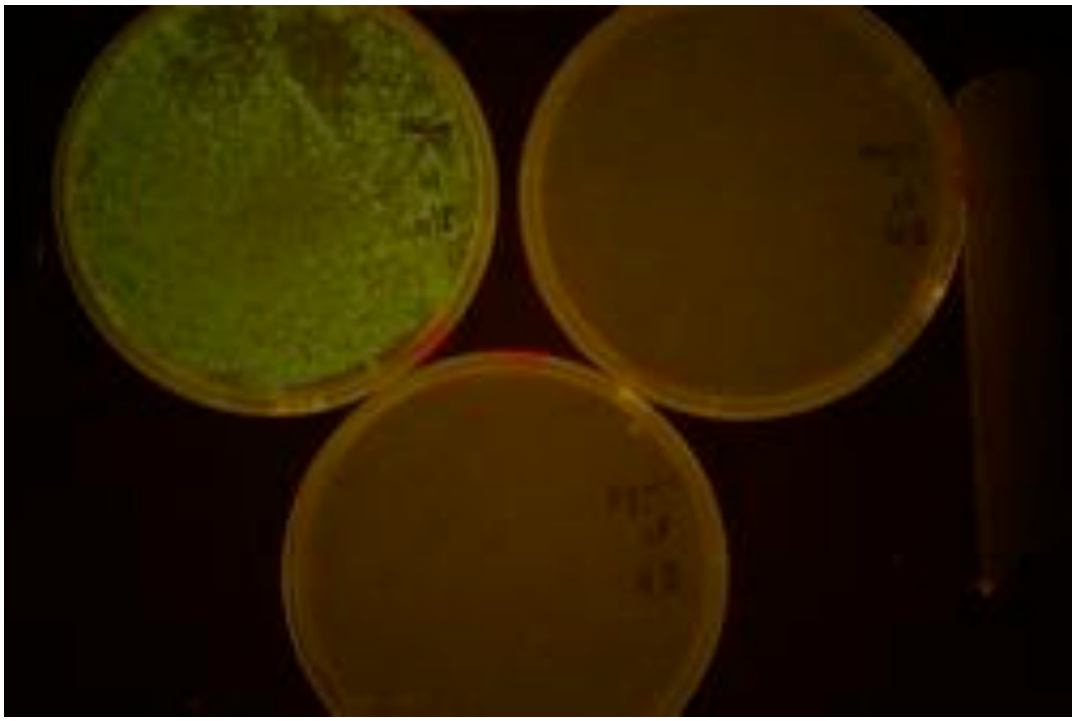
  - Scraped 51/53 (+) Selection and miniprepped 500 uL
  - Diluted all my minipreps for this selection series 1:50 (2uL DNA in 100uL water)
  - Transformed 2uL into Bss52 cells (from Spencer's stocks) - 10 transformations total

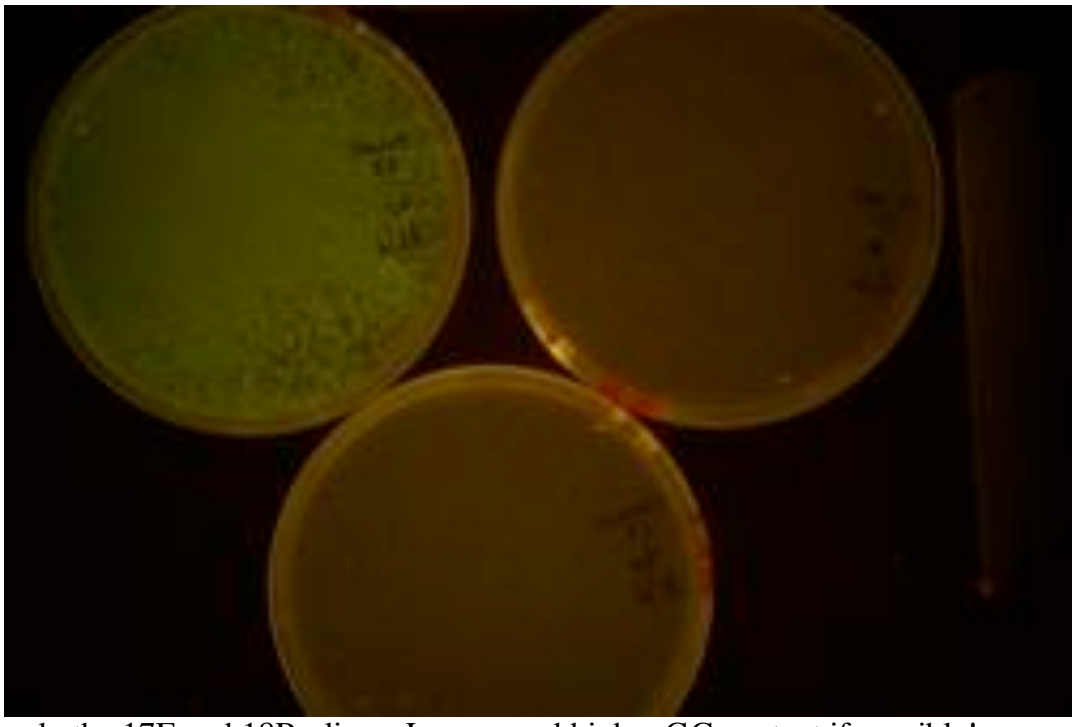
Nikitpatel 23:42, 29 June 2011 (PDT)

- Left plate labelled naive shows portion of entire library (many green and white colonies)
- Right plate labelled "fast (-)" is the result of plating the library straight on the negative selection
- The bottom plates labelled "slow (-)" is the result of plating the library on Trim/Spec/Kan and then moving into the negative selection

- In both the 51 and 53 bOBP library, the "fast" plates has more green colonies (~40) and thus more bleed through of ON members. While the "slow plates" showed much less green colonies (~3)

  - Therefore, plating on the minimum amount of antibiotics before moving into the selections is better!





dearest nikit.. can you please redo the 17F and 18R oligos. Longer and higher GC content if possible!