SpencerScott start 05/23/2011

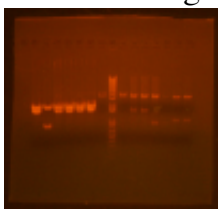
From AndersonLab wiki

HOME

SpencerScott 11:23, 30 June 2011

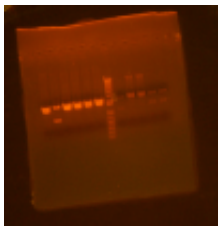
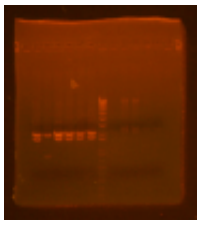
HIV attached to ToxR (9525-Bsrs022) were all white colonies when plated on Spec.
Hopefully, it is inducible!

All 14 overnights were Mini-prepped and Mapped using Eco/Bam digest:

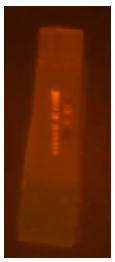


All were good except lane 2 (Srs023 construct 2).

- Srs032's were Nhe1/Bam'd Expected sizes are: 2027 and **1683**
  - Lanes 1-6
- Bxa5 was Bam/xhoi'd but **1051** was lost on re-run, so was ran again by itself.
- 2109 was Bam/xhoi Expected sizes are: **2700** & 2200
- 2051 was BgIII/xhoi'd Expected sizes: **2200** & 1100


--> 

The Bxa5 was lost when it was re-run for longer So it was ran on its own gel:



- Ligations ran below:
  - Bsrs023 to 1834 (pre-digested)
  - Bxa5 to 1601AK-2051
  - bjh2109 & bss52 (labeled Bi in box)

SpencerScott 11:05, 29 June 2011

- 6 colonies from ER1+ER2 (bsrs023) were picked and grown in AMP
- 2 colonies of 1601AK-bjh2051 were picked and grown in AK

- 2 colonies of 1601CA-bjh2109 were picked and grown in CA
- 2 colonies of bss52+b0015 were picked and grown in CK
- 2 colonies of bss52 were picked and grown in CA

Thursday Morning

- Mini-Prep above
- Digest
  - nhe1/Bam Bsrs023 & 1834
  - Bam/xhoi Bxa5 & BgIII/xhoi 2051-1601AK
  - Bam/xhoi bjh2109 & Ligate with (bi from Tuesday) (Bgii/xhoi'd Bss52)
- Ligate
  - Bsrs023 to 1834 (pre-digested)
  - Bxa5 to 1601AK-2051
  - bjh2109 & (Bi)
- Transform into JTK155 and Plate
  - pBca9525-Bca1834-Bsrs023 on SPEC
  - Bxa5+2051 on CK
  - bjk2109 & Bss52 on CA

Thursday Night (~10PM)

- Pick and Grow

Friday

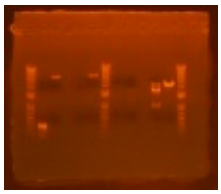
- Mini-Prep

SpencerScott 15:43, 28 June 2011

Today was a day of frustration.
Sequnce of Events:
1. PCR'd 1601KC-Bjh2051 with ca998 & g00101
2. Digested 8 constructs:

```
a. Dropping 2051 into 1601AK
     i. Eco/Bam PCR product (cut out ~250 part) (use NEB2)
     ii. Eco/Bam 1601AK-bxa50 (cut out large part ~3337) (use NEB2)
b. Connecting bss52 with 2109
     i. BgIII/xhoi bss52 (cut out bigger part ~3100) (use NEB3)
     ii. Bam/xhoi bjh2109 (cut out bigger part ~2700) (use NEB3)
c. Adding Terminator to bss52
     i. BgIII/xhoi b0015 (cut out bigger band ~2200) (use NEB3)
     ii. Bam/xhoi/nhe1 bss52 (cut out largest band ~1900) (use NEB2)
d. Assembling Estrogen Receptor
     i. BgIII/xhoi ER2 (2.1(3)) (cut out small band ~900) (use NEB3)
     ii. Bam/xhoi ER1 (1.2(2)) (Cut out large band) (use NEB 3)
```

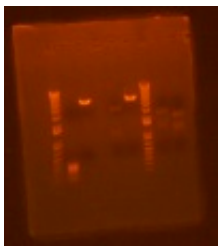
Picture shows that aii, bi, bii, ci, and cii all failed. The Estrogen Receptors worked great.:



3. ai, di, & dii were cut out and gel purified.

4. DIgestions Round two

- The first 6 digestion were re-done and ran on a gel.
- All should work except aii, and bii, because those are marked with GREEN, meaning they cannot be Bam'd.
- As you can see, this is indeed what happened:



- That aii doesn't work it doesn't matter because we got the predigested 1601AK from Josh
- Bjh2109 (AraC-Pbad BRP) needs to be re-transformed into MC1061, grown up and mini-prepped

5. Ligations

- ER1 (dii) and ER2(di) were ligated
- Bjh2051 (ai) Ligated to pre-digested 1601AK Eco/Bam (from Josh!)
- bss52 ligated to b0015 (ci & cii)

6. Transformations & plating

- ER1+ER2 ligation was transformed into JTK155 & plated on AMP
- Bjh2051 in 1601AK was transformed into JTK155 & was plated on AK (after recovery 45 mins)
- bjh2109 was transformed into MC1061 & plated on CA (after recovery 30 mins)
- bss52 by itself was plated on CA (diluted from comp cells)
- bss52 + b0015 was transformed into JTK155 and plated on CK (after recovery 30mins)

7. Constructions to be done Thursday (after above have been mini-prepped):

- ER1+ER2 into 1834
- Bxa5+2051 in 1601AK
- 2109+Bss52 (Bi)
- Bss52-b0015 + "2061" (Will be done next week, have to wait for Bxa5+2051)

SpencerScott 14:05, 27 June 2011

All 8 mini-preps of ER1 were digested (Eco/Bam) and ran on a gel: Lanes 3 and 6 look the best. (ER1.1(3) & ER1.2(2))


All 4 mini-preps of ER2 were digested (Eco/Bam) and ran on a gel: Lane 3 looks good.

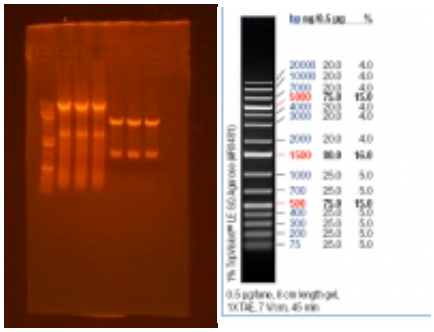
ER1 lanes 3,6, and 8 will be sent in for sequencing. (ca998 &g00101)
ER2 lane 3 will be sent in for sequencing. (ca998 &g00101)
HIV clones 1-8 will all be sequenced using g00101

SpencerScott 14:42, 25 June 2011

Four colonies from each of the 5 plates were picked and grown up in their respective resistances.
ER1.1, ER1.2, ER2.1 in Amp. HIV(1), HIV(2) in Spec.

SpencerScott 15:17, 24 June 2011

9145 was Eco/Bam'd and run on a gel (lanes 2-4) and 1843 was nhe1/Bam'd and run on a gel (lanes 5-7):



HIV PCA Amplification clean up (1&2) was also nhe1/Bam'd
All were Zymo'd.
ER1.1, ER1.2, & ER2.1 were ligated to 9145.
HIV1 (no DMSO), & HIV2 (DMSO) were dropped into 1834.
9145 constructs were plated right away on AMP, and the 1834s were recovered for an hour and plated on Spec.

SpencerScott 15:43, 23 June 2011

Oligos came in:
(Side Note: You have the non-zymo clean-up of **2** (marked in red) in Scott Box 2, as well as the non-zymo

clean-up of **3** in Scott Box 2 (marked in blue); both in PCR tubes. Andd **4** (marked in red but with only 2 tubes))

1. PCA Amplification for ER parts

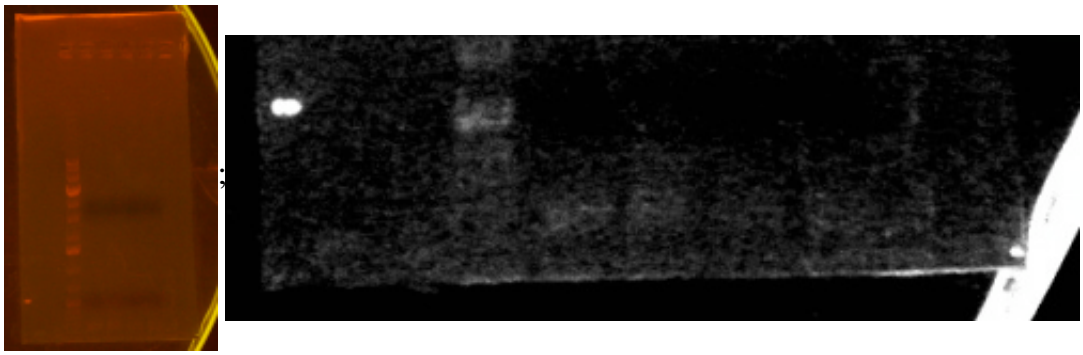
- This was run under the same conditions as **3** (below), However I accidentally used Expand Polymerase instead of Phusion, I also only used 1ul of Template, and 1ul of each oligo. (should've use 2ul)
- This will likely fail.
- It was then Eco/Bam'd and ran on a gel.
- Someone changed the voltage to 270+, so the gel melted. Oh well, Round 2 of Amplification is already under way with the correct recipe. **See 3**

2. PCA Assembly of HIV antigen

```
In well 1:
1. 67ul H20
2. 20ul 5x Phusion bufffer
3. 10ul 2mM dNTPs
4. 1ul Phusion
5. 2ul mix (4 oligos at 100uM)
50ul was taken from 1 and put into 2.
4ul DMSO was added to well 2

In well 3:
1. 76ul H20
2. 10ul 10x Expand bufffer
3. 10ul 2mM dNTPs
4. 1.5ul Expand Polymerase
5. 2ul mix
50ul was taken from 3 and put into 4.
4ul DMSO was added to well 4
```

- All four were run on a gel, with Phusion No DMSO looking the best (lane 1):

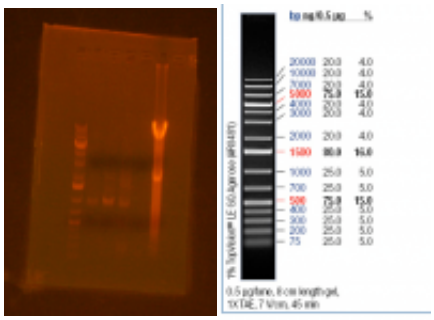


3. PCA Amplification for ER parts: Round 2!

```
In well 1:
1. 67ul H20
2. 20ul 5x Phusion bufffer
3. 10ul 2mM dNTPs
4. 1ul Phusion
5. 2ul Template (PCR cleanup #1)
6. 2ul forward oligo (well A1) (non-diluted; oops!)
7. 2ul reverse oligo (well F4)
50ul was taken from 1 and put into 2.
4ul DMSO was added to well 2

In well 3:
1. 76ul H20
2. 10ul 10x Expand bufffer
3. 10ul 2mM dNTPs
4. 1.5ul Expand Polymerase
5. 2ul Template (PCR cleanup #3)
6. 2ul forward oligo (well G4)
7. 2ul reverse oligo (srs140)
50ul was taken from 3 and put into 4.
4ul DMSO was added to well 4
```

- Eco/Bam'd All four PCA's, as well as 9145-purple part:
- Expected/Desired Sizes: 1. 875 2. 875 3. 837 4. 837 5. 2057 (pBca9145 backbone)



- Only gel cleanups were done on lanes 1-3. 9145 had too much DNA and lane 4 just failed.

4. PCA Amplification of HIV antigen

```
In well 1:
1. 67ul H20
2. 20ul 5x Phusion bufffer
3. 10ul 2mM dNTPs
4. 1ul Phusion
5. 2ul Template (PCR cleanup #1; marked with red in box)
6. 2ul forward oligo (srs136; 10uM)
7. 2ul reverse oligo (srs139; 10uM)
50ul was taken from 1 and put into 2.
4ul DMSO was added to well 2
```

SpencerScott 15:13, 22 June 2011

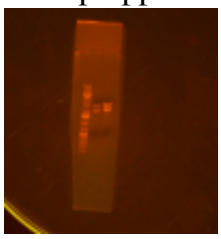
You will be working on QC of libraries (Quality control). You will be running 'colony PCR' after every mutagenesis step of the library creation. Most importantly you will be sequencing after splitting and stuffer ligation with:

- **ca1786** (CTGATAGCGGTCTTACTTCC) which sits at the end of ToxR and reads forward. Use on

'Lefty'. (ca1786 in Miriam's iGem Box)
    - OR **Jkc009_seq** which sits 50 bps from the end of toxR and reads forward. Use on 'lefty'. Get from Jason
- **ca1787** (CTTAGTACGTTAAACATGAG) which sits in the stuffer and reads forwards. Use on 'Righty'.

SpencerScott 12:27, 22 June 2011

Mini-prepped both overnight cultures of 9525-Bca1834. Eco/Bam'd and Ran on Gel:



Oligos never came =\

SpencerScott 14:24, 21 June 2011

No visible DNA in gel cleanup of PCA when run out on gel. This will still be amplified, but backup Assembly PCA reactions were run:

1. Phusion of ER1 (-) DMSO
2. Phusion of ER1 (+) DMSO
3. Phusion of ER2 (-) DMSO
4. Phusion of ER2 (+) DMSO
5. Expand of ER1 (-) DMSO
6. Expand of ER1 (+) DMSO
7. Expand of ER2 (-) DMSO
8. Expand of ER2 (+) DMSO

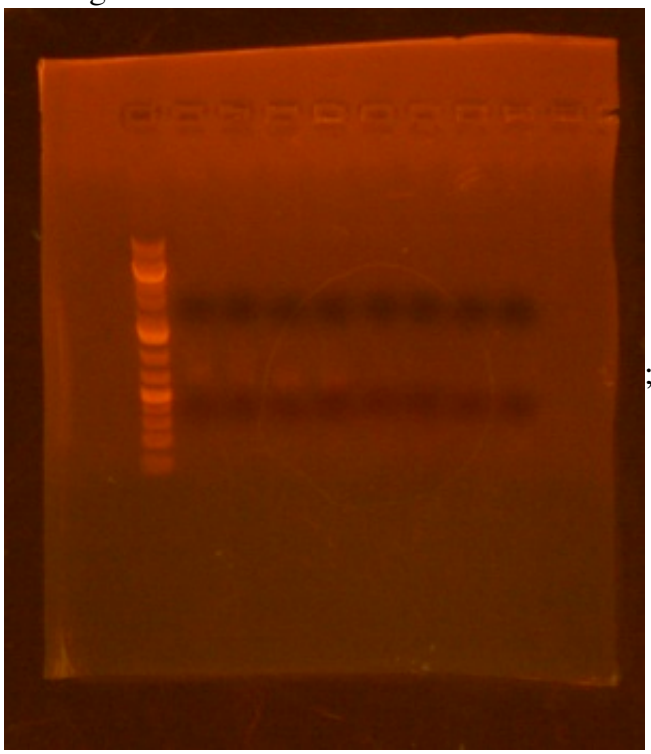
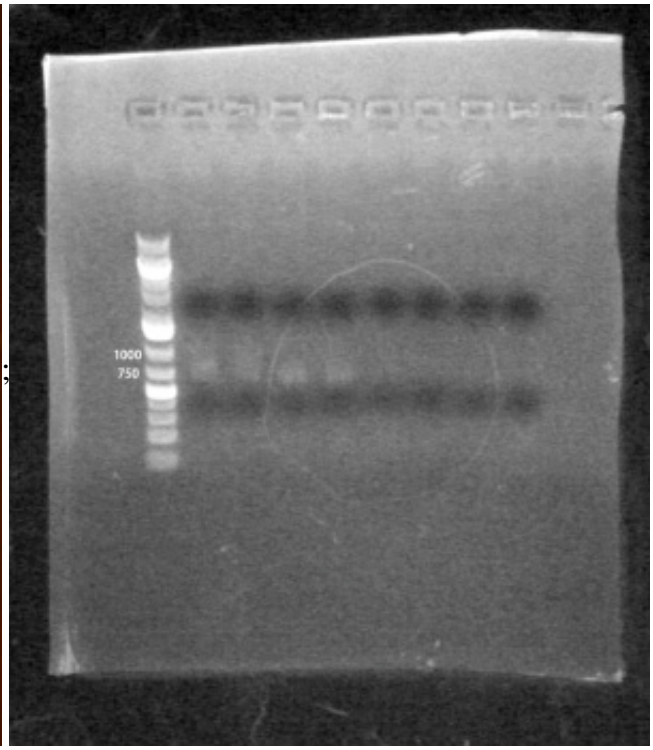
Phusion was as follow:

```
In well 1:
1. 67ul H20
2. 20ul 5x Phusion bufffer
3. 10ul 2mM dNTPs
4. 1ul Phusion
48.5ul was taken from 1 and put into 3.
1ul ER1 mix was added to 1; 1ul ER2 mix was added to 2.
25ul from 1 transferred to 2, 25ul from 3 transferred to 4.
2ul DMSO was added to wells 2& 4
```

Expand was as follow:

```
In well 5:
1. 76ul H20
2. 10ul 10x Expand bufffer
3. 10ul 2mM dNTPs
4. 1.5ul Expand Polymerase
48.5ul was taken from 5 and put into 7.
1ul ER1 mix was added to 5; 1ul ER2 mix was added to 7.
25ul from 5 transferred to 6, 25ul from 7 transferred to 8.
2ul DMSO was added to wells 6& 8
```

All were ran on PCA program in the thermocycler with **Tm set to 59**.
The results of the PCR grid are below; (The non-cleaned up PCA results were ran on a gel; you have reserve of this in the freezer in the PCR rack labeled in orange) Phusion worked for all four with the (-) DMSO looking the best for both ER1 and ER2:

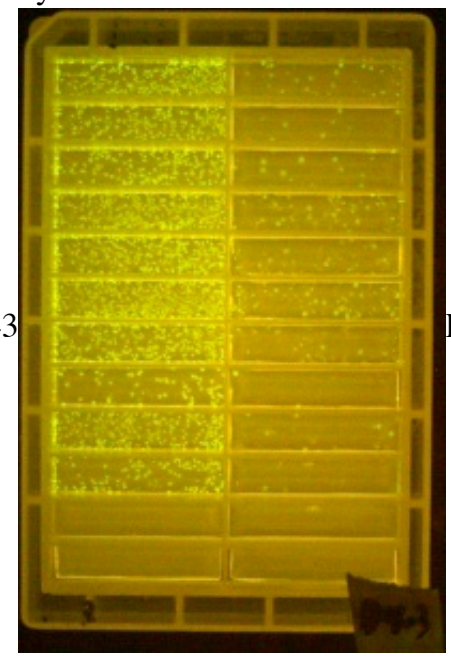
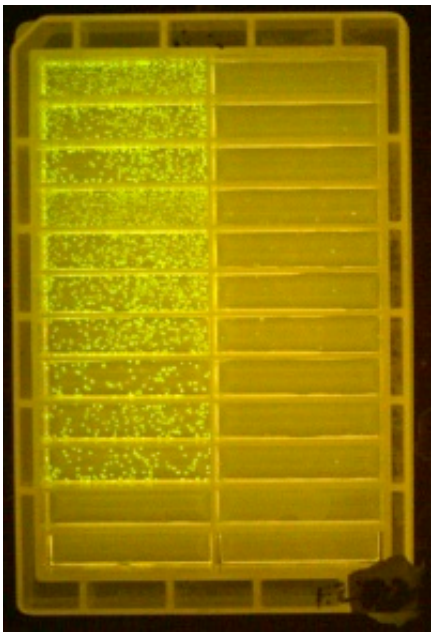
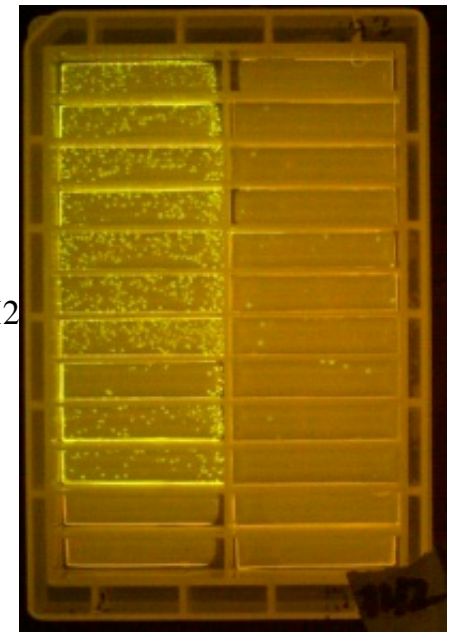
ER1 Should around be 890; and ER2 should be around 850.
Tomorrow, the end oligos will be used to run a PCR Amplicfication on the cleaned up PCR products of 1 and 3.

SpencerScott 17:43, 20 June 2011

Received Estrogen Receptor Oligos
1-30 (ER1) mixed; 3G, 3H, and 4H were all weak because I spilt a lot of the oligos...dunce Cap!
31-58? (ER2) mixed; Mixtures saved
Both were diluted, and put into Assembly PCR reaction, and ran on 'PCA' in thermocycler.
More Bca1834 (dropout) was transformed and plated on Spec so that drop-out ligations will have enough.
Gel Cleanup of PCA reactions labeld in your Box as "ER1 Assemb" and "ER2 Assemb"

SpencerScott 10:18, 13 June 2011

Finally worked with the different GFP:

D43  H21  1H2 

D43 actually had the most green coloneis in the right lanes (T/S/A antibiotic). However, the number of green colonies decreased as the mukF got stronger.
H21 had virtually not green colonies for any mukF mutants. All white colonies
1H2 had very few colonies in general on the right side. Some green some white. no real pattern, except no green colonies in the last row.

SpencerScott 14:01, 9 June 2011

The constructs were plated on Pitri Strips and once again there were no green colonies.
after careful inspection of the plasmids being used we realized it was because the Bca1788 that is already transformed into the MSD002 cells have p15A origin which is the same as the GFP.
We want GFP to compete with the pBca1785 (mukF) and not the Bca1788 which it can't compete with.
All that needs to be done is to use a GFP with the same origin as PbCa1785 which is puc.
puc-GFP will be used to compete instead.
Once again, the three different Bca1788's were transformed into MSD002 cells and plated on Trim+Cam.

SpencerScott 17:08, 6 June 2011

Transformed three different Bca1788 (D4-3, H2-1, 1H2) into MSD002 cells. Plated on Trim+Cam

SpencerScott 14:04, 5 June 2011

For some reason the GFP plasmid didn't transform properly, seemingly only the MukF was transformed as there were virtually no green colonies. There were a few green colonies in the GFP only lane, one at most per lane. Clearly, the GFP didnt get in properly for some reason.

```
Monday:
Transform three different Bca1788 into MSD002 cells. Plate on Trim+Cam
Transform MukF's into Bss52 comp cells from -80? Re-do Tecan experiment??
Tuesday:
Pick colonies from the three Bca1788.
Grow up in overnight of Trim+Cam
Run Tecan Experiment?
Make Pitri Strips!
Wednesday:
Transform mukF mutants into MSD002 cells as per experiment on June 4th.
Plate on Pitri Strips as per experiment on June 4th as well.
Thursday:
Pray for green colonies!
```

SpencerScott 11:00, 4 June 2011

Sequences came back for ToxR's. Will wait for Chris's program to analyze them.
begining small scale competent cell prep for ss52 to be made into -80 stocks.
TODO:

Finishing the Small Scale Competent Cell Prep

- Grown up for 1-2 hours in 37 degree shaker upstairs.
- Spin down to get Pellet (6500 rpm for 6 mins) (Change rotor and cool to 4degreees)
- Pour off
- Add 10mL TSS
- Vortex/Re-suspend
- Aliquot 120ul per tube: should fill 10 PCR strips
- Put all strips into the -80 freezer

Tecan Read of ToxR variants + Bss52

- First two rows will be 150ul LB + 2ul Culture
- Third and fourth rows will be 100ul LB + 50ul Culture
- Fifth and Sixth rows will be 50ul LB + 100ul Culture
- Seventh and Eight rows will be 150ul Culture

Tecan Results at 0 hours are here: [| excel (https://andersonlab.qb3.berkeley.edu/mediawiki/images/6/60/SpencerScottTecan0Hours.xls)]

Small Scale Competent Cell Prep of MSD002 w/ Bca1788 & Transformation of MukF Mutants

- 1:25 dilution done on each Bca1788/MSD002 cells into 2ml eppi
- Grown up for 1.5 hours
- Spun down, poured off, resuspended in 1.6mL TSS
- Added 400ul KCM
- Aliquoted out 100ul of cells per PCR tube (12 tubes) (50 ul per pitri strip: 24 lanes)
- 10 min ice, 90 sec 42, 1 min ice.
- Plate on Pitri Strips

Plating on Pitri Strips required taking 1ul MukF and putting it into 9ul water. Then taking 1ul of that mixture and putting it into a mixture of 1ul GFP+9ul water. Then take 1 ul of that and transform.
When making the comp cell aliquots, draw out the scheme on a PCR tube plate, putting 50ul of comp cells in

When making the comp cell aliquots, draw out the scheme on a PCR tube plate, putting 50ul of comp cells in per well (that way when you add 100ul 2YT you will have enough for 50ul per lane).
Recover for 2 hours.

SpencerScott 14:45, 3 June 2011

Pitri Strips are being prepared for tomorrow's experiment. For all three pitri strip plates, The entire left side needs Trim/Spec, and for the right side, the top 10 rows need Trim/Spec/Amp, and the last two rows just need Trim/Amp.

SpencerScott 09:57, 3 June 2011

Sequences came in:
All the mukF mutants were **not** truncated and matched up with mukF (at least the g00101 side) perfectly, with the exception of F2 (which had a silent mutation), and G1 that had a point mutation from G->R (at protein 430 of ORF of toxR-mukF)
The Bca1788 also aligned:

```
Aligned to:                          AAGCTTAGCCGAATTA...AGGTAAAG.TGCAAGGAGCCAAATCTCTTG
SRS063_CA998_2011-06-03_H10.seq      AAGCTTAGCCGAATTATTTAGGTAAAGATGCAAGGAGCCAAATCTCTTG
```

All that needs to be done today is Picking of mukF constructs + bss52 colonies and plating in Spec-CA. Also, picking of three Bca1788 mutants in the MSD002 and growing up in Trim+Cam.
Lastly, growing plain bss52 cells in CA.

SpencerScott 10:47, 2 June 2011

9 mutants and 1 Bca1788 mutant were mini-prepped and sent off for sequencing. There was a slight mishap in the mini-prepping because too much P1 and P2 were added (750 instead of 250), colonies from yesterday may be picked again and grown up just in case.
Transforming will commence.

SpencerScott 11:09, 1 June 2011

Today, colonies from all 9 pBca1785 mutants were grown up in Spec, and Bca1788-1H2 was grown up in Trim.
We have the library sequence of pBca1785 and,
Bca1788 is to be aligned with sequence below as D4-3 was (using ca998):

```
Aligned to:                          AAGCTTAGCCGAATTA...AGGTAAAG.TGCAAGGAGCCAAATCTCTTG
1788D4-3_ca998_2011-05-13_A08.ab1    AAGCTTAGCCGAATTAATGAGGTAAAGGTGCAAGGAGCCAAATCTCTTG
```

SpencerScott 15:34, 31 May 2011

New Project:
Characterizing select mutants in pBca1785 MukF libraries (attached to ToxR):
C2, C3, A4, D5, A5, A1, F1, F2, G1 from lowest to highest fluorescence.
Also, characterizing very weak (hopefully) rbs Bca1788 mutant 1H2. (contains Pctx, and ExsA to drive activation of Cre)
Today's Work:
1. Mini-prep
2. Dilute (1:50)
3. Transform into MC1061 cells (using 1ul of the dilution)
4. Plate MukF on Spec and Bca1788 on Trymethylcrin

Characterization of MukF Mutants in pBca1785 Library

Background (no ToxR) = 182; Max (bOBP) = 8305

Mutant	Intensity	Percent ((I-background)/(Max-Background))
C2	308	1.55%
C3	658	5.86%
A4	1008	10.17%
D5	1461	15.75%
A5	1874	20.83%
A1	2344	26.62%
F1	3010	34.81%
F2	3697	43.27%
G1	7625	91.60%

SpencerScott 10:38, 30 May 2011

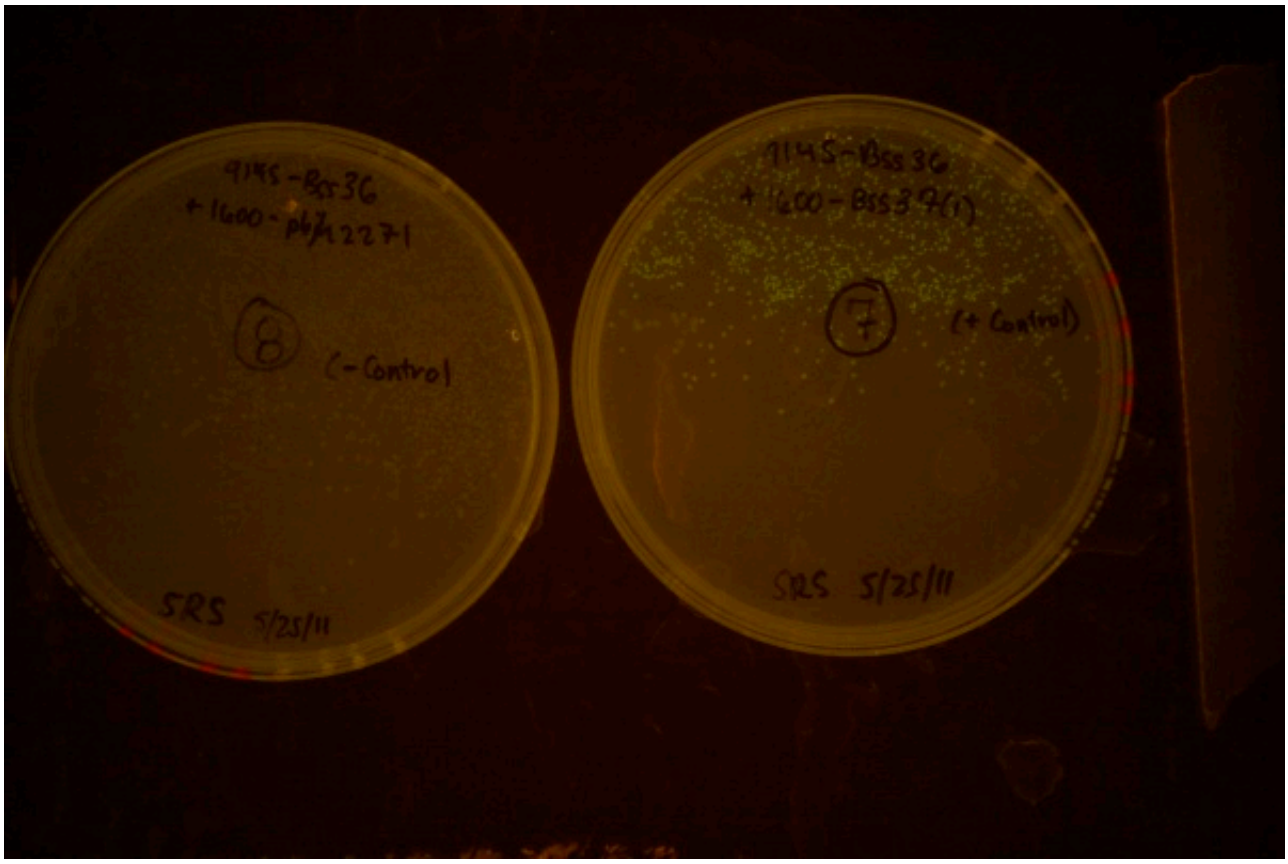
Time course didn't show any growth. New Colonies were picked and grow up in Amp+Spec LB;
New Colonies' Time Course was also insignificant. It worked, but clearly Exsa does not like to be linked.

SpencerScott 13:10, 27 May 2011

Overnight cultures were put into a Tecan Time Course (*1 for some reason didn't grow...) so the plate is as follows:
Bsrs007(1), Bsrs009(3), Bsrs010(4), Bsrs011(5), Bsrs012(6), Bss37(+), Dummy(-)

SpencerScott 10:00, 26 May 2011

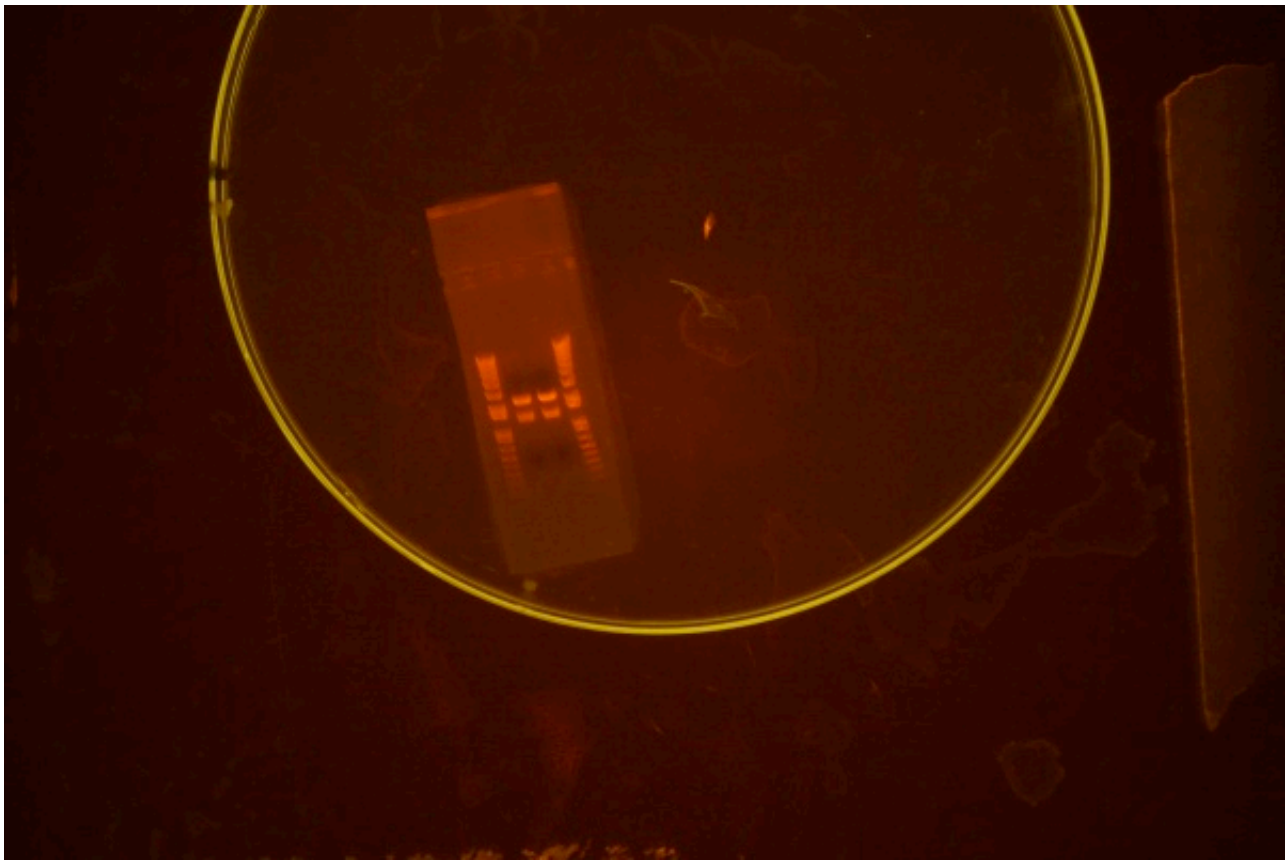
The Positive control had all florescent colonies while the negative control had none:



However, there was heterogeneity in all of the 6 constructs, but they were mostly just **dead**.
Abort experiment?
Colonies were picked from all 8 AmpSpec plates anyway and grown up in AmpSpec LB.
On another note:
9145-Bss36 sequences were confirmed. 1600-Bss37 were a little shotty but mostly okay.

SpencerScott 09:48, 25 May 2011

Made a 1/25 dilution of 9145-bss36 (batch #2) into LB AMP. Now growing up in 37 degree shaker.
Mini-prepped both batches of 9145-bss36.
Also, Mini-prepped both colonies of 1600-bss37. Digested bss36-9145 (both batches) in Eco/Bam:



The Expected sizes were: 2057 and 1273, which matches perfectly
These along with the 1600-bss37 will be sent in for sequencing.

Finishing the Small Scale Competent Cell Prep

- Grown up for 1-2 hours in 37 degree shaker upstairs.
- Spin down to get Pellet (6500 rpm for 6 mins) (Change rotor and cool to 4degreees)
- Pour off
- Add 5mL TSS (8 transformations + 4 extra PCR strips in -80)
- Vortex/Re-suspend
- Aliquot 120ul per tube: should fill 5 PCR strips
- Put Four of those strips into freezer
- Add 30ul KCM to each tube in the fifth strip

Transform 6 different constructs, pBad-Exsa, and dummy 1600 plasmid (8 total)

- Take 100ul of above and pipette into another PCR strip (saving the fifth PCR strip to do a contamination control)
- Add 1ul DNA of constructs 1-6, and + and - control to the Tubes in the 6th PCR strips respectively (the ones you just pippeted into)
- 10 mins ice, 90 sec 42, 1 min ice.
- Recover for 1 hour in 100ul 2YT
- Use half of the liquid to plate on AMP&SPEC plates (remember the contamination control) and the other half to plate on AMP+SPEC+Arabinose plates.

SpencerScott 19:35, 24 May 2011

At 11AM bss37 in 1600 was plated on a SPEC plate.

At 11:11AM 9ss36 in 1ml was plated on a SPEC plate.

At 7:20PM there were many colonies but they were all extremely small. I attempted to pick a colony (and hopefully succeeded) and am growing it up in SPEC LB.

SpencerScott 09:36, 24 May 2011

Monday: 9145-Bss36 tranformed into MC1061 cells

- Diluted 1ul of 9145-Bss36 miniprep into 50ul water
- Transformed into MC1061 cells
  - 30ul KCM to -80 cells
  - 90 ul cell mixture then taken and put into 1ul DNA
  - 10 min ice, 90s at 42, 1 min ice
- Plated on AMP

This procedure was run twice for a total of two separate transformations and plates.

AMP+SPEC LB plates, as well as AMP+SPEC+Arabinose LB plates were poured in preparation for Wednesday.

Tuesday:
One colony was picked from each plate and added to 5mL of AMP+LB.
Observations:
There were many satellite colonies, but colonies were picked that had relatively few or no satellite colonies.
There was homogeneity in the phenotype of all colonies.