SpencerScott start 07/05/2011

From AndersonLab wiki

HOME

SpencerScott 11:15, 6 August 2011 (PDT)

1. HIV Project
    1. Tecan was ran yesterday. Results are in; looks promising.
2. Pir/R6K Project
    1. Mini-prepped R6K-hsvTKs, going to map
    2. Picking off re-transformation of hsvTK; Grew in Spec
3. Pctx.rbs Tuning Project
    1. Ran EIPCR, only (-) DMSO worked, those two were PCR cleanup'd.
    2. Digestion with BsaI & DpnI
    3. There was a BsaI site in Amp, but the whole giant messy band was picked and gel purified
    4. It was then ligated in hopes that they would assemble correctly.
    5. Transformed into MC1061 and plated on CA (plated evenly all 200ul, just used a spreader)
4. BBa Project
    1. Mini-prepped all the different constructs of PSB3K3s (I shouldn't have made Bjc0005's but oh well)
    2. Eco/PstI digested the Bss52s; mapped well; all were sent for sequencing
    3. Transformed 1.1, 2.1, & 3.1 PSB3K3-Bss52's into MC1061 to make comp cells

- Digestion of EIPCR Product w/ BsaI/DpnI

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20uL Scale Digestion:
8uL ddH2O
2uL NEB2
8uL Cleaned EIPCR Product
1uL BsaI
1uL DpnI
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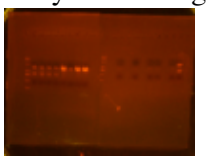
SpencerScott 15:13, 4 August 2011 (PDT)

1. HIV Project
    1. Picked colonies and grew 019 in CAS & Bsrs021 in KS
2. Pir/R6K Project
    1. Picked from the KO-pir plates and grew in Kan, and in parallel picked for making Comp cells
    2. Had to redo the Eco/bam Transfer of Pcon-hsvTK into R6K
    3. Digested (see gel at bottom), hsvTK was a little weak so I did a weak part digest in parallel.
    4. Ligated, Transformed, and plated on Amp after a short recovery.
    5. Used the second digest...
3. Pctx.rbs Tuning Project
    1. Picked from the ligation and grew in CA
4. BBa Project
    1. Mini-prepped all the different constructs as well as the PSB3K3s (which were red)
    2. Eco/PstI'd all of them
    3. Gel Purified the PSB3K3s and ligated with yesterdays Eco/pstI cleanups of Bss52 and Bjc0005 (E-'s)
    4. All PSB1C3s will be sent for sequencesing with ca998 and g00101
    5. the PSB3K3s will be trasnformed and plated on KAN

Ladder, pSB1C3-Bss52 1.1, 1.2, 2.1, 2.2, psB1C3-Bjc0005 1.1, 1.2, 2.1, 2.2 || Ladder, PSB3K3 '10(1), '10(2),

'11(1),'11(2), Pcon-hsvTK, R6K
All of the pSB1C3's were great, however, the PSB3K3s were light, as well as the Pcon-hsvTK. All the light ones were re-digested with 10ul of part.
They were still gel purified and used in ligations however, because the bands were there just faint.



SpencerScott 17:28, 3 August 2011 (PDT)

1. HIV Project
    1. Transformed Bsrs022, Bjc0005, & (-) control into both Bsrs019 and Bsrs021 comp cells
    2. Plated 019 on CAS
    3. Plated 021 on KS or CS
2. Pir/R6K Project
    1. Re-Streaked KO-pirs on Kan Plates
    2. Eco/Bam Transfered Pcon-hsvTK into R6K (JTK2993-2828) (amp resistance)
    3. No Recovery, Plated on AMP
    4. Re-streaked regular pir strains on other antibiotics to check for spec/??? resistance
3. Pctx.rbs Tuning Project
    1. Eco/bam'd Pctx-hsvTK & 1601CA
    2. Gel Purified
    3. Ligated
    4. Transform
    5. No Recovery, Plate on AMP
4. BBa Project
    1. Grew PSB1C3-Bss52 and PSB1C3-Bjc0005 in Cam
    2. Grew PSB3K3 2010 &2011 in Kan

SpencerScott 16:23, 2 August 2011 (PDT)

1. Re-streaked colonies on Kan plates with citrate
2. PCR Purify
3. Digested with proper enzymes (EcoRI/PstI)
4. Ran on Gel; Cut out Bss52 E- and P - and Bjc0005 E- and P- as well as both PSB1C3 E/P digests
5. Gel Purified
6. Ligated (4 total)
7. Transformed above four ligations as well as the 2011 pSB3K3 and the 2010 pSB3K3
8. Ligations were plated on Can and the pSB3K3s were plated on Kan
9. The BBa plasmids use ca998 and g00101 as their sequencing primers. score.
10. Booked flights for iGEM
11. Talked to Gabe about the specifics of the hsvTK project

TODO tomorrow:

1. Pick from KO-pir plates and grow in Kan
2. Pick from BBa plates and grow in Cam & some in Kan
3. Eco/Bam Transfer of Pcon-hsvTK & Pcon-GFP into R6K-hsvTK & R6K-GFP (digest, ligate, transform, plate on ????)
4. Transform Pctx.rbs into JTK164-A or "4" Plate on ????

TODO Thursday:

1. **Pir Project:**

    1. Genome Prep all 10 and MC1061
    2. QC: PCR, map, Send for Sequencing
    3. Restreak on other antibiotics to check for contamination
    4. Pick from R6K plates and Grow in ???
2. **rbs tuning Project:**
    1. Pick from JTK164-"4" and run Negative Selection
    2. Plate, scrape, (-) liquid for 2-3 hours
    3. Plate

TODO Friday:

1. **Pir Project:**
    1. Check sequences of pir genome??
    2. Mini-prep R6K hsvTK & GFP cultures; map, sequence
    3. Transform mini-preps into all pir strains and plate on ???
2. **rbs tuning Project:**
    1. Scrape, comp cells? Run Positive Slection by transforming in constituitve dimerizers?
    2. Plate/ titer/ (scrape on Sat, re plate on + selection?)

TODO Saturday:

1. **Pir Project:**
    1. Pick & Grow
2. **rbs tuning Project:**
    1. Scrape and re-plate on + Selection

TODO Sunday:

1. **Pir Project:**
    1. Mix Green & White overnight cultures 1:1
    2. Put in (+), (-), & no Selection...
2. **rbs tuning Project:**
    1. ?????

SpencerScott 10:33, 1 August 2011 (PDT)

Transduction is being re-done. Donor strain was grown up overnight as well as MC1061 and the 10 different JTK164s.
P1 Lysate being prepared.
Grew up Overnight culture of donor in LB-MgClCaCl for 1 hour. Then added 100ul p1vir.
This will be grown up for 2 hours.
At the same time I am growing up overnight culture in donor in LB-MgclCaCL for 2 hours to have as a backup since I can't tell if it had grown up enough in 1 hour.
p1vir will be added to this as I prepare the transduction into the different strains.
(p1 lysates are both in Scott Box 1)
Everything was tranduced and there were 2 seperate rounds (6 plates each) that were plated on Kan.
Also, primers came in for Bba parts, so those were PCR'd with E/P -,+ DMSO for each. (1k55 & 2k55 for Bss52 and Toxr-Lambdarep)

TODO Tomorrow:

1. Re-streak colonies on plates with citrate
2. Talk to Gabe about the specifics of the hsvTK project
3. PCR Purify
4. Digest with proper enzymes (spe?)
5. Run on gel
6. Gel Purify

7. Ligate
8. Transform
9. Plate on...?
10. Order Sequencing Oligos

SpencerScott 11:30, 29 July 2011 (PDT)

1. Transduction being done on the 10 different JTK164s as well as MC1061 as seen here: https://andersonlab.qb3.berkeley.edu/mediawiki/index.php/JTK_AndersonLab_Techniques#Transduction
2. In parallel I am preparing more donor P1 lysate because I'm scared the ones from yesterday weren't done properly.
3. I will reseed the 10 different JTK164s and MC1061
4. I am growing an overnight of the donor strain because I'm nervous that the lysate won't work allowing it to grow overnight before adding the P1 vir.

SpencerScott 14:40, 28 July 2011 (PDT)

1. The ER-Truncations were non-inducible
2. The colonies picked from ER-4 didn't grow
3. I have therefore decided to give up on the Estrogen Receptor
4. hsvTK project started
    1. The JTK's and the MC1061 were re-seeded for use tomorrow
    2. The donor strain was prepared as a P1 lysate using the protocol for transduction found here:https://andersonlab.qb3.berkeley.edu/mediawiki/index.php/JTK_AndersonLab_Techniques#Transduction
    3. The Benchtop mix is labeled LB + Cacl + MgCl; 2ml of it was taken and grown up for an hour with 20ul donor ON culture.
    4. P1 phage was then added to that and grown up for another hour
    5. 50ul of choloform was added, vortexed, centrigufed, poured into new tube.
    6. 15ul of chloroform was added and it was put in Scott Box 3 for use tomorrow
    7. Tomorrow this lysate will be added to the recipient cells

SpencerScott 11:39, 27 July 2011 (PDT)

5.2 and 6 are very constitutively on, 5.1 & 5.3 not as much, and 8.1 &8.2 are slightly on (constitutively).
Running Tecan +/- Estradiol
Colony PCR ran on ER-Trunc #4

grew up 10 different strains of JTK164 and 1 culture of MC1061 (in Trim and in plain LB respectively).
Also grew up the donor strain in Kan.

SpencerScott 11:01, 26 July 2011 (PDT)

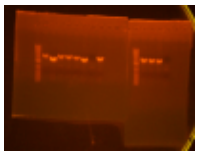
Estrogen Truncations 5.2, 6.2 & 8.1 were fluorescing. They will be grown up again and then Tecan'd to test for induce-ability.
Bss52 Cells large scale competent cell prep done.

SpencerScott 14:21, 25 July 2011

Estrogen assay ran on 7 different truncations (all except Trunc-4). +,- Estradiol with 15ul of 70mM into 3mL CAS LB.

SpencerScott 11:28, 23 July 2011

Colony PCR:

 Clones 2.1, 2.3, 2.4, 3.1, 3.2, 3.4, 5.1, 5.2, 5.3 will be mini-prepped and sequenced.
Put ER transformed into Bss52 plates in refrigerator taped together; will wait for other truncations and sequence confirmation.
TODO:

1. Send above clones in for sequencing
2. Transform them into Bss52 after lunch
3. Pick from all tomorrow and grow in CAS

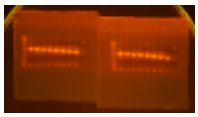
SpencerScott 16:09, 22 July 2011

1. Mini-prepped ER-Truncations, Digested and ran on gel:


Only 1.1,1.2,4.2,6.2,7.2,8.1,8.2 mapped well; these will be sent in for sequencing. (3.1 was red)
2. Transformed above as well as + and - controls into Bss52 and Plated on CAS
3. Ran colony PCR on clones #2, 3, & 5 to find proper clones
4. Dialyzed 500ul antibody in PBS (only for 45 minutes though!)
5. Set up the complex tecan and will run over night.

SpencerScott 16:54, 20 July 2011

1. PCR'd 8 different ER-Truncations (Expand +, - DMSO)
2. PCR Zymo Clean-Up
3. Nhe1/Bam'd (also N/B'd 9525-1834)
    1. 1 Hour in Incubator
4. Ran on gel; Cut out -DMSO since they all looked good and both 9525-1834 digests
5. Gel Purified
6. LIgated the 8 truncations with N/B 9525-1834
    1. 30 min wait
7. Transformed into MC1061 Cells
    1. 45 Min Recovery in 2YT
8. Plated on Spec


Above are the ER-Truncations 1-8 (-) and (+) DMSO. The (-) DMSO's were used for all.

TODO Tomorrow

1. Pick from ER truncations; Grow in Spec
2. Pick from HIV Plates; Grow in respective antibiotics for 6 hours
3. Figure out Dialysis
4. Run the Tecan again! ahhh

TODO Friday

1. Mini-Prep ER Truncations; send for sequencing
2. Transform into Bss52 Comp- Cells

3. Check the HIV Tecan

SpencerScott 11:14, 14 July 2011

1. Picked 2 colonies from each of the HIV plates. Grew the Bsrs019 on the left side in CAS
2. Grew the Bsrs021 on the right side in KS
3. Here is what the Plates looked like; Bsrs021 (pCon which is stronger had much smaller colonies):

 :: 

4. After spinning down the ER Assay, it was clear that ERΔF was still completely dead. However, ER-LBD did turn a little green! Unfortunately, it didn't look like it responded to the Estradiol, since the + estradiol was just as green as the - estradiol.
5. Will wait on Chris, to decide what is the next course of action for the ER.
6.Realized I can't mini-prep the transformations... Since they have two plasmids... Need to transform and plate just the ToxR-Constructs. That will be plated on just Spec.
7. (+) & (-) controls transformed and plated on Spec
8. Set up a Tecan to measure the ER-LBD at different concentrations. (started with 196ul LB-CAS + 4ul DMSO/Estradiol solution, then took 50ul of that and put it into 150ul LB-CAS, mixed, then took 50 of that, etc.) Left the last one with 0M as a control.

SpencerScott 15:58, 13 July 2011

1. +, - controls, ER-LBD #5 & #8 were grown up in CAS
2. Bsrs019 and Bsrs021 were grown up in CA & CK.
3. 4 Aliquots of Comp cells were made from each (2 stored in the -80)
4. Positive and Negative controls, as well as Srs022 (ToxR-HIV) were transformed into different tubes.
    1. 1 Hour recovery, Plated on CAS & SC
5. Clone #8 did not grow up, so Clone 5 was tested under two concentrations of E2. (40ul and 10ul of 70mM E2 in DMSO into 1.96mL LB-CAS)
6. ER1.1 & ER2.1 were also tested under 40ul of 70mM estradiol in DMSO into 1.96mL LB-CAS

(You put your DMSO solution in the freezer top drawer)

'**TODO Tomorrow:**

1. Check Assay!!
2. Meet with Terry!
3. Pick from HIV Plates (check for green!)
4. Mini-Prep + and - Controls to get more DNA stocks

SpencerScott 15:57, 11 July 2011

HIV assay:
Stock solution is at .6 uM!! Today, the two different reporters were transformed into MC1061. Tomorrow they will be made into comp cells so that the ToxR-antigen can be transformed into it as well as the + and negative controls.

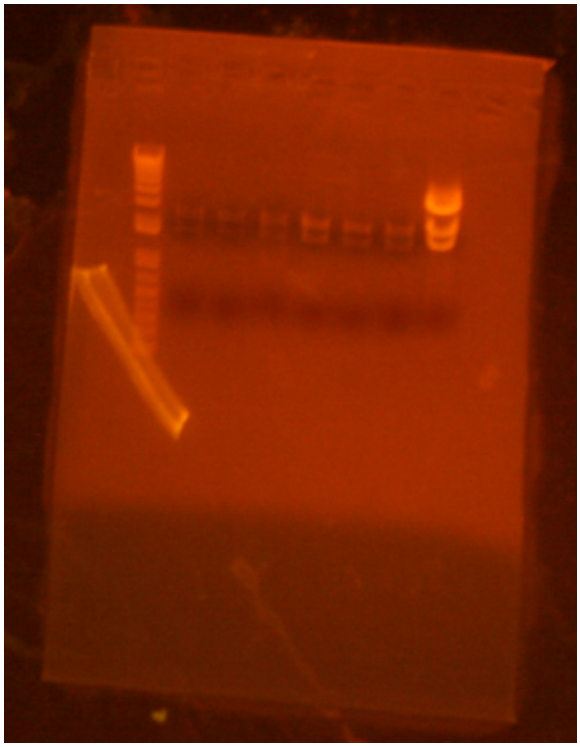
Transform the Two Reporters into MC1061 (to see if they are green and to use as comp cells later as positive and negative controls).
Plate on CA & CK.
Transform Two ER-LBD's and + and - Control. Plate on CAS
ERΔF were picked and grown up in CAS, as well as the + and - controls in Bss52.

TODO tomorrow: Pick off everything very early then wait 5/6 hours:

1. Make Comp cells from reporter cells
2. Transform in HIV-antigen & + and - Control mini-preps. Plate on CAS
3. Put 20mg Estradiol into DMSO
4. Take some culture from ER-LBD's & + and - Controls and put into Estrogen Assay with 40ul and 10ul of 70mM Estradiol in DMSO
5. At the same time do the same thing with the culture from the ERΔF culture from today.

SpencerScott 15:37, 11 July 2011

1. Plates looked contaminated
2. Ligated ER3 digests with 1834 again
3. Transformed them into MC1061; plated on Spec
4. PCR'd Srs021 clones 1.3, and 3.3 and sent in for sequencing after I did a PCR clean-up.
5. Transformed SRS021 1.3 and 3.3, and Srs019 into HIV comp cells; Plated on CAS and Spec-Kan reverse respectively.
6. Digested the 7 mini-preps of the ER3 colonies, ran on gel: They actually mapped well even though The plate looked contaminated.



Sent clone 5 (lane 4) and clone 8 (lane 7) in for sequencing.

TODO Tomorrow:

- Pick from new ER plates; grow in Spec
- Pick from HIV transforms; grow in antibiotics
- Check sequences; If ER is good, transform into Reporter comp cells.

SpencerScott 17:22, 8 July 2011

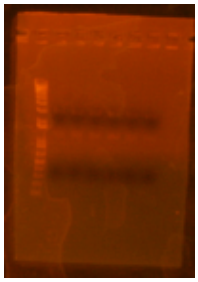
Estrogen Tecan showed the same thing... no green.

TODO Saturday:

1. PCR Domain D (LBD) from Estrogen Receptor.
2. Check Sequences of BRP-reporter (FAIL)
3. Nhe1/Bam Digest PCR & 9525-1834
4. Gel purify
    1. Gel Purifications are in Scott Box 3 in a vertical line down the center.

TODO Sunday:

1. Ligate nhe1/Bam Digests from yesterday
2. Transform into JTK155
3. Plate on Spec
4. Pick from ER-LBD plates, grow in Spec



Above is Eco/Bam'd Bsrs021 clones. They are all veryyy faint, but the best one was clone 3.3. We will do PCR to get sequencing, and continue with the transformation.

TODO Monday:

1. PCR (Expand) up two of the Bsrs021 clones and send in for sequencing.
2. Transform two different reporter/BRP plasmids into 9525-1834-HIV comp cells.
    1. Recover, Plate Bsrs021 on Spec-CK and Bsrs019 on Spec-CA
3. Mini-Prep ER-LBD clones

SpencerScott 11:33, 8 July 2011

Picked from the 1601CK-Srs022 plates and grew up in Spec. (will Make comp cells in 5 hours)
Mini-prepped all Bsrs021 and sent in 1.2, 1.3, 3.1,3.3,3.4, 4.4 in for sequencing.
Estrogen looks dead (no green). Ran a tecan with various amounts of Estrogen.

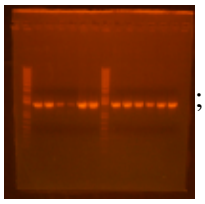
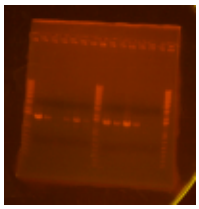
SpencerScott 14:27, 7 July 2011

Estrogen Assay

1. 10mg of Estradiol dissolved into 5mL of DMSO.
2. 40ul of above was put into 3 wells of 2mL of CAS-LB
3. one well was for +, one for -, and one for the ER culture (2ul of culture taken)
4. then the same was grown up but with no Estradiol/DMSO

First round didn't work because the ER-bss52 cells didnt grow up, in fact they seem to get clearer later in the day. However, the ER-bss52 overnight culture used was the one that grew up the least, so two others were chosen and the same experiment was run. Will check at the end of the day.

Colony PCR was run on 6 samples of each of the 4 Bsrs021 plates, and they were grown up in CK in a 24 well block. Results of the PCR on a gel:


;

L, 1.1-1.6, L, 3.1-3.6 || L, 2.1-2.6, L, 4.1-4.6, L
Basically use anything from plates (rows in 24 well block) 1 and 3.
The desired size was 1466, with other possible non-desirable products of 1206 and 428. Any that showed up seemed to be around 1466.
Mini-prep and Sequencing tomorrow.

SpencerScott 10:07, 6 July 2011

Digestions 1 and 2 from yesterday were plated on the wrong antibiotic!!
The digestions were re-ligated but there is no more digested vector anymore, so if this fails we have to redo the digestion and gel purification.
Transformed into MC1061 Plated on CK!

Srs023 into bss52 comp cells grew well, no green colonies. Two of each colony will be chosen from each plate and grown in test tubes with CAS.
The + and - control from Jason were also grown up in CAS.

Everything sent in for sequencing was sequenced confirmed except Bsrs019 clone 3 (as expected).

SpencerScott 15:03, 5 July 2011

TODO Tomorrow:

1. Pick Two of each Transformation of Bsrs024 in Bss52 and grow in CAS.
2. Analyze Sequences; if clone 1 is not good do not do above, and mini-prep whichever ones are good (then transform, plate)
3. Check Bsrs019 sequence, sequence confirm?
4. Make Dilutions of Estradiol for ER Assay
5. Plan HIV Assay
6. CA pouring Party!

TODO Thursday:

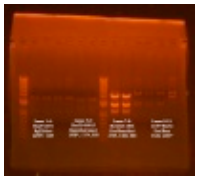
1. Pick six colonies per ligation of Bsrs021 and grow in CK; Run **a colony PCR** on all (6x4 = 24)
2. Start Estrogen Receptor Assay

TODO Friday:

1. Mini-Prep Bsrs021, Map with Eco/Bam expecting 3196, and 1307. Sequence hits.

SpencerScott 11:53, 5 July 2011

- **Digestions**
  - 1. BgIII/xhoi: Bxa5+bjh2051 ("bjh2061"): **2258** & 1205
  - 2. Bam/xhoi/XmnI: Bsrs020 (bss52+b0015): **1958**, 1174, & 958
  - Analytical:
  - 3. Eco/Bam/Xhoi: 9525-1834-Bsrs023: 2705, 1486, 986
  - 4. Eco/Bam: 2109-Bss52: 3328, 2464



Only CLone 4 of 2109-Bss52 looks good, but both 3 & 4 were sent for sequencing;
Clones 1 and 3 of Bxa5+2051 were gel purified; Both Clones of Bss5+b0015 were gel purified

- **Ligations**
  - Bxa5-bjh2051 & Bsrs020, Transform, Plate on CK
- **Transformations**
  - 1. Bxa5-bjh2051 & Bsrs020 into MC1061, Plate on **CK**
  - 2. 1834-Bsrs023 into Bss52 Comp Cells, Plate on **Spec-CA**
  - 3. Positive & Negative Controls (Jason will do these, pick off his plate)
- **Sequencing**
  - 9145-Bsrs023 (Ca998 & g00101), clones 1,3,4,5,& 6
  - 9525-1834-Bsrs023 (ca1787 & g00101)
  - Bsrs019 (ca998 & g00101) clones 3 & 4
- **Picking Srs023 backups**
  - Clones 3,4,5,&6 were picked and grown up in Spec (since only clone 1 was picked last time)