Nikit start 7/1/11

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Nikitpatel 10:21, 2 July 2011 (PDT)

- Design MukF Interface Library #3's EIPCR Oligos (Xin Xin #2)

Nikitpatel 10:21, 3 July 2011 (PDT)

- Design MukF Interface Library #2's EIPCR Oligos (Xin Xin #1)

  - What degenerate codon should I use? Need to email out my ideas on which one we should use.

Nikitpatel 21:17, 9 July 2011 (PDT)

- Finished designing last set of EIPCR oligos yesterday. Waiting for order.
- Wrote up pseudo-protocol of the chemical selection assays on Friday with Spencer

Nikitpatel 10:48, 11 July 2011 (PDT)

- Transformed D4-3 Pctx-ExsA into MSD 2.0 Cells

- Plated on Kan/Trim

Nikitpatel 10:48, 12 July 2011 (PDT)

- Pick and comp D4-3 cells in 2YT/Trim
- Transformed in bOBP Library #53 and MukF Library #1
- Plated one half of each into Spec/Trim/Kan (Slow cells) and the other half into Spec/Trim/Cam (Fast cells)
- Overnight Culture D4-3 Cells

Nikitpatel 13:09, 13 July 2011 (PDT)

- Got phage contamination on plates. Possibly in cultures as well.

Wednesday

- Scrape all 4 plates. Miniprep 500uL of scrape.
- Take Slow cells and plate on Spec/Trim/Cam (with 10E-3 and 10E-6 dilutions)
- Take Fast cells and plate on Spec/Trim/Cam (with 10E-3 and 10E-6 dilutions)

- Large scale comp cell D4-3 cells. x both libraries

Nikitpatel 10:21, 19 July 2011 (PDT)

- Transform D4-3 Pctx-GFP RBS hit into MSD 2.0 cells. Plate on Trim/Cam.
- Digest rbs.TetA and ligate into 9145 and 1600 vector. Plate on Amp and Spec respectively.
- Plate 0, 0.5, 1, 2 mM Ni selection onto Amp plate

Nikitpatel 14:37, 20 July 2011 (PDT)

- Count green to white ration on nickel plates

- 0 mM = 59 White / 49 Green
- 0.5 mM = 41 White / 55 Green
- 1 mM = 59 White / 83 Green
- 2 mM = 6 White / 263 Green
- *Conclusions: 2mM gives around 10 fold enrichment. We can try multiple rounds and see if that helps.*

- Make comp cells out of D4-3 MSD 2.0 cells. Trans in MukF library and bOBP library.

  - Plate half onto T/S/C (-) and half on T/S only
  - Culture half on T/S/C and half in T/S

- Ideas for TetA RBS Screening?

Nikitpatel 16:26, 21 July 2011 (PDT)

- Scrapped and miniprepped solid selections (4 tubes)

  - Replated into negative selection

- Miniprepped liquid selections (4 tubes)

  - Plated some into negative selection
  - Reinnoculated into negative liquid selection

- Miniprepped more pBrc32 plasmid (2 tubes)
- Miniprepped p1600-rbs.TetA and p9145-rbs.TetA colonies (2 tubes each). Sent for sequencing.
- Plated Day 2 round of nickel selection (competition experiment)
- Did large scale comp cell prep of D4-3 plasmid in MSD 2.0 cells

  - Aliquoted into PCR strips and stored in -80 freezer with black stripes

- Scraped TetA Lib #1 and #2 plates and innoculated 4uL into 5mL of LB/Trim/2mM Ni

Nikitpatel 10:49, 22 July 2011 (PDT)

- Miniprep rest of stuff
- Dilute plasmids
- Transform into Bss52. How much do i plate?

  - do transformations separately to avoid confusions
  - enough plates?
  - Plate plain Bss52 onto CA and see what background we get

- Take TetA library and plate onto Tet

Nikitpatel 16:26, 23 July 2011 (PDT)

- Count Green to White. Decide what the next plan of action for MSD 2.0 is

- We get enrichment in liquid after 2 DAYS of negative selection (i.e. go into CAM and reinnoculate into CAM for second day). With this we get around 10000 fold enrichment
- Solid selections mirrored liquid but turned out slightly cleaner. However there was no difference between the two after 2 days of negative selection.

- Read up on Xin Xin's Selection system (hsvTK) and plan out experiments
- Transform fresh batch of D4-3 cells

Nikitpatel 10:59, 24 July 2011 (PDT)

- Pick D4-3 cells into 5 mL of LB+TRIM/CAM
- Prepare equipment for large scale comp cell prep

Nikitpatel 20:40, 25 July 2011 (PDT)

- Large scale comp cell prep of D4-3 cells

  - Clean MukF Library with Zymo and transform in 3,5,7 uL into cells
  - Rescue in LB and SOB for one hour
  - Titer 1/10 uL and 1/100 uL. Grow up a little in plain LB for next day miniprep.
  - Add Trim/Spec/Cam

- Pick and culture Bss52 for large scale comp cell prep

- Digest p1600-pctx with BamHI/XhoI and p1600-rbs.TetA with BglII/XhoI

  - Ligate. Transform into MC1061 plate on Spec.
  - Picked a few colonies at 11pm into LB+Spec

- Talked to Gabe about what I need to do for hsvTK experiment

Tuesday

- Dump saturated batch of Bss52 cells into 500 mL 2YT + CA.

- Miniprep p1600-Bnp008 and cultures from large scale MukF selections.

  - Eco/Bam Digest p1600-Bnp008, TetA RBS Hits, and pjtk2993-2828.
  - Drop parts into jtk2993.
  - Transform into jtk155 and plate on Amp. (no rescue needed).
  - Send minipreps for sequencing. (8 of them)
  - Pick colonies at night.

- Large scale MukF Selections

- look at titer plate and determine how much to reseed the cultures into.
- be sure to oversample (x4) and cover all members of the library. grow in T/S/C again.
- take 2 mL from each flask. miniprep it, seperate plasmids, and transform into Bss 52 along with previous days miniprep.

- Make -80 comp cells of Bss52