Eleanor's Lab Notebook

5/26/15

- Streaked E. Cadherin and CB008DB on YPD

5/29/15

1. Restreak CB008DB
   - Previous attempt did not grow
   - Completely thawed glycerol stock & vortexed before plating.
2. PCR pTET mfAlpha
   - FW: pTET(+tFBA1)
   - RV: mfAlpha(+tFBA1 +tAdh1)
   - lower annealing temperature because AT%
3. PCR tFBA1 (template from Russell)
   - FW: tFBA1(+GFP)
   - RV: tFBA1(+pTET)
   - Standard PCR cycle
4. Transform DH5alpha cells with Hy6E1 (+pTET-GFP) for miniprep to use for transformation
5. attempted to Gel Extract but tFBA1 band was not proper size.

6/1/15

Possible contamination - unusual white spot on plate, but all the plates had colonies

Re-PCRed tFBA1 in 4 different tubes:

- Phusion w/ DMSO
- Phusion w/o DMSO
- GoTaq w/ DMSO
- GoTaq w/o DMSO

Used "Touchdown PCR" - temperature decreases in intervals.

Started liquid cultures of cadherin and HY6E1 Streaked out CB008DB

6/2/15

- Gel Extration for tFBA1 and pTET-mfalpha PCRs

- Yeast DNA extraction of Cadherin

- Miniprep HY6E1

- 10 µl Not1 digest of HY6E1 overnight 37 degrees

6/3/15

.5 µl Antartic Phosphotase in Not1 digest of HY6E1 (37 for 1 hr)

PCR purify HY6E1 digest. LOW CONCENTRATION (MAY NOT WORK)

```
Gibson Assembly:
- Positive
  -5 µl +
  -5 µl Gibson Mix
- Negative:
  -4.25 µl HY6E1
  -.75 µl water
  -5 µl Gibson Mix
- Gibson:
  -4.25 µl HY6E1
  -.5 µl mfalpha  -
  -.2 µl tFBA1
  -5 µl Gibson Mix
*50 for 1 hr, keep everything on ice!*

Transform Gibson Mix in C2987.
```

Start liquid cultures of cadherin in YPD and SD-Trp (Quintara said no DNA in tube)

Start 10 µl Not1 digest of HY6E1.

6/4/15

Dephosphorylate Not1 digest PCR purify Not1 digest

Colony PCR Gibson Transformations. (Used FW (tFBA1) and RV (mfalpha)) FAIL Transformed pGEM39 into DH5a for bootcamp miniprep.

Yeast Miniprep E.cadherin, eluted in 35µl 1x TE Buffer. Diluted 10x water for measuring because salt confuses the Nanodrop.

Redo Colony PCR w/ tFBA1 primers (overnight)

6/5/15

Start liquid culture of pGEM39 (E.coli). Keep in freezer. Miniprep on Monday.

Sequencing of Cadherin failed

Colony PCR failed

Redo-Gibson

```
 Gibson Assembly:
- Positive
  5 µl +
  5 µl Gibson Mix
- Negative
  2 µl HY6E1
  3 µl water
  5 µl Gibson Mix
- Gibson
  2 µl HY6E1
  1 µl mfalpha
  .25 µl tFBA1
  1.75 H20
  5 µl Gibson Mix

50 for 1 hr, keep everything on ice!

Transform Gibson Mix in C2987.
```

Transform Cadherin (SD-Trp) in C2987.

- 5 µl DNA
- 25 µl cells

6/8/15

Colonies too small to PCR

- Let grow at 37 for another night.

Miniprepped pGEM39 (but sequencing shows it is just PJW608)

6/9/15

Gibson/Cadherin colonies: Everything failed but negative controls.

- Cadherin have plausible colonies, start overnight cultures and miniprep tomorrow

-Redo Gibson w/ Seamless. Use same parts as last Gibson.

```
Seamless Assembly:
- Positive
  2.5 µl +
  2.5 µl Seamless Mix
- Negative
  1.5 µl HY6E1
  3.5 µl water
```

```
  5 µl Gibson Mix
- Gibson
  1.5 µl HY6E1
  2.5 µl mfalpha
  1 µl tFBA1
  5 µl Seamless Mix
Room Temperature incubation for 20 min.
Put on ice for 3 min.

Transform in C2987
    25 µl cells
    3 µl DNA
```

6/10/15

Seamless colonies:

-Positives small/slow growing colonies

- Negatives had ~5 colonies

Seamless reaction had ~20 colonies

Colony PCR of Seamless colonies. Started liquid cultures of 8 colonies.

We have 400 bp bands at 1,2,3 8 and (questionable 5,6,7)

Miniprepped cadherin. (5 colonies)

Only 2 had any DNA. Send to sequencing

6/11/15

- Cadherin sequencing came out correct

- Miniprep correct Seamless colony PCRs from yesterday.

- Send to sequencing 1,2,8.

6/12/15

- 1:20 Dilution of CB008DB for transformations

Sequencing correct for 1 & 2.

Pme1 Digests of 1 & 2 Minipreps.

- 4000 ng. 50 µl digestion.

Yeast Transformation- HY6E1+tFBA1+pTET-mfalpha (1 & 2)

6/16/15

- Colony PCR of Yeast Transformations
  - Use RA 145/ RA 146 (His Integration) (#87/89)
    - Should be a 580 bp fragment
  - Zymolase in 50 μl reaction then Colony PCR w/ GoTaq Master Mix

HY6E1+tFBA1+pTET-mfalpha = pGEM41

6/17/15

- Ran new 2% gel for colony PCR - ugly
- Redid Colony PCR. Everything successful.

6/19/15

- Made glycerol stock of yGEM127 (pGEM41 in CB008DB)
- Streaked other clustering proteins from 2011 on Carb (Mach1) and SD-TRP (EBY100)
  - HWP1 (From Candida Albicans)
  - MGFP5 (From mussel feet)
  - In pCTCON plasmid for yeast transformation. Includes galactose promoter and AGA anchors.

6/22/15

- Made liquid cultures of HWP1 and MGFP in LB+ Carb and CB008DB in YPD for transformations tomorrow.

6/23/15

- Miniprep HWP1, MGFP
- Transform HWP1, MGFP, Ecadherin into CB008DB

Plated on SD-TRP

6/25/15

We have colonies on SD-TRP with adhesion proteins in CB008DB

Made S-Raff(1%) and S-Gal(1%)

- 10 ml 10x Yeast Nitrogen Base
- 10 ml 10x Complete Supplement Mixture
- 75 ml ddH2O
- 5 ml 20% Sugar (Either Raffinose or Galactose)

Keep media in fridge.

Started liquid culture of HWP1, MGFP5, Ecadherin in CB008DB in 1% S-Raff overnight

6/26/15

- Pelleted overnight S-Raff cultures
- Induced yeast in 5 ml S-Gal (1%)

```
MGFP5:
    -  5 ml S-Gal
    -  4.5 ml S-Gal
    -  500 µl 1M NaCl
HWP1:
    - 5 ml S-Gal
Ecadherin;
    - 5 ml S-Gal
    - 4.95 ml S-Gal
    - 50 µl .1M CaCl2
```

- Wasn't dense enough to see clustering because we were supposed to grow in YPD overnight, then move to S-Raff for an hour, then move to S-Gal.

6/29/15

- Started overnight cultures of HWP1, MGFP5, Ecadherin in YPD

6/30/15

Started preliminary testing for clustering genes.

1. Pellet overnight cultures of HWP1/Mgfp5/Ecadherin in CB008DB
2. Grow for 1 hr in 5 mls of S-Raff
3. Pellet.
4. Resuspend in 1.5 mls of S-Gal

```
6 different culture tubes with varying inductions

MGFP5 (NaCl):
    0 mM:
        - .5 ml cells
        - 4.5 ml S-Gal
    100 mM:
        - .5 ml cells
        - .5 ml (1M)NaCl
        - 4 ml S-Gal
    200 mM:
        - .5 ml cells
```

```
        - 1 ml (1M) NaCl
        - 3.5 ml S-Gal

ECadherin (CaCl2):
    0 mM:
        - .5 ml cells
        - 4.5 ml S-Gal
    1 mM:
        - .5 cells
        - .05 ml CaCl2
        - 4.45 ml S-Gal

HWP1:
    0 mM:
        - .5 ml cells
        - 4.5 ml S-Gal
```

7/1/15

Made stock solution of 50 ml (5M) NaCl in H20.

1. Pellet overnight cultures.
2. Transfer to 1% S-Raffinose for 1 hr.
3. Pellet.
4. Transfer cells to 1% S-Gal. Spilled hal of Ecadherin, so doubled cells.

```
MGFP5 (NaCl):
    0 mM:
        - .25 ml cells
        - 4.75 ml S-Gal
    200 mM:
        - .25 ml cells
        - .2 ml (5M) NaCl
        - 4.55 ml S-Gal
    400 mM:
        - .25 ml cells
        - .4 ml (5M) NaCl
        - 4.35 ml S-Gal

ECadherin (CaCl2):
    0 mM:
        - .5 ml cells
        - 4.5 ml S-Gal
    1 mM:
        - .5 cells
        - .05 ml CaCl2
        - 4.45 ml S-Gal
    2 mM:
```

```
        - .5 cells
        - .1 ml CaCl2
```

7/2/15

Should've been using 2% Galactose

2% Galactose Media: - 10 ml 10x Yeast Nitrogen Base - 10 ml 10x Complete Supplement Mixture - 70 ml ddH2O - 10 ml 20% Galactose

LOW=.001 OD HIGH= .1 OD

1. Pellet overnight cultures.
2. Transfer to 1% S-Raffinose for 1 hr.
3. Pellet.
4. Transfer cells to 1 ml 2% S-Gal.

Checked OD of 1:10 dilution of 1ml S-Gal resuspension for HWP1, Mgfp5, Cadherin.

```
    MGFP5-  .4661   ACTUAL: 4.6
    ECAD-   .9912   ACTUAL: 7.4
    HWP1-   .9912   ACTUAL: 9.9
```

Diluting cells to LD (Low Density) and HD (High Density)

```
MGFP5 (NaCl):
    LD
        0 mM:
            - 1.08 µl cells
            - 5 ml S-Gal

        400 mM:
            - 1.08 µl cells
            - .4 ml (5M) NaCl
            - 4.6 ml S-Gal
    HD
        0 mM:
            - 108 µl cells
            - 5 ml S-Gal

        400 mM:
            - 108 µl cells
            - .4 ml (5M) NaCl
            - 4.55 ml S-Gal

ECadherin (CaCl2):
    LD
        0 mM:
```

```
            - .5 µl cells
            - 5 ml S-Gal
        2 mM:
            - .5 µl cells
            - .1 ml CaCl2
            - 4.9 ml S-Gal
    HD
        0 mM:
            - 50 µl cells
            - 5 ml S-Gal
        2 mM:
            - 50 µl cells
            - .1 ml CaCl2
            - 4.9 ml S-Gal

HWP1:
    LD
        0 mM:
            - .67 µl cells
            - 5 ml S-Gal
    HD
        0 mM:
            - 67 µl cells
            - 4.9 ml S-Gal
```

Low density cells hardly grow.

High density cells cluster relatively well past 6 hours.

7/8/15

Induced in 2% S-Gal. At time points 0, 1.5, 3, 5, 8.

Diluted to OD=.1, except for one CB008DB.

- CB008DB (OD=.015)
- CB008DB
- CB008DB+HWP1
- HWP1
- Cadherin (0mM)
- CB008DB+Cadherin (0mM)
- Cadherin (2mM)
- CB008DB+Cadherin (2mM)

7/9/15

PCRed tCyc1 and tEno2 from T64M64

- TouchdownPhu protocol
- Phusion 2XMM in 50 µl reaction.

Primers

- 136
- 137
- 138
- 139

Transformed T64M64 into E. Coli for miniprep tomorrow.

Gel for terminator PCR:

- tCyc - correct band size
- tEno2 - is wrong

7/10/15

Gel extracted tCyc1 ~20ng/µl

PCR tEno2 from T64M64 and T64tdTomato

Primers 138/139

TouchdownPhu protocol

Ran gel for tEno2 PCR. Failed

PCR Gibson overhangs for tCyc1

7/14/15

PCR:

- tEno2 from yeast genome
    - 138/139 primers/DMSO/ TouchdownPhu and TouchdownGoTaq
- Aga2-E.cadherin from pCTCON2
    - 142/143 primers/DMSO/ Phusion MM

7/15/15

Ran gel for tEno2/E.Cadherin PCR

FAILED.

- Re-PCR tEno2 w/o DMSO from T64M64
    - Phusion and Gotaq, used TouchdownGo with lower annealing temp
- Re-PCR E.Cadherin from pCTCON2 using Phusion
- Ran gel for tEno2/E.Cadherin PCR

7/16/15

- Re-PCR E.Cadherin from pCTCON2 using Phusion
    - Aga FW/Cadherin RV.
    - Ran gel. -Failed.
- Set up overnight cultures for CBoo8DB, HWP1, Ecadherin, Mgfp5 in YPD

7/17/15

1) Grow overnight culture of cells in YPD

2) Pellet. Respuspend and grow in S-Raff for 1 hr.

3) Pellet. Resuspend and grow in S-Gal. Dilute to OD = 1.04 in 1ml.

4) Add 3 µl beads. Take timepoints.

PCR

```
All:
25 µl Phusion MM
1 µl Template DNA
2.5 FW
2.5 RV
19 µl H2O

Ecadherin (+Sbf1/tAdh)
    142 RV
    144 FW
    pGEM42
Ecadherin (+Not1/pNH605)
    143 RV
    144 FW
    pGEM42
HWP1 (+Sbf1/tAdh)
    145 RV
    144 FW
    pGEM43
HWP1 (+Not1/pNH605)
    146 RV
```

```
    144 FW
    pGEM43
tENO2
    138 FW
    139 RV
    T64M64

Ran clustering PCRs on Phusion PCR protocol.
Ran tENO2 on on TouchdownPhu protocol.

Gel extracted HWP1 because it had the right bands (~3kb).
Cadherin was smaller than 2.6kb
```

7/20/15

- PCR of tEno2 failed.

  - Used two forward primers. Whoops.
- Finished gel extraction of HWP1 and overhangs.

- Ordered Aga2-LexAOps primers, and new tEno2 primers.

7/21/15

- PCR of tEno2 and LexAOp2 with overhangs done
  - tENO2
    - 138 FW
    - 139 RV
    - T64M64
  - LexAOps
    - 150 FW
    - 154 RV
    - LexAOps+mfa+Hy86E

7/22/15

- PCR products ran off of the gel.
- Redo PCR products from yesterday, same protocol.
- More preliminary testing of clustering genes and beads.

  - 5 μl of beads. 5 ml of culture.
- Gel extracted tEno2! (correct)~45ng/μl

  - TOUCHDOWN PHU (50 μl reaction)
- PCR

- tEno2(+Ste2 Overhangs)

    - FW- 141
    - RV- 139
        - DNA- tEno2 Gel Extraction
- LexAOps (+Aga2 Overhangs)

    - FW- 150
    - RV- 154
    - DNA- tdTomato and LexAOps+GFP

7/23/15

- Left out overhang PCRs overnight without running it (whoops)

    - Added .5 µl of Phusion Polymerase and everything was Gucci
    - Ran on gel
    - Gel extracted tCyc1(+mfa), tEno2(+Ste2), LexAOps (+Aga2)
- More preliminary tetsing of clustering with HA-tagged beads.

1) Grow overnight culture of cells in YPD 2) Pellet. Respuspend and grow in S-Raff for 1 hr. 3) Pellet. Resuspend and grow in S-Gal. Dilute to OD = 1.04 in 1ml. 4) Add 5 µl beads. Take timepoints.

- 50 µl of cells in 1 ml of S-Gal
    - CB008DB
    - Ecadherin
    - Hwp1
    - Mgfp5

7/24/15

- Gibson Assembly for LexAOps-Aga2-HWP1

    - Gibson:

        - Hy83 Plasmid: 1µl
        - LexAOps: .5 µl
        - Aga2-HWP1: 6 µl
        - H2O: .5 µl
        - Gibson MM: 8 µl
    - Negative:

        - Hy83 Plasmid: 1µl
        - H2O: 4 µl
        - Gibson MM: 10µl

*Plated. Left in drawer.

7/27/15

- Colony PCR
    - FW- LexAOps (150)
    - RV- Hwp1 (+Not1) (146)
    - GoTaq MM
- Colony PCR worked for colonies 3-6, 8

    - Made overnight cultures of colonies 3 and 8
- Made overnight cultures of CB008DB, yGEM127

- PCR-ed Aga2-Mgfp5 from pCTCON2

    - FW- 144
    - RV- 162 (+Sbf1/tAdh1) and 163
    - (+Not1/tAdh1)
    - template- pGEM44

Gel Extraction

```
Mgfp5(+Sbf1/tAdh1)   75ng/µl
Mgfp5(+Not1/tAdh1)   85ng/µl
```

7/28/15

Dilute overnight cultures:

- CB008DB:
    - 500 µl of cells
    - 9.5 ml of YPD
- yGEM127:
    - 1 ml of cells
    - 19 ml of YPD (2 tubes)

Miniprep overnight cultures of pNH605+LexAOps+Hwp1(Not1)

Gibson Assembly:

- pNH605+LexAOps+Hwp1(+Sbf1)
    - pNH605 1 µl
    - LexAOps .5 µl
    - HWP1 6.5 µl
- pNH605+LexAOps+Mgfp5(+Not1)
    - pNH605 1 µl

- LexAOps .5 µl
- MGFP5 .2 µl

- pNH605+LexAOps+Mgfp5(+Sbf1)
  - pNH605 1 µl
  - LexAOps .5 µl
  - MGFP5 .2 µl

Pme1 Digest of:

```
   pTEF1(m7)+rtTA:: URA3            pGEM20
        2000ng
   pTEF1(m10)+rtTA:: URA3           pGEM21
        2000ng
   pFig2C+LexADBD:: TRP             pGEM48
        5000ng
   LexAOps+GFP:: LEU                pGEM50
        2000ng

Transform with 1000ng of digested DNA
Boil 8 tubes of salmon sperm
```

Transformations

CB008DB:

- pFig2C+LexADBD BASIC
- pFig2C+LexADBD/ LexAOps+GFP POSITIVE
- LexAOps+GFP POSITIVE

yGEM127:

- pTEF1(m10)+rtTA/ pFig2C+LexADBD ME
- pTEF1(m7)+rtTA/ pFig2C+LexADBD ME
- pTEF1(m10)+rtTA ME
- pTEF1(m7)+rtTA ME
- pFig2C+LexADBD BASIC

7/29/15

```
Sequencing for pNH605+LexAOps+Hwp1(+Not1) Colonies 3 and 8 are correct!
We have colonies on our Gibson plates
    Positive and Negative controls are very few colonies
    Experimental plates are a lot (except for Hwp1)

Colony PCR for Gibsons
    Mgfp5 (+Sbf1)
        FW  150
```

```
        RV  162
    Mgfp5 (+Not1)
        FW  150
        RV  163
    Hwp1 (+Sbf1)
        FW  150
        RV  145

Gel showed correct bands for most of the colonies!
    Liquid Culture of 1 & 5 for Mgfp5s
                      1 & 4 for Hwp1
```

7/30/15

```
No colonies on our yeast plates yet. Wait for tomorrow!
Miniprep our overnight cultures.
    Send to sequencing

20 ul reaction with 5 ul plasmid. 37 incubation.
Digest pGEM49 with Not1
Digest pGEM53 with Sbf1
    for Gibsons tomorrow

Set up overnight cultures of CB008DB and yGEM127 for possible redo transformations
```

7/31/15

```
All sequencing for LexAOps+Mgfp5 worked!
    Gave pGEM54 and pGEM55
m10-rtTA, m7-rtTA had colonies on yeast plates
    Low transformation efficiency: so remake reagents and redo transformation
    Re-transformed m7-rtTa (+pFig2C-LexADBD) and m7-rtTa (+pFig2C-LexADBD)

Pme1 Digest of pGEM20 and pGEM21
    pGEM20= m7+rtTA
    pGEM21 = m10 +rtTA
    5 ul DNA
    1 ul Pme1
    1 ul Cutsmart
    3 ul H20
    ---------------- Use 5 ul to transform in yeast
    37C incubation for 1 hr

Remake LiOAc and TE
```

```
    Outsourced to Erika and Squach

Colony PCR for yeast m10 and m7 (URA integration markers)
    Used 50 ul working stock Zymolase
    FW- 158
    RV- 159

PCR out LexAOps+Mgfp5 (+tEno2)
    FW-157
    RV-162
    pGEM55

PCR out LexAOps+Mgfp5 (+tCyc1)
    FW-155
    RV-162
    pGEM55

PCR out LexAOps+HWP1 (+tEno2)
    FW-157
    RV-145
    pGEM52

PCR out LexAOps+HWP1 (+tCyc1)
    FW-155
    RV- 145
    pGEM52

Gel Extract
    high yields (~80 ng/ul)

PCR cleanup Not1/Sbf1 digest of vector

Gibson Assembly
    pGEM47+ tCyc1 + LexAOps-Hwp1
    pGEM47+ tCyc1 + LexAOps-Mgfp5
    pGEM47 negative
        vector: .5 ul
        tCyc1: .2 ul
        Hwp1: .7 ul
        Mgfp5: .3 ul

    pGEM49+ tEno2 + LexAOps-Hwp1
    pGEM49+ tEno2 + LexAOps-Mgfp5
    pGEM49 negative
        vector: 2 ul
        tEno2: .2 ul
        Hwp1: .6 ul
        Mgfp5: .3 ul
```

```
    pGEM53+ tEno2 + LexAOps-Hwp1
    pGEM53+ tEno2 + LexAOps-Mgfp5
    pGEM53 negative
        vector: 5 ul
        tEno2: .2 ul
        Hwp1: .5 ul
        Mgfp5: .3 ul

    Positive control.

Left gibson transformations in drawer over the weekend

Colony PCR for m10 and m7 -rtTA worked!
```

8/3/15

```
Gave yGEM129 and yGEM130 to m7/m10-rtTA in yGEM127
    Streak out on YPD
1:20 dilution of yGEM129/130 to transform in pFig2C-LexADBD
    Pme1 Digest pFig2C-LexADBD
Transform pFig2C-LexADBD in Trp for yGEM129/130

Set up colony PCR for our Gibson transformation overnight
    MM (alpha+Mgfp5):
        FW 131
        RV 162
    MH (alpha+Hwp1):
        FW 131
        RV 145
    SM/MSM
        FW 133
        RV 162
    SH/MSH
        FW 133
        RV 145
    Used Phusion MM instead of GoTaq because they're super big.
Overnight cultures for flow cytometer tomprrow!
    yGEM34, yGEM128, CB008DB
```

8/4/15

```
Ran gel for colony PCR of Gibsons!
    Everything had bands except for MSM/MSH
```

```
    Overnight culture for miniprep tomorrow
Overnight culture of yGEM129/130 for glycerol stock tomorrow
```

Flow Cytometry:

```
Dilute all cultures to .1 OD in shaker plate (1 ml SD)
Grow for 3 hrs.
Induce alpha factor (10ul): 0, .5, 1, 10, 100, 1000, 3000 ng/ml
    (Use serial dilutions and learn how to use a repeater pipette)
3 hrs. Transfer to 96 well plate in triplicate.
Flow data came out eh... no cells?
```

8/5/15

```
Miniprepped overnight culture of positive Gibson Colonies (5 total)
    MM 1
    MM 2
    MH 1
    SM 1
    SH 1

Sent one of each to sequencing
    MM/MH sent with reverse primer and alpha forward
    SM/SH send with reverse primer
        send premixed with tEno2 forward

(All kept in the salmon sperm box in different colored tubes)
Aliquoted 10ul of 10 mM stock alpha factor -YELLOW
Diluted Dox 50mg/ml in 5 ml Ethanol, 15 ml Water
    Aliquots of 130 ul -CLEAR
Aliquoted 15ul of more salmon sperm -PCR CLEAR

Glycerol stock of yGEM129/130:
    use 420ul 50% glycerol, 350ul cells.

Set up overnight cultures for flow tomorrow
```

8/6/15

```
Sequencing came back for Mgfps! (Ste2-Hwp1 was actually Mgfp)
    mFa-Mgfp5 = pGEM56
    Ste2-Mgfp5 = pGEM57
    WE GIVE UP ON HWP1. FOCUS ONLY ON MGFP5
```

```
Flow cytometry:
9:30 dilute to OD .3
1:00 induce
2:15 cyclohex
2:45 take alpha samples
3:00 flow
3:20 take dox samples

    Alpha induction of
        CB008DB
        yGEM34
        yGEM128 (m10-Bar1)
        yGEM132 (pBar1-GFP)
        34+128 mixed
    [Alpha] = 0, .5, 1, 10, 100, 1000 nM

    Dox induction of
        CB008DB
        yGEM129
        yGEM130
    [Dox] = 0, .03, .06, .09, .3, .6, .9, 3, 6, 9 ug/ul

Modeling and learning about nondimensional quantities and finding steady states
```

Basic Circuit

```
Digest pNH604 with Apa1/Not1 for Gibson
    10 ul stcok pNH604
    2 ul cutsmart
    .5 ul each enzyme
    Room temp for an hour, then add Not1 and incubate 37C
PCR out pFig2C from LexADBD
    Template: pGEM48/tdTomato
    FW- 151
    RV- 152
    Phusion MM
    1.5 ul DMSO to each
    Ran overnight
```

8/7/15

```
Yeast Colony PCR of LexAOps-Mgfp5 and pFig2C-LexADBD in m7/m10
    LEU:
        FW- 160
        RV- 87
```

```
    TRP:
        FW- 161
        RV- 87

Mgfp5 worked (colonies 1 and 3), but LexADBD didn't
```

Basic Circuit

```
PCR cleanup of pNH604 Digestion
    ~10 ng/ul
Gel extract pFig2C-LexADBD (tdTomato worked)
    ~18 ng/ul
Gibson Assembly:
    pNH604- 5 ul
    Insert- 4 ul
    Gibson- 9 ul
Seamless Assembly
    pNH604- 5ul
    Insert- 4 ul
    Seamless- 9 ul
Made more pGEM34
```

8/9/15

```
Yeast Transform CB008DB, 127, 129, 130, 131
    with pGEM48 (S1, S2, S3, G1) from Eleanor's colony PCR's on Saturday
    20 total yeasties
Miniprepped overnight cultures of S1, S2, S3, G1
Made streak plate of yGEM133 (LexAOps-Mgfp5)
Yeast Colony PCR of Erika's things:
    CB008DB: pGEM34
    yGEM128: pGEM34
    Trp Locus: so 160 FW, 87 RV (With DMSO and W/o DMSO)
Started overnight cultures of:
    CB008DB
    127
    129
    130
    131
    133
```

8/10/15

```
Made new PEG
Sent in S1, S2, S3, G1 for sequencing
Glycerol stocked LexAOps-Mgfp5
```

8/11/14

```
Sbf1 Digest of pGEM53
PCR purify ~15 ng/ul
Gibson Assembly
    Mfalpha-tCyc1-Ste2-(tEno2-Mgfp5)
    Negative control
    Positive control
We have correct sequencing for all our S1, S2, S3, G1 pGEM48!
We have possible colonies on all our yeast transformations
Started overnight cultures of yGEM133 to transform pGEM48 into!
Photoshoot tomorrow!
```

8/12/15

```
Colony PCR of gibson assembly- Mfa-Ste2-Mgfp5. Colony 8 had correct band!
    Overnight culture for miniprep and sequencing tomorrow
Yeast Colony PCR of:
    CB008DB
    yGEM127
    yGEM129
    yGEM130
    for TRP locus for pFig2C-LexADBD using 160 FW and 87 RV.
Outsourced transformation of pGEM48 into LexAOps-Mgfp5.
```

8/13/15

```
Out Today!
```

To Do:

```
Transform positive feedback strains into basic circuit.
Dilute overnight cultures for flow at 6:00
    OD .015 in 1.5 ml plates
    CB008DB
    yGEM141
    yGEM142
```

```
10 Dox Concentrations.
3 strains in triplicate.
Induce at 9:00.
```

8/14/15

```
Diluted CB008DB, yGEM141, yGEM142 at 7:00
Induced with Dox at 10:00
Made 1:20 dilutions of yGEM141, yGEM142 for positive feedback transformations today.
2000 ng Pme1 Digest of
    pGEM47
    pGEM49
    pGEM62
(Transform positive feedback into basic circuit)
```

8/17/15

```
Colony PCR of Positive Feedback strains (mfa, Ste2, and mfa/Ste2/Mgfp5)
    At least positive colony for each! (yGEM149-154)
Started overnight cultures for glycerol stock, but mixed labels so eh.
```

8/18/15

```
Diluted yGEM148 (Mgfp5 with TF) in SD-Comp to OD=1
4 different controls after 2 hr alpha induction
    148
    148+alpha
    148+beads
    148+alpha+beads
Transformed pGEM62 for more minipreps
Modeling! Yay! Modeled a two cell circuit system.
Started overnight cultures of like everything so we can:
    Transform in Bar1
    Test clustering with alpha induction

It looks like there is leaky expression of a promoter, clustering at 0 induction
    But bigger clusters with induction
```

8/19/15

```
Transformed m7-Bar1 into 147,153,154,155
More clusrering testing
Start overnight culture of pGEM62
```

8/20/15

```
Miniprep pGEM62
```

8/21/15

```
Just worked on the poster. Yay.
```