Jeffrey's Lab Notebook

5/26/15

Eleanor and I started early to set up for bootcamp!

```
- Cleaned lab space
- Bleached and disposed of nasty 2014 project leftovers
- Streaked e-cadherin and CB008DB strains on YPD. (For base-strain and aggregating factor).
```

5/27/15

Did nothing really, cleaned benches; made new agarose gels, TE buffer, LiOAc.

```
Project Notes:
- Continuation of Sense and Secrete-ability
- Focus on divergence
- Use different transcription factor to amplify alpha factor response
- Create positive/negative feedback loops
- Add a separate Bar1 strain
- Combine selection sites to consolidate space
- Microscopy and Flow Cytometry!
```

To do:

```
[] Consolidation of pTET-mfalpha and pTET-GFP
- Design primers for terminator sequence
- PCR out pTET-mfalpha
- Gibson the parts together
- Transform into E. coli
- Colony PCR/ Liquid Culture
- Miniprep plasmids
- Transform into yeast
- Colony PCR/ Streak

[] Presentation of last year's project for new team members
```

5/28/15

Didn't come into lab/ Waiting for primers.

5/29/15

```
Cadherin on YPD grew
Restreak CB008DB on YPD after thawing/vortexing the glycerol stock

PCR pTET-mfalpha (A)
    tFBA1 (B)

1. Diluted stock primers (1ng x 10ul)
2. Then working stock (10ul x 90ul)
    Kept in Jeffrey's Freezer Box
(A) DNA- pTET-mfalpha
    FW- pTET (+tFBA1)
    RV- mfalpha (+tSBF1 +tAdh1)
    (lower annealing temp b/c melting temp was too low due to AT richness)

(B) DNA- tFBA1 (from Russell)
    FW- tFBA1 (+GFP)
    RV- tFBA1 (+pTET)
    (Standard PCR cycle)

Transform DH5a competent cells with HY6E1 (backbone strain w/ pTET-GFP)for miniprep

Attempted Gel extraction, but tFBA1 had a band that was super big! (not 400bp)
```

To Do:

```
- Start liquid cultures of DH5a, CB008DB, e-cadherin in LB+Carb and YPD respectively.
- PCR tFBA1 with new protocol
- Gel extract tFBA1 and pTET-mfa
- Miniprep/Plasmid prep liquid cultures
- Gibson tFBA1/mfa/GFP
- Presentation
```

6/1/15

```
Colonies on all plates! But possible contamination since there was an unusual white spot.

Re-PCRed tFBA1 in 4 different tubes:
    Phusion w/ DMSO
    Phusion w/o DMSO
    GoTaq w/ DMSO
```

```
    GoTaq w/o DMSO

Used "Touchdown PCR" where 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
Made 10x TE Buffer
Yeast DNA extraction of Cadherin
Miniprep HY6E1

Sequencing sent in for cadherin
10 ul Not1 digest of HY6E1 overnight 37degrees

To do:
PCR cleanup HY6E1
Gibson Assembly
Transform into E. Coli.
```

6/3/15

```
.5 ul Antartic Phosphotase in Not1 digest of HY6E1 (37 for 1 hr)
PCR purify HY6E1 digest. LOW CONCENTRATION (MAY NOT WORK)

Gibson Assembly:
- Positive: 5 ul +, 5 ul Gibson Mix
- Negative: 4.25 ul HY6E1, .75 ul water, 5 ul Gibson Mix
- Gibson: 4.25 ul HY6E1,.5 ul mfalpha, .2 ul tFBA1, 5 ul 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 ul Not1 digest of HY6E1.
```

To Do:

```
Phosphotase and PCR purify HY6E1.
(Gibson HY6E1 again.)
(Transform E.Coli with Gibson product.)
```

Colony PCR of Wednesday's Gibson.
Re-Yeast DNA extraction of cadherin cultures.

6/4/15

Dephosphorylate Not1 digest
PCR purify Not1 digest

We have colonies on Wednesday Gibsons!
    (Positive had few colonies, Negative had 1-2 colonies, Gibson product had 20+ colonies).

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

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

Redo Colony PCR w/ tFBA1 primers (overnight)

To Do

Liquid culture of pGEM39 for miniprep
Redo Gibson with new digestion.
Run overnight colony PCR gel

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 ul +, 5 ul Gibson Mix
- Negative: 2 ul HY6E1, 3 ul water, 5 ul Gibson Mix
- Gibson: 2 ul HY6E1, 1 ul mfalpha, .25 ul tFBA1, 1.75 H20, 5 ul Gibson Mix
50 for 1 hr, keep everything on ice!

Transform Gibson Mix in C2987.
Transform Cadherin (SD-Trp) in C2987.
    5 ul DNA. 25 ul cells.

To Do

```
Miniprep pGEM39
Colony PCR of redone Gibson/cadherin
Liquid culture of cadherin (if no primers)
```

6/8/15

```
First day of Bootcamp!
Checked transformation/gibson colonies- too small to colony PCR.
    Let grow at 37 for another night.
Miniprepped pGEM39 (but sequencing shows it is just PJW608)
    Kept in Jeffrey's Freezer Box

Partner: Hunter
    PCRed pTEF1(m7) and Bar1 for cloning later.
```

To Do

```
Colony PCR of Gibsons/cadherin
Apa1/Xho1 Digestion of PCR products
Presentation for Thursday
```

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. (should be in Erika's box)

Seamless Assembly:
- Positive: 2.5 ul +, 2.5 ul Seamless Mix
- Negative: 1.5 ul HY6E1, 3.5 ul water, 5 ul Gibson Mix
- Gibson: 1.5 ul HY6E1, 2.5 ul mfalpha, 1 ul tFBA1, 5 ul Seamless Mix
Room Temperature incubation for 20 min.
Put on ice for 3 min.

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

To Do

```
Miniprep cadherin cultures and send 2 for sequencing
Colony PCR of Seamless transformations
```

6/10/15

```
Checked 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.
    SUCCESS! 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
For Bootcamp: Did gel extractions/ligation/transformation
    pTEF1 (m7)+ Bar1 + PJW608
```

To Do

```
Check sequencing for cadherin and pTEFs (unknown)
Miniprep colonies of Gibsons.
```

6/11/15

```
Colony pCR of ligations with Hunter. pTEF1(m7)-Bar1.
Cadherin sequencing came out correct! (yay!)
    Kept in Jeffrey's freezer box (#5)
Miniprep correct Seamless colony PCRs from yesterday.
    Send to sequencing 1,2,8. Kept questionable 5,6,7 liquid cultures in 4degree freeer.
Presentation of 2014 project.
Start overnight CB008DB yeast cultures for yeast transformations tomorrow.
```

To Do

```
Check sequencing for pTEFs (m3) and Seamless Minipreps.
1:20 yeast dilutions for yeast transformations
Transform yeast with HY6E1+tFBA1+pTET-mfa
NASA field trip.
```

6/12/15

```
1:20 Dilution of CB008DB for transformations
Sequencing correct for 1 & 2.
    8 sequencing reaction failed
Pme1 Digests of 1 & 2 Minipreps.
    4000 ng. 50 ul digestion.
Yeast Transformation- HY6E1+tFBA1+pTET-mfalpha (1 & 2)
    kept at 30degree incubator
Pelleted Cadherin Yeast for PCR of pBar1 in Jeffrey's Freezer Box
```

To Do

```
PCR pBar1 from Cadherin Strain
Yeast Colony PCR of transformations
Group Meeting Presentations
```

6/15/15

Group Meeting

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 ul reaction, then Colony PCR w/ GoTaq Master Mix
PCR pBar1 from Ecadherin pellet.
    Use Primers #119/120
    ERIKA AND JOSH WILL DO THIS. NOT BEGUN.
Logged HY6E1+tFBA1+pTET-mfalpha as pGEM41
Multiple gels of Colony PCR- weird bands. Run a lot of gels.
```

To Do

```
Run more gels
Possibly more colony PCRS.
```

6/17/15

```
Ran new 2% gel for colony PCR - UGLY AND STREAKY
Redid Colony PCR. Everything successful.
    Changing Buffer in gel chamber is important
```

6/18/15

```
Streak pGEM41 #2.3 on YPD.
Make overnight cultures for glycerol stocks tomorrow.

Log pGEM42 for Cadherin.
```

Clustering Idea:

```
Clustering proteins are in plasmids in yeast, not integrated in chromosome.
Try the mussel protein, fungal protein, and cadherin in CB008DB.
Add galactose. See which induces most effective clustering.
Then proceed with project from there.
PROBLEM WITH CADHERIN: No primers for sequencing. Weird translation because stop codon in sequence.
```

Hyun's Paper

```
Our project is different.
Although we are both trying to achieve bimodal response in sense and secret circuits,
Our project is able to read out the "individual" (BEFORE) and "community" (AFTER).
Hyun's only reads out community response.

We are providing a model for understanding community behaviors.
```

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.
```

To Do

```
Miniprep clustering proteins.
Transform clustering genes into CB008DB
```

6/23/15

```
Miniprep HWP1, MGFP
Transform HWP1, MGFP, Ecadherin into CB008DB
     Plated on SD-TRP
Made new PEG 3350
Send to sequencing CLG-Bar1 in PJW608 (2 and 3), HWP1, MGFP, Ecadherin
```

To Do

```
Research clustering experiments and quantitative analysis
Finish paper for journal club
Find sequences for clustering proteins
Finish researching FRET method
```

6/24/15

```
No wetlab today.
Researched clustering proteins
```

Goals

```
1. Find functional clustering gene from 2011
2. Develop/find quantitative data analysis
3. Research non-specific clustering problems
4. Design microscopy experiments.
5. Transfer clustering gene (probably MGP5)
```

HWP1

```
- Hyphal wall protein
- Comes from opportunistic C. albicans
- Natural function is to bind to oral epithelial cells
```

- Should be hyphal specific
- 2011 used homologous recombination instead of pCTCON for this
- May induce random clustering (nonspecific)
- 2.6 kb

MGFP5

- Mytilus galloprovincialis foot protein type-5
- Naturally secreted as a glue to help mussel feet stick to rocks
- Essentially a "plaque" protein
- Type 5 better than Type 1 b/c has high amino acid bias
- Studies show Mgfp5 may be toxic to E. coli (?)
    Proteolytic degradation. Slowed growth.
- BioBrick from Chris Anderson (2009 Berkeley iGEM)
- Clustering should increase with salt concentration
- Best clustering according to Veronica
- 200 bp

Ecadherin

- Epithelial calcium dependent adhesion protein
- Should only cluster specific subtypes
- Mixing speed can affect specificity
- Ca ion dependent
- Clustering reversible with EDTA
- Has a bunch of stop codons (not functional gene)
- 1.7 kb

Data Analysis

- CellProfiler
- ImageJ
    Stack pictures. Time lapsed. Analyze cell area?
- Matlab Image Processing
- Statistical Analysis
- Use plate reader to correlate cloudiness w/ clustering

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
```

Primer Design

```
Designed different primers for feedback combinations.
Combined different pFig2C-gene-terminator in ApE.
```

To Do

```
Induce all clustering strains in 1% S-Gal and visually observe clustering
    Check different timepoints while in liquid culture.
    May have to induce MGFP5 with salt/ Ecadherin with Calcium
Check out image processing software and develop experiments.
Optimize primers for melting temperatures
```

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 ul 1M NaCl
    HWP1- 5 ml S-Gal
    Ecadherin- 5 ml S-Gal
            4.95 ml S-Gal, 50 ul .1M CaCl2

(We were supposed to grow in YPD and transfer to S-Raff then S-Gal. Whoops)
```

Observe at different time points

```
Not dense enough to tell any difference in clustering
```

6/29/15

```
Brainstormed wiki themes and graphic ideas.
Started overnight cultures of HWP1, MGFP5, Ecadherin in YPD
```

6/30/15

```
Moved microscope from Nanodrop room to lab bench. (It's ours!)
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
                                  0 hrs       2 hrs       4 hrs    24 hrs
MGFP5 (NaCl):
    0 mM:   .5 ml cells           none        none        none     clumped
            4.5 ml S-Gal
    100 mM: .5 ml cells           none        none        none     clumped
            .5 ml (1M)NaCl
            4 ml S-Gal
    200 mM: .5 ml cells           none        none        none     clumped
            1 ml (1M) NaCl
            3.5 ml S-Gal

ECadherin (CaCl2):
    0 mM:   .5 ml cells           none        none        none     clumped
            4.5 ml S-Gal
    1 mM:   .5 cells              none        SOME        SOME     clumped
            .05 ml CaCl2                      (large)     (some)
            4.45 ml S-Gal

HWP1:
    0 mM:   .5 ml cells           none        none        none     clumped
            4.5 ml S-Gal



Give up on HWP1.
Try higher salt concentrations for Mgfp5.
Somehow cadherin is working relatively well.
Try a 2mM concentration of calcium.
Try longer timepoints to see clustering.

Start overnight cultures in YPD to test again tomorrow.
```

7/1/15

```
Did more preliminary testing
```

Compiled all microscopy photos into a photo grid on a pdf.
Turns out, all cells tend to clump at high cell densities (24 hrs).
Same protocol as before.
Made stock solution of 50 ml (5M) NaCl in H20.

Pellet overnight cultures.
Transfer to 1% S-Raffinose for 1 hr.
Pellet.
Transfer cells to 1% S-Gal. Spilled hal of Ecadherin, so doubled cells.
This time, checked hourly.

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
            4.4 ml S-Gal

7/2/15

Discovered that 2% Galactose media is needed to induce the promoter.

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

We tried to see how clustering would appear in our timeframe (0-8 hrs) of induction)
We also wanted to test it under Hyun's "low" and "high" cell densities.

LOW=.001 OD
HIGH= .1 OD

Pellet overnight cultures.
Transfer to 1% S-Raffinose for 1 hr.
Pellet.
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 ul cells
                5 ml S-Gal

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

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

ECadherin (CaCl2):
    LD
        0 mM: .5 ul cells
                5 ml S-Gal
        2 mM: .5 ul cells
                .1 ml CaCl2
                4.9 ml S-Gal
    HD
        0 mM: 50 ul cells
                5 ml S-Gal
        2 mM: 50 ul cells
                .1 ml CaCl2
                4.9 ml S-Gal

HWP1:
    LD
        0 mM: .67 ul cells
                5 ml S-Gal
    HD
        0 mM: 67 ul cells

```
                4.9 ml S-Gal

We checked every 2 hours from 0 hours to 8 hours.

Discovered that low density cells hardly grow, let alone cluster.
High density cells tend to cluster relatively well past 6 hours.
    It is possible that Mgfp5 clusters better without salt (?)
    Calcium definitely helps clustering for ecadherin, however. (In HD)

8 hrs (10PM #lolatlife)
    Mgfp5 had no clustering at low density. High density had small-medium clusters.

    Cadherin had all cells make small clusters at low density, calcium.
        HD had small in both calcium/non-calcium.

    HWP1 had small clumps in LD and giant clumps in HD.
```

7/3/15

```
Came in to miniprep Nick's HyX 2nd. (1,2,7,8)
    All had high concentrations. Stored in Nick's freezer box
Checked 24 hour timepoint for clustering. Everything clusters.
```

7/7/15

```
Compiled images from preliminary experiments.
Set up overnight cultures for more testing.
    Gave up on Mgfp5.
    Testing to see mechanism of clustering and whether natural clumping occurs.
Designed more primers for feedback combos, OK-ed by Kara.
```

To Do

```
PCR out terminators and clustering genes
Do more microscopy experiments with mixed strains.
Find more design ideas for the website.
```

7/8/15

```
Helped other people clone and such.
```

```
Ran mixed clustering experiment for random vs specific clustering.
Did some P&P video things.

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

```
Finished up the mixed clustering experiment.
Help Erika transform pTEF1(m10)+Bar1 into CB008DB.
PCRed tCyc1 and tEno2 from T64M64
    Used TouchdownPhu protocol and Phusion 2XMM in 50 ul reaction.
    Used primers 136,137,138,139.
Transformed T64M64 into E. Coli for miniprep tomorrow.

Ran gel for terminator PCR:
    tCyc seems to be correct. tEno2 is wrong.
```

7/10/15

```
Gel extracted tCyc1 ~20ng/ul
PCR tEno2 from T64M64 and T64tdTomato using 138/139 primers.
    Used TouchdownPhu protocol.
Ran gel for tEno2 PCR.
    Failed. Had a bright band at >1kb.
PCR Gibson overhangs for tCyc1
```

7/13/15

Project Ideas from Wendell

```
Integrate Bar1 into our basic circuit
Make cells cluster to beads instead of to each other (HA tag)
```

To Do

```
Make style sheet for wiki ideas
    Find font
    Find color scheme
    Play with logo
PCR out tEno2 from yeast genomic DNA
PCR out E.Cadherin/HWP1 from pCTCON2
Research cell clustering with beads
```

7/14/15

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

Style Sheet:

```
Dark background (black with dark grey)
Some pages of white contrast
Light/white font
Muted pastels as accent colors
Loud neon for intense accent
Simple geometric shapes to attract the eye.
```

Things found out in various papers:

```
Bar1:
    Transmembrane/ periplasmic protease
    Aspartyl protease with homology to pepsin
    Receptive to alpha factor (5x more expression than basal level)
    Helps a cells recover from mating cell arrest
    Sharpens the concentration gradient to help cells find soulmates
    Allows a cells to shmoo toward a single alpha cell
    Alpha factor specific.
    Lyses based on the primary/secondary structure of alpha factor
    Cleaves alpha factor between 6-Leu/7-Lys
    Testing can be done using protein assays on Bar1 or alpha factor

Ste2:
    Part of a protein complex.
```

```
    Alpha factor receptor.
    May be downregulated by alpha factor, but resynthesizes in time.
```

7/15/15

Parts Meeting

```
Parts:
    1) Submit a part
        -terminators
    2) "Validate/characterize" a part
        -Bar1, pBar1
    3) Improve an existing part
        -Ste2
            -alpha factor binding
            -localization
            -yeast w/o Ste2
        -Clustering
            -more timepoints

To Do:
    1) Clone pBar1-GFP construct
    2) Order gene fragments (Bar1, terminators)
    3) Clone all into pSB1C3/ design primers
    4) Find Ste2 reagents
```

Wetlab things

```
Ran gel for tEno2/E.Cadherin PCR
    FAILED.
Re-PCR tEno2 w/o DMSO from T64M64
    Used 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 Phusions
    Aga FW/Cadherin RV.
Ran gel. -Failed.
Set up overnight cultures for CBoo8DB, HWP1, Ecadherin, Mgfp5 in YPD
    GETING BEADS!!
```

7/17/15

> Bead adhesion assay. Adhesion of protein-coated beads to cells was assayed by a modification of the method of Gaur et al. (14). Overnight cultures of yeast strains to be combined with the coated beads were diluted to 2 × 108 cells/ml, and 500-μl aliquots were pelleted and washed twice with an equivalent volume of deflocculation buffer. Washed cells were then resuspended in deflocculation buffer containing 20 mM calcium, to promote adhesion (2), combined with 2.5 μl of coated beads, or 1 × 106 beads, in a total volume of 1 ml. Reaction mixes were vortexed vigorously, and wet mounts were prepared on glass slides for immediate microscopic viewing. Adhesion of yeast cells to beads was assessed by counting beads, utilizing a light microscope with a 40× objective (Leica Microsystems, Inc., Allendale, NJ), and scoring them as belonging to one of two categories, i.e., beads bound to yeast cells and beads not bound to yeast cells. Values for each category were calculated as percentages of the total number of beads counted with respect to that category. To assay adhesion of Σ1278b cells, the cells were first incubated in SC medium plus 0.1% glucose because this strain requires glucose starvation for maximum Flo11-dependent adhesion (36).
>
> To assess the ability of d-mannose to affect the binding of Flo11p-coated beads to yeast cells, the adhesion assay was modified so that 1 × 108 S. cerevisiae var. diastaticus YIY345 cells in 1 ml were placed in a microcentrifuge tube with 2.5 μl beads coated with column-purified Flo11p derived from Saccharomyces cerevisiae var. diastaticus. Beads and cells were pelleted and resuspended in 1 ml deflocculation buffer, vortexed, and centrifuged for 5 min at 3,000 × g. Buffer was pipetted off, and the procedure was repeated once. d-Mannose at a concentration of 1 M in deflocculation buffer was then added to produce a total volume of 1 ml, and the washed beads and cells were incubated in the sugar for 5 min at 30°C with shaking. Calcium was then added to 20 mM, the tube was vortexed vigorously, and a wet mount was prepared.
>
> "Expression and Characterization of the Flocculin Flo11/Muc1, a Saccharomyces cerevisiae Mannoprotein with Homotypic Properties of Adhesion" (Douglas 2007)

Magnetic bead adhesion assay:

```
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 ul beads. Take timepoints.
```

PCR

```
All:
25 ul Phusion MM
1 ul Template DNA
2.5 FW
2.5 RV
19 ul 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 should be around ~2.6kb, but it was way smaller.
```

7/20/15

```
PCR of tEno2 failed.
    DISCOVERED WE WERE USING 2 FW PRIMERS
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
To Do: Run PCRs on gel.

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 ul of beads. 5 ml of culture.

Went to Med School Workshop.

Gel extracted tEno2! (IT'S CORRECT!)~45ng/ul

TOUCHDOWN PHU (50 ul 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 ul of Phusion Polymerase and everything was Gucci
Ran on gel, YAY! Bands for everything.
Gel extracted tCyc1(+mfa), tEno2(+Ste2), LexAOps (+Aga2)
    Good concentrations (~30-80 ng/ul)

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 ul beads. Take timepoints.

50 ul of cells in 1 ml of S-Gal

CB008DB

```
Ecadherin
Hwp1
Mgfp5

(Also yay for differential equations and modeling workshop)
```

7/24/15

```
Made new Agarose
Made new TAE Buffer
Replaced TAE in Gel boxes
Made new Buffer PE
    Add 200 Proof Ethanol to Packaged PE Buffer
Made new Buffer P1
    Add 200 ul RNAase to 20 ml of Buffer P1

Checked 24 hour timepoint for clustering
    HWP1 had baby clusters
    Mgfp5 had Josh-sized clusters
    Everything else was meh.


Gibson Assembly for LexAOps-Aga2-HWP1
Gibson:
    Hy83 Plasmid: 1ul
    LexAOps: .5 ul
    Aga2-HWP1: 6 ul
    H2O: .5 ul
    Gibson MM: 8 ul

Negative:
    Hy83 Plasmid: 1ul
    H2O: 4 ul
    Gibson MM: 10ul

Plated. Left in drawer.
```

7/27/15

```
Transformation of Gibson plates had hella colonies!
    Negative control had ~2 colonies
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
Cleaned out the fridge!
    (EW)
Made overnight cultures of CB008DB, yGEM127 for tomorrow's transformation.

PCR-ed Aga2-Mgfp5 from pCTCON2
    FW- 144
    RV- 162 (+Sbf1/tAdh1) and 163 (+Not1/tAdh1)
    template- pGEM44

GEL HAD BANDS! (Gel extraction!)
    Mgfp5(+Sbf1/tAdh1)   75ng/ul
    Mgfp5(+Not1/tAdh1)   85ng/ul
```

7/28/15

```
Dilute overnight cultures:
    CB008DB: 500 ul 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)
    Sent to Sequencing

Gibson Assembly:
    pNH605+LexAOps+Hwp1(+Sbf1)
        pNH605      1 ul
        LexAOps     .5 ul
        HWP1        6.5 ul
    pNH605+LexAOps+Mgfp5(+Not1)
        pNH605      1 ul
        LexAOps     .5 ul
        MGFP5       .2 ul
    pNH605+LexAOps+Mgfp5(+Sbf1)
        pNH605      1 ul
        LexAOps     .5 ul
        MGFP5       .2 ul

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.
```