6/8/15

Lectures:

1. Molecular Biology & Cloning Crash Course
2. Synthetic Biology

6/9/15

PCR and Gel for Promoter and Bar1:

- PCR:
  - 2 tubes:
    - P = Promoter
    - B = Bar1

Promoter PCR Mix: Refer to **PCR for Constitutive Promoters** below

Bar1 PCR Mix: Refer to **PCR for Constitutive Promoters** below

Observations:

- One PCR tube holding Promoter PCR Mix cracked, transfered to another tube
- All else went according to procedure

- Gel:

  - Take 5ul Promoter Mix and 5ul Bar1 mix
  - Add 1ul Gel Loading Dye Purple 6x
  - Loaded onto gel

Observations:

- Promoter Mix completely loaded
- Bar1 Mix: may have lost some in gel, may need someone elses Bar1 mix?
- 2nd ladder leaked into someone elses well

PCR for Constitutive Promoters

```
Promoter PCR Reaction:
    - Your forward primer (10uM)***     2.5ul
    - Your reverse primer (10uM)***     2.5ul
    - Template DNA                      0.5ul
    - 2x Phusion MM                      25ul
    - H20                              19.5ul
```

```
        - TOTAL:                          50ul


Bar1 PCR Reaction:
    - Primer #92 (10uM)***              2.5ul
    - Primer #93 (10uM)***              2.5ul
    - Bar1 Template DNA                   1ul
    - 2x Phusion MM                      19ul

        - TOTAL:                          50ul

*** Your stock primers are 100uM. You must make a tube of
 diluted primer to use for your cloning!***

1. Mix the above reaction sin PCR tubes on ice. Make sure to mix
    well since the enzyme is viscous and sinks to the bottom.

2. Put in the thermocycler for the following cycle:
```

> Initial Denaturation: 98^o , 30s
>
> > 35 cycles of
> >
> > > Denaturation: 98^o , 10s
> > >
> > > Annealing: 55^o , 20s
> > >
> > > Extension: 72^o , 1m
> >
> > Final Extension: 72^o , 5m
>
> Hold: 4^o , Forever

```
3. Keep samples for gel extraction on the next day.
```

Enzyme Digestion

-pTEF1 Digest

```
-Added 5.0uL CutSmart Buffer
-Added 0.5uL ApaI. Vortexed
-Incubated at room temperature for at least 1 hour
-Added 0.5uL XhoI. Vortexed
-Incubated at 37'C for at least 1 hour
```

-Bar1 Digest

```
-Added 5.0uL CutSmart Buffer
```

```
-Added 0.5uL XhoI and 0.5uL NotI. Vortexed
-Incubated at 37'C for at least 1 hour
```

-pJW608 Digest

```
-Transferred 10.0uL of plasmid from stock into a new tube
-Added 3.0uL CutSmart Buffer and 16uL water
-Added 0.5uL ApaI. Vortexed
-Incubated at room temperature for at least 1 hour
-Added 0.5uL NotI. Vortexed
-Incubated at 37'C for at least
```

6/10/15

Lectures:

- Primer on Flow Cytometry

Enzyme Digestion for Bar1, pJW608, pTEF1:

1. Added 10.0uL of Purple Loading Dye (6x) to pTEF1 and Bar1 Digest Reactions
2. Added 6uL Purple Loading Dye (6x) to pJW608 Digest Reaction
3. Transferred 60uL of pTEF1 and Bar1 Digest Reactions to gel
4. Transferred 36uL of pJW608 Digest Reaction

Gel Extraction of Digestion:

1. Excised gel bands 2 and 3 and transferred to 1.5mL tube
2. Excised gel bands 4 and 5 and transferred to 1.5mL tube
3. Excised gel band 6 and transferred to 1.5mL tube
4. Weighted each tube
    - pTEF1 Tube: 201.9mg
    - Bar1 Tube: 355.1mg
    - pJW608 Tube: 198.1mg
5. Added Buffer QG in a 3:1 ratio (100mg ~ 100uL)
    - pTEF1 Tube: +600uL
    - Bar1 Tube: +1050uL
    - pJW608 Tube: +600uL
6. Incubated at 50'C for 10 minutes
7. Added isopropanol in a 1:1 ratio
    - pTEF1 Tube: +200uL
    - Bar1 Tube: +355uL
    - pJW608 Tube: +198uL
8. Transferred each sample to a QIAquick column in a 2mL collection tube

9. Centrifuged for 1 minute
10. Discarded flow-through, returned column to collection tube
11. Added 0.5mL of Buffer QG to column and centrifuged for 1 minute
12. Discarded flow-through, returned column to collection tube
13. Added 0.75mL of Buffer PE to column and centrifuged for 1 minute
14. Discarded flow-through, returned column to collection tube
15. Centrifuged column empty for 1 minute
16. Transferred column to new 1.5mL tube
17. Added 50uL of water to the column to elute DNA
18. Centrifuged for 1 minute

pTEF1, Bar1, and pJW608 Nanodrop:

- Used Nanodrop to measure 1.5uL of each sample for concentration
    - pTEF1: -XXXng/uL
    - Bar1: -XXXng/uL
    - pJW608: 25.0ng/uL
        - ERROR
            - pTEF1 and Bar1 were reported with negative concentrations of ng/uL
            - Possibly due to residual ethanol from Buffer PE

pTEF1, Bar1, and pJW608 Ligation:

1. Since Bar1 was reported with a negative concentration, we used Erik Lamp's Sample (15.44ng/uL) as a substitution
2. Added the following to a 1.5mL tube:
    - 10x T4 DNA Ligase Buffer: 2.0uL
    - pJW608 DNA (50ng): 2.0uL
    - Bar1 DNA (75ng): 5.0uL
    - pTEF1 DNA (???): 10.0uL
    - Nuclease-Free Water: 0.0uL
    - T4DNA Ligase: 1.0uL Total: 20.0uL
    - Mixed reaction by pipetting up and down
    - Incubated at room temperature for 1 hour

pTEF1, Bar1, and pJW608 Transformation:

1. Transferred 25uL of NEB 5-alpha Competent E. coli cells into a 1.5mL tube. Placed on ice
2. Added 5uL of ligased pJW608 to tube. Flicked tube 5 times to mix
3. Placed mixture on ice for 30 minutes
4. Heat Shocked at 42'C for 30 seconds
5. Placed on ice for 5 minutes
6. Added 950uL of SOC into mixture
7. Incubated at 37'C for 60 minutes. Shaken at 250rpm
8. Warmed two plates (LB + Carb) to 37'C

9. Mixed cells by inverting
10. Spread 100uL cells onto one plate
11. Centrifuged remaining mixture to concentrate cells
12. Discarded excess liquid and resuspended cells
13. Spread 100uL (8x) cells onto second plate
14. Incubated both plates overnight at 37'C

6/11/15

Lectures:

- Working with Bacteria and Yeast
- Introduction to Immunology
- UCSF iGEM 2014 - Sense and Sense-ability

pTEF1, Bar1, pJW608 E.coli Colony PCR

```
1. Added 25uL of water to 6 PCR tubes
2. Transferred a single colony from the plate to each PCR tube
3. Transferred 5uL of each cell/water mixture into a new PCR tube
4. Created a GoTaq Green Master Mix for 7 reactions:
    -2x GoTaq Green PCR Mix:            70.0uL
    -10uM pTEF1 FW Primer (+ApaI):      7.0uL
    -10uM Bar1 RV Primer #93 (+NotI):   7.0uL
    -Water:                             21.0uL
                           Total:       105.0uL
5. Added 15uL of GoTaq Green Master Mix to each PCR tube
6. Vortexed and centrifuged tubes
7. PCR tubes placed in thermocycler:
    -Initial Denaturation       95'C    5m
        -30 Cycles
            -Denaturation           95'C    45s
            -Annealing              55'C    30s
            -Extension              72'C    1m
    -Final Extension            72'C    10m
Observations:
    -Very small colonies, difficult to see if picked up correctly
```

pTEF1, Bar1, pJW608 E.coli Colony PCR Gel

```
-Added 10uL of PCR reaction to 1% Agarose Gel
```

6/12/15

pTEF1, Bar1, pJW608 E.coli Miniprep

```
1. Resuspended cells in 250uL Buffer P1
2. Transferred mixture to 1.5mL tube
3. Added 250uL Buffer P2. Mixed by inverting 6 times
4. Added 350uL Buffer N3. Mixed by inverting 6 times
5. Centrifuged for 10 minutes
6. Poured supernatant into a QIAprep spin column
7. Centrifuged for 30 seconds. Discarded flow-through
8. Added 0.75mL Buffer PE to column to wash. Centrifuged for 30 seconds
9. Discarded flow-through. Centrifuged empty for 1 minute
10. Placed column in a new 1.5mL tube. Added 50uL water to column. Let stand for 1 minute
11. Centrifuged for 1 minute.

Observations:
    -When pouring supernatant in step 6, possible cell debris leak
```

6/15/15

Lectures:

- Introduction to Genetic Ciruits

Assignments:

- Project Role: **Basic Circuit** (With Nick and Jasmine)
- Team Role: **Parts Registry**

To Do:

1. Begin Basic Circuit
    - Transform rtTA into yeast
    - Build the LexA TF part
        - Transform into yeast
        - (A few options for promoter)
2. Make sure parts are sent in and entered to the parts registry by deadline (closer to end of summer)
3. Brainstorm with team: Policy & Practices
4. Review article: Sense & Secrete by Youk and Lim

6/16/15

Lecture:

- Mathematical and Computational Modeling

- Applying Mathematics to Biological Circuits

6/17/15

Work Done:

- MATLAB Day 1
- Overview and plans for Basic Circuit
- Begin design of Basic Circuit Primers

6/18/15

Work Done:

- MATLAB Day 2
- Work on next weeks group meeting presentation
- Finish design of primers
- Ordered primers

Journal Club #1:

Secreting and Sensing the Same Molecule Allows Cells to Achieve Versatile Social Behaviours by Hyun Youk and Wendell A. Lim*

6/19/15

Lecture:

- Adaptive Immune System Overview

Work Done:

- MATLAB Day 3 - Final Day
- Meeting - P&P ideas finalization
- Recieved ordered primers

Transformation of MiniPrep Plasmid - For positive feedback group

- Plasmid: Hy83E4
- Add 0.5ul plasmid to 25ul cells
- Incubate on ice for 10 min
- Heat shock at 42^o for 30s

- On ice for 2-5 min
- Add 450ul SOC media to the tube
- Shake at 37^o for 45 min
- Plate 100ul on prewarmed LB/Carb plates
- Leave in drawer over weekend

6/22/15

Work Done:

1. PCR
    - ^Apa1 pFig2c - TF-RFP ^Not1
    - ^Xho1 TF-RFP ^Not1
2. Run gel
3. Digests - PCR and plasmid backbone
4. Gel purification

NanoDrop Results:

- LexA DBD FW (126) -> 0.26mg, 24.8nM
- LexA DBD RV (127) -> 0.31mg, 31.2nM
- pFig2c (128) -> 0.35mg, 31nM

PCRs:

```
Two PCR reactions:
126 / 128       2.5ul
127             2.5ul
Template        0.5ul
DMSO            1.5ul
Phusion MM       25ul
H20              18ul
---------------------
TOTAL:           50ul
```

1st reaction: LexA FW and LexA RV

2nd reaction: pFig2c and LexA RV

- Ran PCR ~2 hrs
- Ran gel - 5ul PCR product + 1ul Purple Loading Dye

Observations: - Faint band -> re-PCR our PCR product

6/23/15

Work Done:

- Checked PCR from 6/22
  - DID NOT WORK -> PCR again from scratch
    - Same setup as 6/22's PCR reaction (ONLY FOR Lex FW and LexA RV)
- Poured gel for 2nd PCR of LexA
  - 25ml gel + 2.5ml SYBERSAFE

Mini-Prep of Hy83E4 for positive feedback group:

```
1. Get from incubator
2. Centrifuge at 3000rpm for 5 min
3. Resuspend pellet in 250ul Buffer P1 and transfer to microcentrifuge tube
4. Add 250ul Buffer P2 and invert 4-6 times
5. Add 350ul Buffer N3 and invert 4-6 times
6. Centrifuge for 10 min at 13,000 rpm
7. Apply suppernatants from step 6 to spin column
8. Centriguge for 30s and discard flow-through
9. Wash by adding 750ul Buffer PE and centrifuge for 30s
10. Discard flow-through and centrifuge additional 1 min
11. Place spin column in a clean 1.5ml microcentrifuge tube and elute by adding 30ul H2O to the center of the
column, and let sit for 1 min. Centrifuge for 1 min.
```

Observations: - Possible over/under elution in last step - Given to positive feedback group

Positive Feedback group did not use :(

(They used Hy83E3)

6/24/15

Work Done:

- Ran new PCR on LexA
- Ran gel -> Band appeared!
- Digest
  - pGEM22 plasmid
  - LexA +DMSO
  - LexA -DMSO
    - All cut with Xho1/Not1
    - Incubator 1 hr+
- Group presentation on methods of data collection
  - Notes/presentation on Screening siRNA Knockdown

Gel:

- Lane 1 - Ladder
- Lane 2 - LexA + DMSO
- Lane 3 - LexA -DMSO
- Lane 4 - LexA plasmid
- Lane 5 - X
- Lane 6 - LexA plasmid pGEM22
- Lane 7 - pFig2c
- Lane 8 - X
- Lane 9 - pFig2c plasmid
- Lane 10 - X
- Lane 11 - pFig2c plasmid pGEM22

6/25/15

Work Done:

- Plasmid digestion
- Gel extraction
- Gel extraction #2
- Nanodrop
- Ligation

Plasmid Digestion:

1. Hy130E +Apa1 +Not1
    - 10ul plasmid
    - 3ul Cutsmart
    - 1ul H2O
    - 0.5ul Apa1
    - 0.5ul Not1
2. Hy130E +Xho1 +Not1
    - 10ul plasmid
    - 3ul Cutsmart
    - 1ul H2O
    - 0.5ul Xho1
    - 0.5ul Not1

See **6/9/15** for digestion procedure

Gel Extraction:

1. LexA
    - 231mg LexA
    - 93ul BufferQG
    - 231ul Isopropanol
2. pFig2c
    - 6mg pFig2c
    - 300ul BufferQG
    - 100ul Isopropanol

See **6/10/15** for extraction procedure

Observations:

- Super low pFig2c concentration -> New gel extraction

New Gel:

Lanes:

1. Ladder
2. pGEM22 +Xho1 +Not1 (LexA)
3. X
4. pGEM22 +Apa1 +Not1 (pFig2c)
5. X
6. Hy130E +Xho1 +Not1 (LexA)
7. X
8. Hy130E +Apa1 +Not1 (pFig2c)

Only Hy130E worked!

Gel Extraction #2:

See **6/10/15** for extraction procedure

- Hy130E +Xho1 +Not1 (LexA)
    - 128mg
    - 384ul BufferQG
    - 128ul Isopropanol
- Hy130E +Apa1 +Not1 (pFig2c)
    - 115mg
    - 345ul BufferQG
    - 115ul Isopropanol

NanoDrop Data:

1. Hy130E +Xho1 +Not1 (For LexA)
    - 44.13ng/ul
2. Hy130E +Apa1 +Not1 (For pFig2c)
    - 39.76ng/ul
3. LexA part
    - 23.97ng/ul
4. pFig2c part
    - 13.53ng/ul

Ligation:

1. Hy130E +Apa1 +Not1 (pFig2c)
    - Tube 1:
        - 2ul plasmid
        - 8ul pFig2c part
        - 2ul T4 Ligase Buffer
        - 1ul T4 Ligase
        - 7ul H2O
        - 20ul TOTAL
    - Tube 2 (-):
        - 2ul plasmid
        - 15ul H2O
        - 2ul T4 Ligase Buffer
        - 1ul T4 Ligase
        - 20ul TOTAL
2. Hy130E +Xho1 +Not1 (LexA)
    - Tube 3:
        - 1.5ul plasmid
        - 3ul LexA part
        - 2ul T4 Ligase Buffer
        - 1ul T4 Ligase
        - 12.5ul H2O
        - 20ul TOTAL
    - Tube 4 (-):
        - 1.5ul plasmid
        - 15.5ul H2O
        - 2ul T4 Ligase Buffer
        - 1ul T4 Ligase
        - 20ul TOTAL

See **6/10/15** for Ligation procedure

- Change:

    - Step #3 -> rm temp. for 1 hr, not 10 min
    - No heat inactivation

6/26/15

Work Done:

- Transform Hy130E Ligation

Transformation of Hy130E Ligations:

- 1x and 8x plates -> LB/Carb
    - Spin 7-8 thousand rpm for 30 sec
    - Dump supernatant and resuspend
- Total of 8 plates, one 1x and one 8x for each of:
    - Hy130E +Apa1 +Not1
    - Hy130E +Apa1 +Not1 (-)
    - Hy130E +Xho1 +Not1
    - Hy130E +Xho1 +Not1 (-)
- Plates set in drawer to grow over the weekend

See **6/10/15** for transformation protocol

6/29/15

Work Done:

- Colony PCR #2 of 6/26/15 ligation
- Phosphotase treat Hy130E plasmid

Colony PCR #2:

- Picked 6 colonies total from Hy130E +Apa1 +Not1 8x and Hy130E +Xho1 +Not1 8x plates
- 3 minutes extension at 72 C

See **6/11/15** for procedure

Phosphotase treatment:

- Add 1ul SAP to Hy130E
- Incubate in 37 C for 1 hr
- Move to 65 C for 5 min

6/30/15

Work Done:

- Gel for colony PCR from 6/29
- Ligation of Hy130E +Apa1 +Not1 and Hy130E +Apa1 +Not1 (-)
- Colony PCR #2 of Hy130E +Apa1 +Not1
- Gel for Colony PCR #2

Ligation of Hy130E +Apa1 +Not1 and its negative control:

```
HyA
    2ul vector HyA
    8ul insert pFig2c
    2ul T4 Ligase Buffer
    1ul T4 Ligase
    7ul H2O
    20ul Total

HyA (-)
    2ul HyA
    2ul T4 Ligase Buffer
    1ul T4 Ligase
    15ul H2O
    20ul Total
```

Ligation protocol on **6/10/15**

Colony PCR #2 for Hy130E +Apa1 +Not1:

See **6/11/15** for procedure

- 8 colonies
- Made liquid culture -> Shake overnight

Observations:

- liquid cultures checked, tubes fell off shaker, colonies 1/4 lost

7/1/15

Work Done:

- Miniprep of Hy130E +Apa1 +Not1 and Hy130E +Xho1 +Not1
- Nanodrop

Miniprep of Hy130E +Apa1 +Not1 and Hy130E +Xho1 +Not1:

See **6/12/15** for procedure

- 4 tubes:
  - "HyA8" - Hy130E +Apa1 +Not1 colony 8
  - "HyX2" - Hy130E +Xho1 +Not1 colony 2
  - "HyX5" - Hy130E +Xho1 +Not1 colony 5
  - "HyX6" - Hy130E +Xho1 +Not1 colony 6

Nanodrop results:

1. HyA8 - 739.6ng/ul
2. HyX5 - 474.0ng/ul
3. HyX6 - 699.6ng/ul
4. HyX2 - 549.9ng/ul

7/2/15

Work Done:

- Sequencing of Minipreps
- Colony PCR #3
- Gel for Colony PCR #3
- Miniprep Colony PCR #3
- Primer Reconstitution: pNH604F

Sequencing of Minipreps:

- HyA8 - Has LexA
- All other colonies do not have LexA

Colony PCR #3:

See **6/11/15** for procedure

- Colonies from HyX 8x plate

Gel for Colony PCR #3:

```
Lanes:
1. Ladder - 10ul
2. X
3. Hy130E tdTom. LexADBD - 5ul Colony 1
4. Hy130E tdTom. LexADBD - 5ul Colony 2
```

```
5. Hy130E tdTom. LexADBD - 5ul Colony 3
6. Hy130E tdTom. LexADBD - 5ul Colony 4
7. Hy130E tdTom. LexADBD - 5ul Colony 5
8. Hy130E tdTom. LexADBD - 5ul Colony 6
9. X BROKEN WELL
10. X BROKEN WELL
11. X
12. Hy130E tdTom. LexADBD - 5ul Colony 7
13. Hy130E tdTom. LexADBD - 5ul Colony 8
```

Miniprep Colony PCR #3:

```
Colony 1 HyX 2nd - 643.9ng/ul
Colony 2 HyX 2nd - 699.7ng/ul
Colony 7 HyX 2nd - 748.4ng/ul
Colony 8 HyX 2nd - 415.5ng/ul
```

Primer Reconstitution: pNH604F:

1. Reconstitute primer w/ water like normal
2. label with 135 and store in primers box
3. Instead of making a 10 fold dilution, take 50ul of primer and 950ul H2O. Label with "pNH604F"
4. Put tube with primer in with your seq. reaction (5ul of #1)
5. Put in seq. box

7/6/15

Work Done:

- Plasmid Digest #2

Plasmid Digest #2:

- HyA - Several hours Apa1, room temp., end of day add Not1 and make HyX digest - 37 degrees

```
1. HyA
10ul plasmid
3ul Cutsmart
1ul H2O
0.5ul Apa1
0.5ul Not1

2. HyX
10ul plamsid
3ul Cutsmart
```

```
1ul H2O
0.5ul Xho1
0.5ul Not1
```

Notes: - Accidentally re-digested old HyA digest - Also made new HyA digest - 2 HyA digest tubes, old and new

See **6/9/15** for enzyme digest protocol

7/7/15

Work Done:

- Phosphotase treatment of miniprep tubes
- Gel Extraction of digest #2
- New digest #3
- Gel of digest #3

Phosphotase Treatment of Miniprep tubes:

- 0.5ul phosphotase
- Incubate at 37 degrees for 1 hr
- Incubate at 65 degrees for 10 min

Gel Extraction of digest #2:

```
Lanes:
1. Ladder
2. X
3. HyX
4. X
5. HyA New
6. X
7. HyA Old/Re digest
8. X
```

NO CUTS -> Did not extract -> New digest

New Digest #3:

- See **6/9/15** for enzyme digest protocol
- See **7/6/15** for digest recipe

Gel of Digest #3:

- Faulty agarose - GEL BROKE

- Lost digest #3

7/8/15

Work Done:

- New Digest #4

New Digest #4:

- See **6/9/15** for enzyme digest protocol

```
1. HyA
10ul plasmid
3ul Cutsmart
16ul H2O
0.5ul Apa1
0.5ul Not1
30ul TOTAL

2. HyX
10ul plamsid
3ul Cutsmart
16ul H2O
0.5ul Xho1
0.5ul Not1
30ul TOTAL
```

Digest Overnight

7/9/15

Work Done:

- Gel Extraction of digest #4
- Nanodrop digest #4
- Ligate digest #4
- Transform digest #4

Gel Extraction of Digest #4:

```
Lanes:
1. Ladder
2. X
3. HyA
```

```
4. HyA
5. X
6. HyX
7. HyX
8. Ladder
```

See **6/10/15** for gel extraction procedure

Weights:

- HyA - 214mg
- HyX - 257mg

NanoDrop of Digest #4:

- HyA - 97.15ng/ul
- HyX - 76.54ng/ul

Ligation of Digest #4:

See **6/10/15** for ligation procedure

- LexA part - 23.97ng/ul
- pFig2C part - 13.54ng/ul

```
1. HyA
    - 2ul HyA
    - 7ul pFig2C
    - 2ul Ligase Buffer T4
    - 1ul T4 Ligase
    - 8ul H20
- 20ul TOTAL

2. HyX
    - 2ul HyX
    - 2ul LexA
    - 2ul Ligase Buffer T4
    - 1ul T4 Ligase
    - 13ul H20
- 20ul TOTAL

3. HyA (-)
    - 2ul HyA
    - 2ul Ligase Buffer T4
    - 1ul T4 Ligase
    - 15ul H20
- 20ul TOTAL
```

```
1. HyX (-)
    - 2ul HyX
    - 2ul Ligase Buffer T4
    - 1ul T4 Ligase
    - 8ul H20
- 20ul TOTAL
```

See **6/10/15** for transformation protocol

7/10/15

Work Done:

- Colony PCR of Digest #4

Colony PCR of Digest #4:

- Plates have very low colony count - HyX
- Let them incubate longer

Colony PCR of HyA performed:

- 6 colonies from 8x plate
- 1 colony from 1x plate

INCONCLUSIVE RESULTS - SCRAPPED

7/13/15

Work Done:

- PCR of old inserts/T64 tdTom.

PCR of old inserts/T64 tfTom.:

See **6/9/15** for PCR protcol

```
1. LexA Old
    - 2.5ul FW Xho1 (primer 126)
    - 2.5ul RV Not1 (primer 127)
    - 1.5ul DMSO
    - 2ul LexA insert
    - 25ul Phusion MM
    - 16.5ul H2O
        - 50ul TOTAL
```

```
2. LexA New
    - 2.5ul FW Xho1 (primer 126)
    - 2.5ul RV Not1 (primer 127)
    - 1.5ul DMSO
    - 0.5ul T64 tfTom. Template
    - 25ul Phusion MM
    - 18ul H2O
        - 50ul TOTAL

3. pFig2C Old
    - 2.5ul FW Apa1 (primer 128)
    - 2.5ul RV Not1 (primer 127)
    - 1.5ul DMSO
    - 2ul pFig2C insert
    - 25ul Phusion MM
    - 16.5ul H2O
        - 50ul TOTAL

4. pFig2C New
    - 2.5ul FW Apa1 (primer 128)
    - 2.5ul RV Not1 (primer 127)
    - 1.5ul DMSO
    - 0.5ul T64 tdTom. Template
    - 25ul Phusion MM
    - 18ul H2O
        - 50ul TOTAL
```

7/14/15

Work Done:

- NanoDrop of inserts
- Insert Digest
- Gel Extraction of 7/13 PCR
- Ligation of L1/F1 into HyA/HyX

NanoDrop of Inserts:

- LexA Old - 377.8ng/ul
- LexA New - 414.0ng/ul
- pFig2C Old - 446.5ng/ul
- pFig2C New - 434.2ng/ul

Insert Digest:

```
1. pFig2C Old
    - 5ul Cutsmart
    - 0.5ul Apa1
    - 0.5ul Not1

2. LexA Old
    - 5ul Cutsmart
    - 0.5ul Xho1
    - 0.5ul Not1
```

Gel Extraction of 7/13 PCR:

See **6/10/15** for Gel Extraction procedure

Weights:

- L1 (LexA old) - 147mg
- F1 (pFig2C old) - 228mg

Ligation of L1/F1 into HyA/HyX:

See **6/10/15** for Ligation procedure

```
1. L1
    - 1ul HyX
    - 3.5ul L1
    - 2ul T4 Ligase Buffer
    - 1ul T4
    - 12.5ul H2O
        - 20ul TOTAL

2. F1
    - 1ul HyA
    - 6ul F1
    - 2ul T4 Ligase Buffer
    - 1ul T4
    - 10ul H2O
        - 20ul TOTAL
```

7/15/15

Work Done:

- Colony PCR of 7/14 Transformation

Colony PCR of 7/14 Transformation:

See **6/11/15** for procedure

Plates:

- HyA/F1 - Looks promising...
- HyX/L1 - Very few colonies, on both + and - plates

Total of 8 colonies from HyA/F1 and HyX/L1 to be PCR'd

Made liquid cultures to shake in 37 degrees O/N

Notes:

- Although a 7x MM stock was made (by accident), there seemed to be enough for 9 reactions
- Possible miscalculation?

7/16/15

Work Done:

- Gel of Colony PCR
- Miniprep Colonies
- NanoDrop
- Primer Reconstitution
- PCR of 151/152/153

Gel of Colony PCR:

- 16 samples loaded
- Successful Colonies:
  - HyA colony 2 and 6 - Strong bands
  - HyX colonies 1,2,4 and 5 - faint bands

Miniprep Colonies/NanoDrop

```
1. HyA
    Colony 2 - 848ng/ul
    Colony 6 - 9.698ng/ul
2. HyX
    C1 - 374ng/ul
    C2 - 761.7ng/ul
    C4 - 23.92ng/ul
    C5 - 466ng/ul
```

Primer Reconstitution:

- 151 - pFig2C +pNH604 FW - 23.4nMol, 234ul
- 152 - LexADBD +pNH604 RV - 28.5nMol, 285ul
- 153 - LexADBD +pAga1 FW - 23nMol, 230ul

7/17/15

Work Done:

- Gel of 7/16 PCR
- New PCR with New Primers

Gel of 7/16 PCR:

- Ran Gel - No visible DNA
- Ran 2nd Gel - leakage and crack

New PCR With New Primers:

- Same set up as 7/16

7/20/15

Work Done:

- Gel extraction of inserts for Gibson
- Nanodrop
- Gibson Assembly

Gel Extraction of Inserts for Gibson:

- Only L4 and F3 were visible

```
Weight:
    L4-18mg
    F3-45mg
```

Nanodrop:

- L4-56.29ng/ul
- F3-15.40ng/ul

Gibson Assembly:

- Did not work, no colonies

7/21/15

Work Done:

- New PCR of inserts

New PCR of Inserts:

```
1. F5x2
    -2.5ul #151 FW
    -2.5ul #152 RV
    -0.5ul F1
    -1.5ul DMSO
    -25ul Phusion MM
    -18ul H20
50ul Total

2. F6x2
    -2.5ul #151 FW
    -2.5ul #152 RV
    -0.5ul T64 tdTom.
    -1.5ul DMSO
    -25ul Phusion MM
    -18ul H20
50ul Total

3. L5x2
    -2.5ul #153 FW
    -2.5ul #152 RV
    -0.5ul L1
    -1.5ul DMSO
    -25ul Phusion MM
    -18ul H20
50ul Total

4. L6x2
    -2.5ul #153 FW
    -2.5ul #152 RV
    -0.5ul T64 tdTom.
    -1.5ul DMSO
    -25ul Phusion MM
    -18ul H20
50ul Total
```

7/22/15

Work Done:

- Gel extraction of 7/21 PCR
- Nanodrop
- Yeast culture

Gel Extraction of 7/21 PCR:

- Ran gel
  - First L5 and L6
  - Second F5 and F6
- Only L6 worked-extract
- Weight of L6-294mg

Nanodrop:

- L6-30.37ng/ul

Yeast Culture:

- 5ml of YPD in glass tubes
- Pick from yeast culture plate
- Place culture in yeast shaker

7/23/15

Work Done:

- Yeast culturing
- Gibson for L6

Yeast Culturing:

- 250ul overnight culture + 4.75mlYPD
- Put in 30 degree fridge for 3 hours
- Take plasmid 4ul + 0.5ul pME1

```
1. CB008DB (Spilled after 3 hours)
    - Add 4ml YPD
    - Incubate 1.5 hours
    - Take 2.5ml cells
2. PGEM41
```

```
    - Add 2ml YPD
    - Incubate 1.5 hours
    - Take all cells
```

For the culture that spilled, treat it as a 2.5ml culture, and the other as a 5ml culture.

- Placed in 30 degree incubator until Monday

7/24/15

Work Done:

- Gibson Assembly for LexA

Gibson Assembly for LexA:

```
1. (-)Control L6
    - 1.5ul Hy130E
    - 5ul GibMM
    - 3.5ul H20
    Total 10ul
2. (+)Control L6
    - 5ul (+)Control
    - 5ul GibMM
    Total 10ul
3. L6
    - 2ul L6 insert
    - 1.5ul Hy130E
    - 5ul GibMM
    - 1.5ul H20
    Total 10ul
```

Transformed Gibson, left in drawer over weekend.

7/27/15

Work Done

- Check Transformations
- Colony PCR Gibson
- Digest pGEM34
- Gel Extraction pGEM34
- Transformation of more pGem48
- Gibson Assembly #2
- Yeast transformation

Check Transformations

- Yeast-No colonies
- Gibson-Small colonies
  - Put in 37 degree room for a few hours
  - More colonies on (-) plates

Colony PCR for Gibson

- Forward primer of pAga1-#98
- Reverse primer for LexADBD-#127/#151

Digest pGEM34

- Plasmid template for pAga1- +Xho1 +Not1
- About 2 hours - Gel extract
- BFP-700bp cut out
- Extract rest of plasmid

```
10ul pGEM34
3ul CutSmart
0.5ul Xho1
0.5ul Not1
16ul H20
    30ul Total
```

Set in 37 degree incubator for 2 hours

Gel Extraction of pGEM34

- Weight-171mg
- pGEM34 pure - 31.34ng/ul

Gibson Assembly #2

```
1. (-)Control
    - 15ul pGEM34
    - 5ul GibMM
    - 3.5ul H20
        10ul Total
2. (+)Control
    - 5ul (+)Control
    - 5ul Gib MM
        10ul Total
3. LexA/pGEM34
```

```
- 2ul pGEM34
- 2ul L6
- 5ul Gib MM
- 1ul H2O
    10ul Total
```

- pGEM34 - 31.34ng/ul
- L6 - 30.37ng/ul

Yeast Transformation:

- Jeffrey making O/N culture
- Dilute 7/28

7/28/15

Work Done:

- Digest pGEM48 for Yeast Transformation
- Yeast Transformation
- Colony PCR: Gibson #2
- New colony PCR

Digest pGEM48 for Yeast Transformation:

```
6ul pGEM48
2.5ul Cutsmart
1.5ul pME1
15ul H20
    25ul Total
    5ul/Transformation
```

- Liquid cutures pGEM48

Colony PCR: Gibson #2:

- 8 colonies - 9x GoTaq MM
- Colonies 1-5 - 8x plate Gibson L6
- Colonies 6-8 - 1x plate Gibson L6
- Liquid culture in incubator O/N

New colony PCR:

- 5ul cells - New MM with different pAga1 FW primer

7/29/15

Work Done:

- Miniprep of Colony PCR
- Gel of Colony PCR

Miniprep of Colony PCR:

- Colony 1 - 703.4ng/ul
- Colony 2 - 579.5ng/ul