Penn iGEM 2015 Daily Digest

Week 2 (6/8 - 6/12)
Week 3 (6/15 - 6/19)
Week 4 (6/22 - 6/26)
Week 5 (6/29 - 7/3)
Week 6 (7/6 - 7/10)
Week 7 (7/13 - 7/17)
Week 8 (7/20 - 7/24)
Week 9 (7/27 - 7/31)
Week 10 (8/3 - 8/7)
-Skip Week 11-
Week 12 (8/17 - 8/21)

Week 2 (6/8 - 6/12)

6/8/2015

Aims for the day:

1. Run a gel after digestion of lacZ and pDawn
2. Gel extraction & digest cleanup
3. Ligation
4. Transformation

Accomplishments:

1. Ran a gel after digestion
2. Performed gel extraction and digestion cleanup
3. Measured concentrations of pDawn and lacZ with nanodrop

Aims for tomorrow:

1. PCR clean-up for new PCR of lacZ
2. Digestion of lacZ and pDawn with new Nde1 and BamHI
3. Digest clean-up
4. Ligation of lacZ and pDawn
5. Lux box transformation into E. coli

6/9/2015

Aims for today:

1. PCR cleanup for lacZ
2. Digest lacZ and pDawn
3. Digest cleanup
4. Ligation of pDawn and lacZ
5. Lux box transformation
6. Design one plasmid system with Lux box/lacZ and design two plasmid system, one with Lux box and the other with the lacZ reporter

Accomplishments:

1. PCR cleanup for lacZ
2. Digest of lacZ and pDawn
3. Digest cleanup of lacZ and pDawn
4. Ligation
5. Lux box transformation
6. Began working on plasmid design
7. Inoculated pDawn

Aims for tomorrow:

1. Potentially design primers for cloning Lux box into pDawn/lacZ
2. Miniprep pDawn
3. Design SapI primers
4. Design compatibility system
5. Pick colonies for BioBrick, inoculate, glycerol stock
6. Colony PCR pDawn + lacZ, glycerol stock, miniprep, sequencing
7. Induce lux box w/ arabinose

Questions:

In the "empty" region of pDawn (the only space we think a biobrick can be cloned) there's only one restriction enzyme that cuts once in that region and nowhere else, SapI.

1. Are we right in assuming that empty space is the only place we can clone in a biobrick?
2. Would it be okay if we used only one restriction enzyme for the ligation instead of two?

6/10/2015

Aims for today:

1. Miniprep pDawn
2. Design SapI primers
3. Design Compatibility Systems
4. Pick Colonies for Biobrick, inoculate, glycerol stock
5. Colony PCR, pDawn + LacZ, Glycerol stock, miniprep
6. Redigest pDawn (with extreme controls)
7. Reattempt Ligation of pDawn+LacZ
8. Colony PCR of BioBrick Transformation
9. Run gel of colony PCR products
10. Run gel of newly digested pDawn, Overnight ligation.

Accomplishments:

1. Miniprep pDawn
2. Re-digest of pDawn (with extreme controls)
3. Re-attempt Ligation of pDawn+LacZ
4. Colony PCR of pDawn + LacZ
5. Gel extract of newly digested pDawn
6. Transformed lux box BioBrick, pDawn + lacZ, just pDawn backbone (for miniprep and
7. making our own glycerol stock) into NEB turbo again [had just cut pDawn backbone and just digested lacZ insert transformed as well as controls]
8. Plated transformed colonies and placed into incubator (will look at results tomorrow)

Aims for tomorrow:

1. Run gels of colony PCR from 6/10/15 and see if the lacZ was ligated into pDawn backbone
2. Finish designing cloning of luxbox biobrick into pACBB-eGFP (plasmid that is compatible with pDawn)
3. Pick colonies for most recent BioBrick Transformation, Inoculate, glycerol stock, miniprep
4. Colony PCR of new pDawn + LacZ transformation
5. Induce luxbox transformed E. coli with arabinose (growing up transformed E. coli to saturation, back diluting 1:100, growing up to OD600 of 0.3, then inducing with low to high arabinose concentrations)
6. If transformation results not looking good, we will need to re-digest lacZ insert and pDawn, and re-ligate pDawn into lacZ

Questions:

1. How exactly does the two-plasmid transformation work? Do we transform the NEB turbo with one and then make it competent again and transform it with the second?
2. Is there any way we can check for incomplete digestion (because the gel resolution doesn't allow us to see that, since the part we cut out is only a few bases big)?

6/11/2015

Aims for today:

1. Inoculate pDawn
2. Run gel of colony PCR
3. Check plates from yesterday
4. Colony PCR of re-transformed plates (after we miscalculated the ligation of the first plates)
5. Digest and ligate pACBB-eGFP and lux box
6. Grow up luxbox
7. Inoculate pACBB-eGFP

Accomplishments:

1. Inoculated pDawn
2. Ran gel of first colony PCR
3. Checked plates grown overnight
4. Performed second colony PCR of newly transformed plates with pDawn + lacZ
5. Grew up luxbox transformants in LB+chlor (see double check in transformation worked because Chlor diffused in plate)
6. Inoculated DH5alpha with pACBB-eGFP (in prep for miniprep and glycerol stock tomorrow)
7. Retransformed lux box biobrick into NEB turbo

Aims for tomorrow:

1. Miniprep and glycerol stock pACBB-eGFP inoculants
2. Check inoculated colonies from luxbox transformation attempt #2 to see if any grew in LB+Chlor
3. Look at transformation results on plates (for attempt #3 of transforming lux box biobrick)
4. Look at results for colony PCR of pDawn + lacZ (run gel + image)

Questions:

None, we might have some after we get the colony PCR results.

6/12/2015

Aims for today:

1. Miniprep pACBB-eGFP
2. Glycerol stock of pACBB-eGFP
3. Back dilute/ induce lux box→ Back dilute @ 12:57 PM
4. Colony PCR lux box→ run on a gel
5. Glycerol stock lux box
6. Miniprep pDawn
7. Glycerol stock of pDawn
8. 3 hr digest of pDawn + lacZ
9. Overnight ligation of pDawn + lacZ
10. Run colony PCR on a gel for pDawn + lacZ ligation product.

Accomplishments:

1. pCR lacZ
2. Miniprep pACBB-eGFP
3. Glycerol stock of pACBB-eGFP
4. Colony PCR lux box→ run on a gel
5. Miniprep pDawn
6. Glycerol stock of pDawn
7. Run colony PCR on a gel for pDawn + lacZ ligation product.

Aims for Monday:

1. Induce lux box
2. Glycerol stock lux box
3. 3 hr digest of pDawn + lacZ (didn't do before because needed more lacZ)
4. Overnight ligation of pDawn + lacZ
5. Clone lux box into pACBB-eGFP

Questions:

1. Does completing PCR on LacZ as a positive control using the Colony PCR Protocol (a.k.a using Taq instead of Phusion) have a significant effect on the results of the PCR? [If not clear, this question is better explained in person]
2. Should we purchase the enzymes and dntps? How long after the expiration date are the enzymes good for? Should we get rid of enzymes/ dntps that are months/years past their expiration date?
3. What happens if you use a buffer for restriction digest that is not the optimal one?

Week 3 (6/15 - 6/19)

-6/15/2015 SKIPPED-

6/16/2015

Aims for today:

1. Innoculate pACBB
2. Make M9 media
3. Perform gel extraction on pDawn, lacZ, pACBB-eGFP, and luxbox from gel we ran yesterday.
4. Ligate luxbox and pACBB
5. LIgate pDawn and lacZ
6. Transform luxbox and pACBB
7. Transform pDawn and lacZ
8. Miniprep pDawn
9. Back dilute and induce luxbox
10. Measure luminescence of induced luxbox on the hour
11. PCR cleanup on lacZ
12. 3 hour digest of lacZ

Accomplishments:

1. Made M9
2. Gel extracted pDawn, lacZ, luxbox, and pACBB
3. Ligated luxbox and pACBB
4. Ligated pDawn and lacZ
5. Transformed luxbox and pACBB
6. Transformed pDawn and lacZ
7. Miniprep pDawn
8. Back diluted and induced luxbox
9. Measured luminescence of induced luxbox on the hour
10. PCR clean up on lacZ
11. Digested lacZ
12. Innoculate pACBB

Aims for tomorrow:

1. Glycerol stock luxbox and pSB1C3
2. Miniprep more pACBB-eGFP
3. Look at transformed plates, pick colonies, innoculate

4. Colony PCR lacZ from pDawn and luxbox from pACBB
5. Column purify digested lacZ
6. Dilute luxbox to OD600 .3, take measurements at 1 hr, 3 hr, 5 hr, etc.
7. Take 24th hour time point for lux box trial 1 (the one we started today)
8. Potentially do overnight ligations if the transformations from today don't work
9. Miniprep and glycerol stock ligated plasmids pDawn and lacZ and luxbox and pACBB.

6/17/2015

Aims for Today:

1. Glycerol stock LuxBox + PSB1C3
2. Miniprep more pACBB-eGFP
3. Look at plates, pick colonies, inoculate at 4pm LuxBox+pACBB
4. Colony PCR luxbox from pACBB
5. Column purify digested lacZ
6. Transform overnight ligation (& prep plates)
7. miniprep and glycerol stock ligated pDawn+LacZ and Luxbox+pACBB
8. Transform BL21 with luxbox biobrick.

Accomplishments:

1. Glycerol stock of LuxBox+PSB1C3
2. Miniprep more pACBB-eGFP

Aims for Tomorrow:

1. Design primer for lacZ RE site extensions
2. Design luxbox colony PCR primers
3. Design luxbox sequencing primers
4. Design lacZ sequencing primers
5. Look at plates (ligation products lacZ + pDawn and lux + pACBB), pick colonies, innoculate.
6. Plot data of lux
7. Inoculate BL21 w/ lux biobrick (to back dilute on Fri)

Aims for Friday:

1. Miniprep + glycerol stock lacZ + pDawn and lux + pACBB
2. Dilute BL21 w/ lux biobrick to OD600 .3
3. Take measurements at 1 hr, 3 hr, 5 hr, etc.
4. Plot data ^

Questions:

1. Is N = 2 enough for NEB Turbo for measuring luminescence experiment (as opposed to BL21 which we will do later)? Should we do more trials?

2. Should we do the interlab study this year? (http://2015.igem.org/Tracks/Measurement/Interlab_study)
3. We should discuss human practices

6/18/2015

Aims for Today:
1. Inoculate BL21 LuxBox + PSB1C3
2. Colony PCR of pDawn + LacZ transformation
3. Inoculate pACBB + LuxBox, pDawn + LacZ
4. Transform pDawn + LacZ from Mike into Turbo Cells
5. Digest test for pDawn + LacZ; pACBB + Lux
6. Glycerol Stock BL21 with LuxBox
7. Order LacZ Assay Kit
8. Order pDawn & pACBB insert primers
9. Order Lux Sequence primers.
10. Make more arabinose, autoclave flasks, prep for BL21 vs. Turbo experiment.
11. Make LB Chlor. plates

Accomplishments:
1. Inoculate BL21 LuxBox + PSB1C3
2. Inoculate pACBB + LuxBox, pDawn + LacZ
3. Transform pDawn + LacZ
4. Order pDawn & pACBB insert primers
5. Find LacZ Assay kit we want to order (See links below)
6. Order Lux Sequence primers.
7. Make more arabinose, autoclave flasks, prep for BL21 vs. Turbo experiment.

Aims for Tomorrow:
1. Glycerol Stock BL21 + LuxBox
2. Digest Test of pDawn + LacZ & pACBB + LuxBox
3. Autoclave LB Agar & pour plates (Add Chlor)!!
4. Back dilute & induce BL21 and NEB Lux strains
5. Take luminescence measurements.

6/19/2015

Aims for Today:
1. Glycerol Stock BL21 + LuxBox
2. Digest Test of pDawn + LacZ & pACBB + LuxBox
3. Autoclave LB Agar & pour plates
4. Pick colonies pDawn + lacZ; inoculate
5. Glycerol stock pDawn + lacZ

6. Glycerol stock 6 colonies for BL21 vs turbo experiment

Accomplishments:
1. Glycerol Stock BL21 + LuxBox
2. Digest Test of pDawn + LacZ & pACBB + LuxBox
3. Autoclave LB Agar & pour plates
4. Pick colonies pDawn + lacZ; inoculate
5. Glycerol stock pDawn + lacZ
6. Glycerol stock 6 colonies for BL21 vs turbo experiment

Aims for Monday:
1. Back dilute & induce BL21 and NEB Lux strains
2. Take luminescence measurements.
3. Inoculate 10 ml cultures

Week 4 (6/22 - 6/26)

Aims for Today:
1. Back-dilute to 0.3
2. Measure luminescence at 1h, 3h, etc.
3. Presentation
    a. lacZ expression as a result of light
    b. pACBB vs. PSBIC3
    c. BL21 vs. turbo in lux exp.
    d. lux exp. high vs low AHL →
    e. questions
    f. GANT for summer
    g. 20 kb from IDT
    h. nonhydrolyzable arabinose
4. Josh + Danny at 1 PM
5. Decide if digest test worked

Accomplishments:
6. Back-dilute to 0.3
7. Measure luminescence at 1h, 3h, etc.
8. Presentation
    a. lacZ expression as a result of light
    b. pACBB vs. PSBIC3
    c. BL21 vs. turbo in lux exp.
    d. lux exp. high vs low AHL →
    e. questions
    f. GANT for summer

    g. 20 kb from IDT
    h. nonhydrolyzable arabinose
9. Josh + Danny at 1 PM
10. Decide if digest test worked

Aims for Monday:

1. Analyze luxbox in BL21 versus NEB Turbo
2. Transform ligated pDawn+lacZ and pACBB+lux, then induce with arabinose
3. Explore IDT 20kb options
4. Figure out our goals (given our knowledge of Peking)
5. Work on website design
6. Work on human practices

6/23/15

Aims For Today:

7. Analyze luxbox in BL21 versus NEB Turbo
8. Transform ligated pDawn+lacZ and pACBB+lux, then induce with arabinose
9. Explore IDT 20kb options
10. Figure out our goals (given our knowledge of Peking)
11. Work on website design
12. Work on human practices

Accomplished Today:

1. Analyzed luxbox in BL21 versus NEB Turbo data
2. Transformed pDawn+lacZ and pACBB+lux into BL21
3. Looked into other past iGEM team projects involving communication through light
4. Started thinking about human practices and figuring out the logistics of sorting past projects by topic/team/year

Aims For Tomorrow:

1. Compare fluorescent output for BL21 at different ODs by taking measurements 1hr, 3hr, 5hr, etc.

Questions/Concerns:

1. Discuss what the other teams are doing/have done and how are goals should shift accordingly

6/24/15

Aims For Today:

1. Back dilute luxbox cultures to different OD's, induce, measure OD until 7 hrs
2. Decide on 20 kb from IDT

3. Listen to IDT webinar @2 pm
4. Look @ transformed plates and innoculate
5. Glycerol stock ligated pDawn + lacZ and pACBB + lux
6. Look over lacZ kit
7. Grow up cultures for both (ours and Mike's) pDawn lacZ experiment
8. Glycerol stock Mike's pDawn+lacZ
9. Grow up lux BL21 cultures for arabinose spiking experiments
10. Order d-fucose

Aims For Tomorrow:

1. pDawn+lacZ experiment (varying blue light intensity)
2. back dilute lux BL21 to .3 and induce with arabinose
3. Measure lux BL21 and add arabinose every hour
4. pick up dNTPs

6/25/15

Aims For Today:

1. Re-do yesterday's experiment
2. pick up dNTPs
3. order more SOC
4. make color chart _ plan prospective experiments
5. IDT → $$
6. plan lacZ + pDawn experiment

Accomplishments:

1. Research previous team projects
2. Created a spreadsheet detailing all sender-xreceiver cell relationships
3. Rethought lux box experiment
4. Set up tomorrow's pDawn + lacZ experiment
5. Inoculated cultures for ^ experiment

Aims for Tomorrow:

1. Complete pDawn + lacZ experiment
2. Pick up dNTPs
3. Order more SOC
4. IDT → $$
5. Karol's iGEM database project

6/26/15

Aims For Today:

1. iGEM database (human practices)
2. B-Galactosidase assay
3. Finalize plan for the summer
4. OD + luminescence experiment

Accomplishments:

1. iGEM database (human practices)
2. B-Galactosidase assay
3. OD + luminescence experiment

Aims for Monday:

1. Co-transforming pDawn + lacZ
2. Redoing standardized curve for B-Galactosidase assay
3. Design exp for different blue light intensity on B- Galactosidase activity experiment.
4. pACBB + pSB1C3 experiment
    a. Did pACBB + lux ligate properly? (we learned that pDawn + lacZ cloning did not work, but we still have Mike's which turned yellow when we did the assay yay!)
5. Decide which parts to order from IDT (but actually though)
6. Examine more of the different color systems that we have been discussing; talk to advisors about this more; decide which we want to do

Questions:

1. https://docs.google.com/spreadsheets/d/1HosOnFdv6kmtn_Q1y98bH6RwkHVTM65OsZQLPafrFRg/edit?usp=sharing
    a. (Just a quick conclusion) Finished the assay and noticed that only Mike's pDawn + lacZ turned yellow after adding WS; our ligation was not successful
    b. But, when we tried to make the standard curve, we got a graph that didn't make any sense.
        i. We think it could be because:
            1. The equation includes OD660 for something that's not a cell?
            2. We waited too long to add the stop solution (too yellow)
            3. We diluted many times to get the OD420 in the range of 0.2 - 1.0; we may have not mixed the solutions enough (the ODs were all pretty much 0.6)

Week 5 (6/29 - 7/3)

6/29/15

Aims For Today:

1. Finish presentation
2. DIscuss project ideas; goals and data with advisors during Monday Meeting

3. Figure out trajectory for the summer.

Accomplishments:

1. Finish presentation
2. DIscuss project ideas; goals and data with advisors during Monday Meeting
3. Figure out trajectory for the summer.

Aims for Tomorrow:

1. Co transform pDawn + lacZ + luxbox (fail-fast)
2. Redo standard curve for B- Gal assay
3. Design experiment for blue-light intensity for assay
4. Did lux box + pACBB ligate properly?
5. Decide which parts to order from IDT (but actually)
6. Talk about different color circuits

6/30/15

Aims For Today:

1. grow up pACBB-lux
2. dilute B-Gal
3. Grow up BL21 lux
4. Grow up lux for spiked arabinose experiment
5. Fill out safety form
6. Set up incubator for blue light vs. dark pDawn experiment
7. Get primers
8. Sequence pACBB-lux
9. Inoculate pDawn-lacZ for blue light inducibility experiment (dark)
10. Set up sequencing tubes for pACBB-lux part
11. do colony PCR at pACBB-lux
12. redo B-gal assay standardized curve

Accomplishments:

1. Grow up BL21 lux
2. Grow up pACBB-lux
3. Grow up lux for spiked arabinose experiment
4. Fill out safety form
5. Set up incubator for the blue light vs. dark pDawn experiment
6. Get primers
7. Set up sequencing tubes for pACBB-lux part

Aims for Tomorrow:

1. back dilute pDawn - lacZ 1:100 (dark)
2. at OD of around .4, separate→ 3 blue; 3 dark

3. miniprep pACBB lux (2 tubes)
4. Sequence miniprepped pACBB-lux
5. PCR test of miniprepped pACBB-lux
6. Redo B-gal assay standardized curve
7. Redesign the illuminated incubator

Aims for Friday:

1. After 20 h of growth, do OD600 and miller assay for pDawn lacZ light vs. dark

7/1/15

Aims for Today:

1. Autoclave flasks
2. Set up blue light incubator
3. At OD of .4, induce pDawn-lacZ
4. Back dilute pDawn-lacZ 1:100 (dark) in 25 mL LB Kan
5. Dilute B-Gal for curve
6. Do B-Gal curve w/ BL21
7. Miniprep pACBB-lux (4 tubes)
8. Back-dilute 1:100 lux BL21 for arabinose experiment
9. Induce lux w/ arabinose every hour; measure OD + luminescence
10. Autoclave flasks
11. Colony PCR
12. Sequencing.

Accomplishments:

1. Autoclave flasks
2. Set up blue light incubator
3. At OD of .4, induce pDawn-lacZ
4. Back dilute pDawn-lacZ 1:100 (dark) in 25 mL LB Kan
5. Dilute B-Gal for curve
6. Do B-Gal curve w/ BL21
7. Miniprep pACBB-lux (4 tubes)
8. Back-dilute 1:100 lux BL21 for arabinose experiment
9. Induce lux w/ arabinose every hour; measure OD + luminescence
10. Autoclave flasks
11. Colony PCR

Aims for Tomorrow:

1. Run PCR of pACBB lux on gel
2. Pour gel
3. Send in sequencing of pACBB-lux

4. At II AM, miller assay of pDawn light vs. dark
5. If pACBB gel good, do pACBB vs. pSB1C3 experiment

7/2/15

Aims for Today:

1. Sequencing of pACBB-lux
2. Pour gel
3. Run pACBB on gel
4. At 11 AM, Miller Assay of pDawn light vs. dark
5. Co-culture pSB1C3 + lux and pDawn + lacZ. You have to cotransform the opposite antibiotic resistance into each plasmid before transformation.
    a. Dialysis Tube (ask Mike for this) _ controls for them growing at different rates
    b. Just pipet them into the same tube
6. Grow up a giant culture of pSB1C3 + lux; induce at the right times for visible luminescence
    a. reach out to the iGEM teams who did similar experiments to see what they did for a visible glow.

Accomplishments:

1. Sequencing of pACBB-lux
2. Pour gel
3. Run pACBB on gel
4. At 11 AM, Miller Assay of pDawn light vs. dark

Aims for Tomorrow:

1. Run PCR of pACBB-lux on gel
2. Pour gel
3. Send in sequencing of pACBB-lux
4. At II AM, miller assay of pDawn light vs. dark
5. If pACBB gel good, do pACBB vs. pSB1C3 experiment

Week 6 (7/6 - 7/10)

7/6/15

Aims for Today:

1. Autoclave flasks
2. Miller assay of samples from light vs. dark experiment (samples were stored in the fridge for 4 days, so if it doesn't work, restart experiment)
3. Send in sequencing
4. 1:100 pDawn-lacZ in 25 mL LB-Kan

5. Redo PCR of lux-pACBB; troubleshoot?
6. Inoculate pACBB-lux from glycerol stock
7. At an OD of 0.3, induce with arabinose measure luminescence every hour (controls: uninduced pACBB-lux and pACBB-eGFP)
8. At an OD of 0.3, induce with blue light
9. Analyze arabinose spiking data.

Accomplishments:

1. Autoclave flasks
2. Miller assay of samples from light vs. dark experiment attempted_ experiment restarted
3. *Send in sequencing*
4. 1:100 pDawn-lacZ in 25 mL LB-Kan
5. Redo PCR of lux-pACBB
6. Inoculate pACBB-lux from glycerol stock
7. At an OD of 0.3, induce with arabinose measure luminescence every hour (controls: uninduced pACBB-lux and pACBB-eGFP)
8. At an OD of 0.3, induce with blue light
9. Analyze arabinose spiking data.

Aims for Tomorrow:

> Debrief from monday meeting

- sequence Mike's pDawn+lacZ
- research lacZ degradation rate (decide if we need to characterize)
    - if we can't find much, expose pDawn lacZ to blue light for 2h then measure lacZ expression every 2 hours until 20 h
- clone pDawn essentials onto pACBB backbone (make primers to PCR off pDawn portion needed)
- adjust beta-galactosidase curve for concentration
- discuss Karol database stuff (we disagree with Monday's discussion)
- be more careful with what we put in the Monday meeting slide
    - don't put in tentative data
    - keep it short
    - focus on the questions you have
    - focus on graphs, experimental designs, conclusions
- data into excel spreadsheet
- talk to Victor about lacZ, decide whether it's worth it to maybe switch to GFP
    - ask Victor about how quickly it degrades
- as soon as sequencing results come back, cotransform
    - either with pACBB or other compatible plasmids with the opposite antibiotic resistance
- do an experiment with
- do the poster 2 weeks before the July 31st deadline
    - present it at the meeting, so we can nip any potential problems in the butt
- talk about restructuring our day and integrating most poster/website stuff
    - include laying out experimental designs and sending them out

Questions:

7/7/15

Aims for Today:

1. Miller assay
2. Arabinose spiking experiment
3. Research on lacZ degradation
4. Pour gel
5. Grow up from glycerol stock *B2* BL21 lux for OD experiment
6. Run gel of pACBB PCR
7. Talk to Seville about equipment
8. Sequence pDawn+lacZ
9. Plan lacZ experiment
10. Look into XGal
11. Grow Mike's pDawn *get from Mike
12. Figure out luminometer
13. Grow up pDawn-LacZ (A1)

Accomplishments:

1. Miller assay
2. Pour gel
3. Run gel of pACBB PCR
4. Plan lacZ-degrading experiment

Aims for Tomorrow:

1. 1:100 for OD experiment
2. Induce with arabinose at dif OD and take luminescence measurements
3. Illuminometer meeting with Mike
4. 1:100 of pDawn-lacZ in 25 mL
5. At OD of 0.4, induce with blue light
6. Measure lacZ every 3 hours

7/8/15

Aims for Today:

1. Sequence rest of pACBB-lux
2. Sequence pDawn-lacZ (Mike's) with primers that bind to outside
3. Pour gel
4. Run gel of pDawn+lacZ PCR
5. Do pDawn+mRFP light vs. dark experiment (1:100 at 11 AM and induce at 2 PM)
6. 1:100 BL21 lux in 25 mL LB+chlorom
7. Design primers to swap out promoter in lux

8. pACBB vs. pSBIC3 experiment

Accomplishments:

1. Backdiluted BL21 and DH5-alpha with pDawn RFP 1:100 for blue vs. dark experiment
2. Induced pDawn RFP strains at OD ~.3-.4 with blue light
3. Poured gel, ran gel of pDawn lacZ PCR check → success! lacZ amplified, next step if to sequence pDawn lacZ to make sure the band is what we think it is
4. Inoculated BL21 lux from glycerol stock for arabinose induction at different OD experiment
5. Designed primers to change lux operon promoter to T7 (not pBAD) by cloning into pRSFDuet and pET-26b(+) backbones
6. Backdiluted pACBB lux 1:100, induced with arabinose at OD ~.4, took OD measurements every hour for 7h (to characterize pACBB lux expression vs. pSCB1C3 lux expression)

Aims for Tomorrow:

1. Backdilute BL21 lux 1:100 in LB Chlor (9 AM)
2. Measure RFP expression in pDawn blue light vs. dark experiment at 20 h (10 AM)
3. Backdilute pDawn lacZ 1:100 in 25 mL LB Kan to redo blue vs. dark experiment
4. induce pDawn lacZ with blue light at OD ~.4
5. Miniprep pDawn lacZ
6. Send of pDawn lacZ for sequencing with primers (2) that bind to outside pDawn
7. Analyze pACBB vs. pSB1C3 luminescence results
8. Think about swapping out pDawn backbone for pACBB and redoing lacZ cloning (decide what to do moving forward)

Questions:

1. Should we move forward with changing the lux operon promoter to T7 ASAP, and should we order the primers for the cloning tomorrow?

7/9/15

Aims for Today:

1. Glycerol stock pDawn RFP
2. Induce pDawn lacZ at OD .4 for light vs dark experiment
3. Miniprepped pDawn RFP and pACBB lux
4. Induce BL21 with arabinose at different OD and measured luminescence
5. Sequence miniprepped pDawn lacZ with pDawn binding primers
6. Sequence miniprepped pACBB lux
7. Analyze pACBB vs. PSB1C3 lux results
8. Think about swapping out pDawn backbone
9. Analyze pDawn RFP light vs. dark results

Accomplishments:

1. Cotransformed pDawn RFP and pACBB lux into BL21
2. Glycerol stock pDawn RFP

3. Miniprepped pDawn RFP and pACBB lux
4. Analyzed pACBB vs. PSB1C3 lux results
5. Analyzed pDawn RFP blue light vs. dark results
6. Looked into h-ns mutant E. coli and effects on lux operon expression
7. Looked into other plasmids that would luminesce better than the lux operon biobrick

Aims for Tomorrow:

1. Backdilute BL21 lux 1:100 in 40 mL LB Chlor for OD arabinose induction experiment
2. Sequence miniprepped pDawn lacZ with pDawn binding primers
3. Sequence miniprepped pACBB lux
4. Think about swapping out pDawn backbone
5. Induce BL21 with arabinose at different OD and measured luminescence (started today but issues with Tecan readings)
6. Pick colonies from transformation plates, inoculate, glycerol stock (glycerol stock later in day)
7. Look into ordering h-ns mutant E. coli and ordering plasmids off Addgene that may luminesce brighter than lux operon BioBrick

Questions:

7/10/15

Aims for Today:

1. Back dilute BL21 lux 1:100 for temperature experiment
2. Send off pDawn lacZ, pDawn RFP, and pACBB lux for sequencing
3. induce BL21 with arabinose and grow at different temperature
4. Pick colonies from cotransformation of pACBB lux and pDawn RPF
5. Set up experiment to see if pACBB lux induced RPF expression
6. organize data
7. prepare BL21 competent cells
8. glycerol stock pDawn lacZ/RFP + pACBB lux BL21 strains from cotransformation (4 colonies per cotransformation)

Accomplishments:

1. Back dilute BL21 lux 1:100 for temperature experiment
2. Send off pDawn lacZ, pDawn RFP, and pACBB lux for sequencing
3. Induce BL21 with arabinose and grow at different temperature, measure every hour
4. Pick colonies from cotransformation of pACBB lux and pDawn RPF
5. Set up experiment to see if pACBB lux induced RPF expression

Aims for Tomorrow:

1. Finalize what to spend the 20kb on from IDT
2. Order primers for changing lux operon promoter
3. Inoculate pDawn RFP + pACBB lux colonies for fail fast attempt number two, backdilute 1:100 after grown to saturation, induce with arabinose at OD ~.4

4. After 20h, measure RFP expression for all fail fast tubes (Tuesday)
5. Analyze lux and pDawn sequencing results
6. Email asking for the split lux plasmid from this (http://link.springer.com/article/10.1007/s00216-010-4266-7/fulltext.html); specifically asking for Sy102 and SY104
7. Cotransform opposite antibiotic resistence for coculturing experiment (pUC57 Kan, find something for pDawn RFP)
8. Prepare BL21 competent cells (grow up cells in the morning to OD .3-.4 is the first step)

Questions:

1. How do you make BL21 competent cells?
2. What white microwell plates should we order?
3. Can we order primers to move ahead with replacing the pBAD promoter in lux with a T7 promoter?
4. How should we plan the fail fast experiment? Right now, we have it set up so each cotransformed colony will grow in 1 tube with light (not induced with arabinose to check for pDawn presence/activity alone) and two tubes in the dark (one induced with arabinose as our "test" tubes and one uninduced to show that pDawn is not induced without lux operon expression). All of these are done in 1:1 LB kan LB Chlor media. We also have a negative control of BL21 with the lux operon alone growing in LB kan+chlor to show that it will not grow. This tube will remain in the light and idk if we should induce it with arabinose or not. Anyway, are there any other controls we should seriously consider including?

Week 7 (7/13 - 7/17)

7/13/15

Aims for Today:

1. Plan RFP-lux experiment #2
2. Innoculate from glycerol stock
3. Plan Tecan calibration experiment
4. Ask Mike + Spencer to grant us access to BE undergrad lab
5. Analyze sequencing for pDawn lacZ and lux

Accomplishments:

1. Innoculate from glycerol stock
2. Plan Tecan calibration experiment
3. Prepare presentation.

7/14/15

Aims for Today:

1. Autoclave flasks
2. Make BL21 competent cells (6; grow up 1)
3. Make LB

4. Make LB + chlor
5. Co transform w/ opposite antibiotic
    a. Ask Mike for pet26B
    b. Order primers to swap out GFP
6. Tecan test
    a. inoculate A5 and B2/B3
7. Print a toucan
8. 1:100 pDawn + lacZ; At an OD of 0.4 induce
9. 1:100 cotransformed stuff; At an OD of 0.4 induce
10. Make arabinose
11. Order luminol + email iGEM team
12. Analyze sequencing results.

Accomplishments:

1. Autoclave flasks
2. Analyze sequencing results.
3. Make arabinose
4. 1:100 cotransformed stuff; At an OD of 0.4 induce
5. Inoculate
    a. Tecan test-
        i. **A1** - Mike's ligated pDawn + lacZ (as a negative control)
        ii. **B2** - pSB1C3 + lux box (to test luminescence)
    b. Miller Assay (light vs. dark)-
        i. **A1** - Mike's ligated pDawn + lacZ
        ii. **B2** - pSB1C3 + lux box (negative control)

BL21 + lux - USE B2
Mike's ligated pDawn +lacZ - USE A1
pACBB + lux - USE A7

Aims for Tomorrow:

1. Pick up LB from the autoclave
2. Make BL21 competent cells
3. Make BL21 glycerol stock
4. Tecan test to make sure that luminescence measurements are correct
5. Co transform w/ opposite antibiotic
    a. Ask Mike for pet26B
    b. Order primers to swap out GFP
6. 1:100 pDawn + lacZ.
7. Induce with blue light at an OD of 0.4
8. Inoculate cotransformed pACBB + lux box & pDawn + lacZ for experiment tomorrow.
9. Analyze data for cotransformed mRFP experiment
10. START POSTER

Aims for Thursday:

1. Run two experiments simultaneously
    a. Nikita/Hannah
        i. Miller assay for pDawn + lacZ
            1. After 20 hours, measure lacZ expression
            2. Analyze data
    b. Jane/Karol
        i. Repeat cotransformed mRFP experiment with cotransformed lacZ
2. Based on results from the first experiment, validate the second experiment.

7/15/15

Aims for Today:

1. Make new LB (since we messed it up)
2. Make BL21 competent cells
3. Make BL21 glycerol stock
4. Grow up pet26B
5. 1:100 pDawn + lacZ.
6. Induce with blue light at an OD of 0.4
7. Inoculate cotransformed pACBB + lux box & pDawn + lacZ for experiment tomorrow.
8. Analyze data for cotransformed mRFP experiment

Accomplishments:

1. Make new LB (since we messed it up) + LB chlor
2. Grow up pet26B
3. 1:100 pDawn + lacZ.
4. Induce with blue light at an OD of 0.4
5. Inoculate cotransformed pACBB + lux box & pDawn + lacZ for experiment tomorrow.
6. Analyze data for cotransformed mRFP experiment
7. Pla big flask culture experiment

Aims for Tomorrow:

1. 20 hr of pDawn-lacZ
2. 20 hr of RFP
3. Run big flask culture experiment
4. Analyze RFP
5. pDawn-LacZ Failfast Experiment
    a. At 9AM do a 1:100 dilution
6. Set up PET primers
7. Design high GC-lux
8. Email Cambridge iGEM
9. Black well plates
10. Order luminol

11. Order primers to swap out GFP and RFP

7/16/15

Aims for Today:

1. 20 hr of pDawn-lacZ
2. 20 hr of RFP
3. Run big flask culture experiment
4. Analyze RFP
5. pDawn-LacZ Failfast Experiment
    a. At 9AM do a 1:100 dilution
6. Set up PET primers
7. Design high GC-lux
8. Email Cambridge iGEM
9. Black well plates
10. Order luminol
11. Order primers to swap out GFP and RFP

Accomplishments:

1. 20 hr of pDawn-lacZ light vs. dark experiment
2. 20 hr of RFP reading
3. Run big flask culture experiment
4. Analyze RFP
5. pDawn-LacZ Failfast Experiment
    a. At 9AM do a 1:100 dilution
    b. Miller assay at 3 time points (2,5 and 7 hours)
    c. OD and luminescence readings every hour
6. Set up PET primers
7. Email Cambridge iGEM
8. Order black well plates

Aims for Tomorrow:

1. Analyze data from pDawn-lacZ failfast experiment
2. Analyze data from pDawn-RFP failfast experiment
3. Determine if the failfast worked
4. Start the Monday Meeting presentation
5. Talk about final goals

7/17/15

Aims for Today:

1. Analyze data from pDawn-lacZ failfast experiment
2. Analyze data from pDawn-RFP failfast experiment

3. Determine if the failfast worked
4. Start the Monday Meeting presentation
5. Talk about final goals

Accomplishments:

1. Analyze data from pDawn-lacZ failfast experiment
2. Analyze data from pDawn-RFP failfast experiment
3. Determine if the failfast worked
4. Start the Monday Meeting presentation
5. Talk about final goals

Aims for Monday:

1. Determine direction
2. Finish Monday Meeting presentation

Week 8 (7/20 - 7/24)

7/20/15

Aims for Today/Accomplishments:

1. Finish analyzing data
2. Prepare for Monday Meeting presentation
3. Talk more about our goals and direction now that the failfast didn't "work"
4. How do we get it to glow?

Aims for Tomorrow:

1. Transform pACBB+lux and pSB1C3 + lux in Top10/ NEB10
2. Make the photon counter <- HIGHEST PRIORITY
3. Order the inverter
4. Order T7 promoters
5. Email Goulian about H-NS
6. Email about split lux
7. Call Glowell
8. Order high GC lux

7/21/15

Aims for Today:

1. Transform pACBB+lux and pSB1C3 + lux in Top10/ NEB10
2. Make the photon counter
3. Order the inverter
4. Order T7 promoters
5. Email Goulian about H-NS
6. Email about split lux

7. Call Glowell
8. Order high GC lux

Accomplishments:

1. Make the photon counter
2. Order T7 promoters
3. Email about split lux
4. Call Glowell
5. Order high GC lux

Aims for Tomorrow:

1. Transform pACBB+lux and pSB1C3 + lux in Top10/ NEB10
2. Finish the photon counter
3. Order the inverter
4. Email Goulian about H-NS

7/22/15

Aims for Today:

1. Grow up NEB10
2. Finish the photon counter
3. Email Goulian about H-NS
4. Poster deadline is approaching
5. Work on the presentation for Seville's talk

Accomplishments:

1. Make the photon counter
2. Heard back about split lux: going to send it in two weeks if we want it
3. Emailed Goulian and the lab Cambridge got their hns mutant strains from

Aims for Tomorrow:

1. Transform into Top10/ NEB10
    a. 1:100 OF NEB10
    b. Grow to an OD of 0.4
    c. Spin them down
    d. Pour out the LB
    e. Resuspend in TSS buffer ⟵ look in the Chow lab
    f. Transform
        i. NEB Turbo (- control)
        ii. B9, B1, B4, G2 and cotransform G2 and F4
    g. Next week
        i. Leave plates out on the counter? On Monday, pick from them and ultimately make glycerol stock

        ii. Redo lux experiments

7/22/15

Aims for Today:

6. Grow up NEB10
7. Finish the photon counter
8. Email Goulian about H-NS
9. Poster deadline is approaching
10. Work on the presentation for Seville's talk

Accomplishments:

4. Make the photon counter
5. Heard back about split lux: going to send it in two weeks if we want it
6. Emailed Goulian and the lab Cambridge got their hns mutant strains from

Aims for Tomorrow:

2. Transform into Top10/ NEB10
    a. 1:100 OF NEB10
    b. Grow to an OD of 0.4
    c. Spin them down
    d. Pour out the LB
    e. Resuspend in TSS buffer ⟵ look in the Chow lab
    f. Transform
        i. NEB Turbo (- control)
        ii. B9, B1, B4, G2 and cotransform G2 and F4
    g. Next week
        i. Leave plates out on the counter? On Monday, pick from them and ultimately make glycerol stock
        ii. Redo lux experiments

7/23/15

-Skip-

7/24/15

Aims for Today:

1. Make NEB10 competent
    a. 1:100 OF NEB10
    b. Grow to an OD of 0.4
    c. Spin them down
    d. Pour out the LB
    e. Resuspend in TSS buffer ⟵ look in the Chow lab
2. Finish working on the photon counter experiment

3. Pick up primers from cell center
4. Finish sending emails to source parts

Accomplishments:

1. Make NEB10 competent
    a. 1:100 OF NEB10
    b. Grow to an OD of 0.4
    c. Spin them down
    d. Pour out the LB
    e. Resuspend in TSS buffer ← look in the Chow lab
2. Finish working on the photon counter experiment

Aims for Tomorrow:

3. Transform into Top10/ NEB10
    i. NEB Turbo (- control)
    ii. B9, B1, B4, G2 and cotransform G2 and F4
        1. pSB1C3 + lux (B9)
        2. miniprepped pSB1C3 + lux (B1)
        3. pACBB + lux (B4)
        4. pDawn + RFP (G2)

    b. Next week
        i. Leave plates out on the counter? On Monday, pick from them and ultimately make glycerol stock
        ii. Redo lux experiments

Week 9 (7/27 - 7/31)

7/27/15

Aims for Today:

1. Transform parts into NEB10 Competent Cells
    a. B9, B1, B4, G2 and cotransform G2 and F4
    b. NEB Turbo (- control)
        i. pSB1C3 + lux (B9)
        ii. miniprepped pSB1C3 + lux (B1)
        iii. pACBB + lux (B4)
        iv. pDawn + RFP (G2)
2. Finish Monday Meeting presentation
3. Finalize information for the website/poster
4. Plan experiment to measure luminescence with NEB10 tomorrow.

    a. "Big-ass culture" -->3 g/ 20 mL arab
5. Finish photon counter (determine fate?)
    a. Call Tecan
6. Ask Jane
    a. T7_pickup cell center
    b. Codon optimized
    c. Inverter strain
7. Send Fedex for Split Lux

Accomplishments:

8. Transform parts into NEB10 Competent Cells
    a. B9, B1, B4, G2 and cotransform G2 and F4
    b. NEB Turbo (- control)
        i. pSB1C3 + lux (B9)
        ii. miniprepped pSB1C3 + lux (B1)
        iii. pACBB + lux (B4)
        iv. pDawn + RFP (G2)
9. Finish Monday Meeting presentation
10. Finalize information for the website/poster
11. Plan experiment to measure luminescence with NEB10 tomorrow.
    a. "Big-ass culture" -->3 g/ 20 mL arab
12. Determine fate of photon counter
    a. Call Tecan
13. Ask Jane
    a. T7_pickup cell center
    b. Codon optimized
    c. Inverter strain
14. Emailed Yolanda about Fedex for Split Lux

Aims for Tomorrow:

1. Plan out entire summer
2. Plan future experiments

7/28/15

Aims for Today:

1. Flesh out goals for toolbox "story"
2. Transform BBa_K525998 T7 + RBS Biobrick into competent cells (lux gene will be transformed into it to make pSB1C3 + lux)
3. Innoculate cultures
    a. pDawn + mRFP in NEB10 (F4 dna)
    b. pACBB + lux in NEB10 (G2 dna) X 4 (three from same colony to run big ass culture) ::::: (the other is to eventually use for pCR of part)
    c. pSB1C3 + lux in NEB10 (B2 dna)

d. Miniprepped pSB1C3 + lux in NEB10 (B9 dna)
e. Cotransformed (pDawn + mFRP + pACBB + lux) in NEB10
f. pACBB + lux in BL21 (DONE)
4. Obtain HNS strain form Goulian

Accomplishments:

1. Flesh out goals for toolbox "story"
2. Transform BBa_K525998 T7 + RBS Biobrick into competent cells (lux gene will be transformed into it to make pSB1C3 + lux)
3. Innoculate cultures
    a. pDawn + mRFP in NEB10 (F4 dna)
    b. pACBB + lux in NEB10 (G2 dna) X 4 (three from same colony to run big ass culture) ::::: (the other is to eventually use for pCR of part)
    c. pSB1C3 + lux in NEB10 (B2 dna)
    d. Miniprepped pSB1C3 + lux in NEB10 (B9 dna)
    e. Cotransformed (pDawn + mFRP + pACBB + lux) in NEB10
    f. pACBB + lux in BL21 (DONE)
4. Obtain HNS strain form Goulian

Aims for Tomorrow:

1. Figure out how to make T7 + pACBB + lux
2. pACBB + lux in NEB10 big ass culture to test for visible luminescence
3. Glycerol stock
    a. pDawn + mRFP in NEB10 (F4 dna)
    b. pACBB + lux in NEB10 (G2 dna) X 4 (three from same colony to run big ass culture) ::::: (the other is to eventually use for pCR of part)
    c. pSB1C3 + lux in NEB10 (B2 dna)
    d. Miniprepped pSB1C3 + lux in NEB10 (B9 dna)
    e. Cotransformed (pDawn + mFRP + pACBB + lux) in NEB10
4. Pick
    a. HNS that Goulian gave us
    b. BBa_K525998 T7 + RBS Biobrick
5. Research
    a. If we can use the T7 promoter in the HNS strain
    b. If the HNS strain mutant (for AraD) so that the arabinose is not eaten up
6. Creation of T7 + pSB1C3 + lux system
    a. Miniprep pACBB + lux in BL21 to later be used for pCR
    b. Miniprep BBa_K525998 T7 + RBS Biobrick
    c. Plan for digestion, ligation and transformation on Thursday
7. Give presentation to PFP scholars
8. Grow up pACBB + lux and cotransformed pACBB + lux & pDawn + mRFP for fail fast experiment

TOOLBOX STORY → bolded combinations are prepared, but not tested. Italicized combinations are completed; nothing has been tested with nonanol; yellow are knocked out because T7 promoter does not function in NEB10

Possible combinations: TOOLBOX 1 LUMINESCENCE

1. **Lux operon + T7 + high pSB1C3 + BL21 (test with/without nonanol)**
2. Lux operon + T7 + high pSB1C3 + NEB10 (test with/without nonanol)
3. Lux operon + T7 + high pSB1C3 + HNS (test with/without nonanol)

1. Lux operon + T7 + low pACBB + BL21 (test with/without nonanol)
2. Lux operon + T7 + low pACBB + NEB10 (test with/without nonanol)
3. Lux operon + T7 + low pACBB + HNS (test with/without nonanol)

1. ***Lux operon + pBAD + high pSB1C3 + BL21*** (test with/without nonanol)
2. **Lux operon + pBAD + high pSB1C3 + NEB10 (**test with/without nonanol)
3. **Lux operon + pBAD + high pSB1C3 + HNS (test with/without nonanol)**

1. ***Lux operon + pBAD + low pACBB + BL21*** (test with/without nonanol)
2. **Lux operon + pBAD + low pACBB + NEB10** (test with/without nonanol)
3. **Lux operon + pBAD + low pACBB + HNS (test with/without nonanol)**

Possible combinations: TOOLBOX 2 SENDER RECEIVER SYSTEM (Simultaneously done)

4. Lux operon + T7 + high pSB1C3 + BL21 (test with/without nonanol)
5. Lux operon + T7 + high pSB1C3 + NEB10 (test with/without nonanol)
6. Lux operon + T7 + high pSB1C3 + HNS (test with/without nonanol)

4. Lux operon + T7 + low pACBB + BL21 (test with/without nonanol)
5. Lux operon + T7 + low pACBB + NEB10 (test with/without nonanol)
6. Lux operon + T7 + low pACBB + HNS (test with/without nonanol)

4. ***Lux operon + pBAD + high pSB1C3 + BL21*** (test with/without nonanol)
5. **Lux operon + pBAD + high pSB1C3 + NEB10 (**test with/without nonanol)
6. Lux operon + pBAD + high pSB1C3 + HNS (test with/without nonanol)

4. ***Lux operon + pBAD + low pACBB + BL21*** (test with/without nonanol)
5. **Lux operon + pBAD + low pACBB + NEB10** (test with/without nonanol)
6. Lux operon + pBAD + low pACBB + HNS (test with/without nonanol)

IF TIME, TOOLBOX 2

1. Lux AB + CDE
2. Lux AB
3. Lux CDE

HUMAN PRACTICES

1. Two inverter strains

7/29/15

Aims for Today:

1. Figure out how to make T7 + pACBB + lux
2. pACBB + lux in NEB10 big ass culture to test for visible luminescence
3. Glycerol stock
    a. pDawn + mRFP in NEB10 (F4 dna)
    b. pACBB + lux in NEB10 (G2 dna) X 4 (three from same colony to run big ass culture) ::::: (the other is to eventually use for pCR of part)
    c. pSB1C3 + lux in NEB10 (B2 dna)
    d. Miniprepped pSB1C3 + lux in NEB10 (B9 dna)
    e. Cotransformed (pDawn + mFRP + pACBB + lux) in NEB10
4. Pick
    a. HNS that Goulian gave us
    b. BBa_K525998 T7 + RBS Biobrick
5. Research
    a. If we can use the T7 promoter in the HNS strain
    b. If the HNS strain mutant (for AraD) so that the arabinose is not eaten up
6. Creation of T7 + pSB1C3 + lux system
    a. Miniprep pACBB + lux in BL21 to later be used for pCR
    b. Miniprep BBa_K525998 T7 + RBS Biobrick
    c. Plan for digestion, ligation and transformation on Thursday
7. Give presentation to PFP scholars
8. Grow up pACBB + lux and cotransformed pACBB + lux & pDawn + mRFP for fail fast experiment

Accomplishments:

1. Figure out how to make T7 + pACBB + lux
2. Plan for pACBB + lux in NEB10 big ass culture to test for visible luminescence (there weren't enough flasks to do it yesterday)
    a. Autoclave flasks
3. Glycerol stock
    a. pACBB + lux in NEB10 (G2 dna) X 4 (three from same colony to run big ass culture) ::::: (the other is to eventually use for pCR of part)
    b. pSB1C3 + lux in NEB10 (B2 dna)
    c. Miniprepped pSB1C3 + lux in NEB10 (B9 dna)
    d. Cotransformed (pDawn + mFRP + pACBB + lux) in NEB10
4. Pick
    a. HNS that Goulian gave us
    b. BBa_K525998 T7 + RBS Biobrick
    c. pDawn + mRFP in NEB10 (F4 dna)
5. Research
    a. If we can use the T7 promoter in the HNS strain
    b. If the HNS strain mutant (for AraD) so that the arabinose is not eaten up
6. Creation of T7 + pSB1C3 + lux system

    a. Miniprep pACBB + lux in BL21 to later be used for pCR
    b. Plan for digestion, ligation and transformation on Thursday

Aims for Tomorrow::

1. Finish the poster
2. pACBB + lux in NEB10 in big ass culture
3. Miniprep BBa_K525998 T7 + RBS Biobrick
4. 1:100 HNS at 11AM
    a. Make HNS cells competent
5. Glycerol Stock pDawn + mRFP in NEB10 (F4 dna)
6. Cleanup pCR product (lux)
7. Digestion of pCRed lux + miniprepped Biobrick
8. Transform HNS with pBAD + pACBB + lux & pBAD + pSB1C3 + lux
9. Overnight ligation

7/30/15

Aims for Today:

1. Finish the poster
2. pACBB + lux in NEB10 in big ass culture
3. Miniprep BBa_K525998 T7 + RBS Biobrick
4. Cleanup pCR product (lux)
5. Digestion of pCRed lux + miniprepped Biobrick
6. Overnight ligation
7. 1:100 HNS at 11AM
    a. Make HNS cells competent
    b. Grow up HNS for big culture tomorrow
    c. Glycerol stock HNS
8. Transform HNS with pBAD + pACBB + lux & pBAD + pSB1C3 + lux
9. Glycerol Stock pDawn + mRFP in NEB10 (F4 dna)
10. Autoclave BE lab flasks and flasks for big ass HNS culture tomorrow
11. Bleach everything on the table in the back room
12. Talk more about switching out the promoter; ask the advisors if we should bother choosing

Accomplishments:

1. pACBB + lux in NEB10 in big ass culture
2. Miniprep BBa_K525998 T7 + RBS Biobrick
3. Cleanup pCR product (lux)
4. Digestion of pCRed lux + miniprepped Biobrick + cleanup
5. 1:100 HNS at 11AM
    a. Make HNS cells competent
    b. Grow up HNS for big culture tomorrow
    c. Glycerol stock HNS
6. Transform HNS with pBAD + pACBB + lux & pBAD + pSB1C3 + lux

7. Glycerol Stock pDawn + mRFP in NEB10 (F4 dna)
8. Autoclave BE lab flasks and flasks for big ass HNS culture tomorrow
9. Bleach everything on the table in the back room
10. Talk more about switching out the promoter; ask the advisors if we should bother choosing

Aims for Tomorrow:
1. Digestion + Cleanup
2. Overnight ligation
3. Big ass culture for HNS
4. Glycerol stock HNS
5. Transform HNS with pBAD + pACBB + lux & pBAD + pSB1C3 + lux
6. Autoclave BE lab flasks
7. Talk more about what final goals will be
8. Finish poster
9. Figure out luminometer
10. If time, transform T7 promoter construct into BL21; also cotransform with pDawn + mRFP (GROW UP IN THE MORNING)
11. 1:100 cotransformed cultures
12. Measure OD, luminescence and RFP every hour

Questions:
1. Should we use the T7 promoter if we cannot put it into HNS or NEB10? Does it detract from the comprehensiveness of the toolbox?

7/31/15

Aims for Tomorrow:
1. Digestion + Cleanup
2. Overnight ligation
3. Big ass culture for HNS
4. Glycerol stock HNS
5. Transform HNS with pBAD + pACBB + lux & pBAD + pSB1C3 + lux
6. Autoclave BE lab flasks
7. Talk more about what final goals will be
8. Finish poster
9. Figure out luminometer
10. If time, transform T7 promoter construct into BL21; also cotransform with pDawn + mRFP (GROW UP IN THE MORNING)
11. 1:100 cotransformed cultures
12. Measure OD, luminescence and RFP every hour

Accomplishments:
1. Digestion + Cleanup
2. Glycerol stock HNS

3. Transform HNS with pBAD + pACBB + lux, pBAD + pSB1C3 + lux, pBAD + pACBB, pDawn + mRFP and cotransformed pACBB + lux and pDawn + mRFP
4. Autoclave BE lab flasks
5. Complete poster (or just generate an idea to be completed on the weekend)

Aims for Monday:

1. Do the cotransformed mRFP experiment
2. 1:100 cotransformed cultures
3. Measure OD, luminescence and RFP every hour
4. Talk more about final goals
5. Iron out the experiments that we have planned for the rest of the summer
    a. tracking
    b. proving orthogonality
6. Prepare final poster presentation to send out to the advisors on Sunday night
7. Prepare for Tuesday Advisor Meeting

Week 10 (8/3 - 8/7)

8/3/15

Aims for Today:

1. Do the cotransformed mRFP experiment
2. 1:100 cotransformed cultures
3. Measure OD, luminescence and RFP every hour
4. Talk more about final goals
5. Iron out the experiments that we have planned for the rest of the summer
    a. tracking
    b. proving orthogonality
6. Prepare final poster presentation to send out to the advisors on Sunday night
7. Prepare for Tuesday Advisor Meeting

Accomplishments:

1. Talk more about final goals
2. Iron out the experiments that we have planned for the rest of the summer
3. Prepare for Tuesday Advisor Meeting
    a. Right now
        i. Orthogonality→ proving for sure that light and chemicals are orthogonal to each other
        ii. Tracking → chemical vs. light
            1. This experiment will demonstrate hopefully that light tracks better than chemicals which would mean it has better spatiotemporal control
        iii. These two principles can be used to build a major experiment (Pim's idea)

1. A bidirectional communication system in which one cell communicates with the second cell with chemicals and the same second cell communicates with the first using light. This should create a "stable" system which oscillates around a line because the K2 (light communication) is higher than K1 (chemical communication). It is a form of negative feedback.

Aims for Tomorrow:

1. Make adjustments to the presentation for advisor meeting with Dr. Chow
2. Iron out more of the cloning details for Pim's bidirectional idea
3. Make changes based on advisor feedback

8/4/15

Aims for Today:

1. Make adjustments to the presentation for advisor meeting with Dr. Chow
2. Iron out more of the cloning details for Pim's bidirectional idea ⟵ Too complicated; not necessarily based on an engineering principle; our abstract talks about this bidirectional communication, but our experiments are not necessarily aligning with it.
3. Rework the presentation to fit a more probable system that we can use.

Accomplishments:

1. A semi-completed poster
    a. What we thought was bistable communication, but instead was just a system that would permanently exist in the second state after addition of light

Aims for Tomorrow:

1. Prepare a distance experiment in which you have six tubes and place tubes inside them so that you can run "co-tubing experiment"
    a. Grow up NEB10 glycerol stock one in the dark (pDawn + mRFP) and one in the light (pACBB + lux)
    b. 1:100 dilute into flasks after reaching saturation
    c. Measure at 20 hours after separating into individual tubes
    d. Adjust experiments based on if you can get this to work
2. Prepare a list of principles that we want to base our entire project on; come up with a list of experiments that complement this list
    a. Ideas discussed during the meeting:
        i. Pim's tracking idea in which we mutate the lov domain to create different tracking curves
        ii. Possibly a distance idea⟶ further iron out the application of this type of a project

8/5/15

Aims for Today:

1. Prepare a distance experiment in which you have six tubes and place tubes inside them so that you can run "co-tubing experiment"
   a. Grow up NEB10 glycerol stock one in the dark (pDawn + mRFP) and one in the light (pACBB + lux)
   b. 1:100 dilute into flasks after reaching saturation
   c. Measure at 20 hours after separating into individual tubes
   d. Adjust experiments based on if you can get this to work
2. Prepare a list of principles that we want to base our entire project on; come up with a list of experiments that complement this list

Accomplishments:

1. Prepare a list of principles that we want to base our entire project on; come up with a list of experiments that complement this list
   a. Current ideas on the table
      i. Light compatible with strains or environment
      ii. Tuneable photoreceptors with the lov mutation
      iii. Luminescence signal → less persistent signal
      iv. Chemical and light → different rates of production; orthogonality
      v. Light driven spatiotemporal control of chemical communication
2. Ironed out specific experiments for the above
3. Prepared the "co-tubing" experiment to run tomorrow

Aims for Tomorrow:

1. Go through the principles/project ideas with advisors and narrow down to the ones we want
   a. Finish the co-tubing experiments and find out which ones are feasible ^
2. Finish cloning the T7 promoter
3. Characterize individual sender + receiver parts
   a. Find out what the sender cell curve looks like in NEB10
   b. Find out if the receiver responds to the sender cell; simulate the light curve to test this???
4. Plan experiments for the chosen project idea more thoroughly
5. Redo poster for next Monday Meeting with Dr. Chow

8/6/15

Aims for Today:

1. Finish ranking the projects/principles
2. Take flasks out of autoclave at 12 PM
   a. 1:100 dilute them
   b. Induce at OD of 0.3
   c. Take 20 hr measurement tomorrow
3. Finish pACBB + lux in NEB10 luminescence curve
4. Inverter
   a. Identify 3 with different strengths

5. Email advisors about projects; remind them that the abstract is due tomorrow

Accomplishments:

1. Email advisors about projects; remind them that the abstract is due tomorrow

Aims for Tomorrow:

1. Finish cloning T7
2. Retransform into HNS
    a. F4
    b. B9
    c. G2
    d. F4 + G2

-Skip Week 11-

Week 12 (8/17 - 8/21)

8/17/15

Aims for Today:

1. inoculate pdawn rfp (dh5alpha and BL21) for receiver characterization
2. inoculate psb1c3 lux in NEB10
3. backdilute psb1c3 lux at 6 PM
4. induced lux psb1c3 with arabinose at 10 PM (when OD at ~.4)
5. order LB plates
6. finish soldering circuit for pDawn receiver characterization
7. setup tubes for pDawn receiver characterization experiment (to start tomorrow) (program arduino to turn off blue light at certain times)
8. plate agar stab with split lux operon
9. plate agar stabs with each antibiotic only to separate plasmids
10. transform psb1c3 lux operon into h-ns
11. regraph pelleting volume luminescence data with photons on x-axis

Publication for reference on split lux:
http://link.springer.com/article/10.1007/s00216-010-4266-7/fulltext.html

Accomplishments:

1. inoculate pdawn rfp (dh5alpha and BL21) for receiver characterization
2. inoculate psb1c3 lux in NEB10
3. backdilute psb1c3 lux at 6 PM
4. induced lux psb1c3 with arabinose at 10 PM (when OD at ~.4)
5. order LB plates
6. finish soldering circuit for pDawn receiver characterization
7. plate agar stab with split lux operon

8. plate agar stabs with each antibiotic only to separate plasmids
9. transform psb1c3 lux operon into h-ns

Aims for Tuesday:

1. backdilute pDawn RFP 1:100 for receiver characterization
2. induce pDawn RFP with light at 10 AM (when OD at ~.4)
3. turn off blue light at appropriate times for pdawn receiver characterization (or check that arduino is working)
4. 8 AM → take a 10 h timepoint for luminescence of psb1c3 lux
5. take hourly luminescence and OD readings for psb1c3 lux up to 17 h (3PM)
6. pick split lux operon colonies, inoculate (at least 3 colonies)
7. pick and replate colonies for attending to separate out plasmids
8. pick h-ns lux transformed colonies, inoculate

8/18/15

Aims for Today:

1. glycerol stock h-ns transformed with psb1c3 lux (at least 3 colonies)
2. glycerol stock split lux operon colonies (at least 3 colonies)
3. inoculate h-ns lux from glycerol stock for time course experiment
4. pick and replate colonies for attending to separate out plasmids for second time
5. Replan NEB10 pSB1C3 + lux/ HNS pSB1C3 + lux experiment

Accomplishments

1. Website

- Make LB Media
    1. chlor
    2. amp
    3. chlor + amp
    4. kan
- To Make Glycerol stocks:
    1. Grow up SY102 in Chlorom to isolate CDE
    2. Grow up SY104 in Chlorom to isolate CDE
    3. Grow up SY102 in Amp to isolate AB
    4. Grow up SY102 in Amp + Chlorom to isolate split lux
    5. Grow up SY104 in Amp + Chlorom to isolate split lux
- To see if plasmid has been lost
    - Grow up SY102 plated on Chlor in Amp to see if it survives
    - Grow up SY104 plated on Chlor in Amp to see if it survives
    - Grow up SY102 plated on Amp in Chlor to see if it survives
- For the timecourse experiment:
    - Take the following upstairs:
        - pSB1C3 + lux in HNS (x2)

- pSB1C3 + lux in NEB10
    - pACBB + lux in HNS (x2)
    - pDawn + mRFP in BL21 → should be in foil
    - pDawn + mRFP in DH5alpha→ should be in foil
    - Take aluminum foil upstairs just in case you cannot find it.
- At 9PM,
    - Measure OD of cultures below to ensure that they have reached saturation (see if one of the two tubes has not grown properly)
        - pACBB + lux in HNS
        - pACBB + lux in HNS
    - 1:100 in 100 ml media culture flasks (so we add 1000 ul of culture into the appropriate flask)
        - pSB1C3 + lux in HNS
        - pSB1C3 + lux in NEB10
        - pACBB + lux in HNS
    - 1:100 in 20 ml media culture flasks(so we add 200 ul of culture into the appropriate flask) COVER GIANT FLASKS IN FOIL
        - pDawn + mRFP in BL21 → should be in foil
        - pDawn + mRFP in DH5alpha→ should be in foil
    - Place all flasks in the incubator; make sure they are closed; make sure that the flasks are not falling out
- At 11:30-12:00PM,
    - Measure the OD of ALL cultures; make sure you use conversion to make sure it is appropriate to induce.
    - Separate the following cultures into three tubes with 5 ml of liquid in each one. Then add 50 ul of arabinose to each tube.
        - pSB1C3 + lux in HNS
        - pSB1C3 + lux in NEB10
        - pACBB + lux in HNS
    - Also add arabinose to the big flasks and place them in teh incubator
    - Clean the inside of the culture tube with Karol's set up with isoproponol. Add 3 mL of the appropriate culture into each tube. Turn on the light by making sure the switch is in - position and that the yellow is connected to red and black is connected to black.
    - Once the light turns on, cover the lids with aluminum foil and make sure controls are also properly covered.

Aims for Tomorrow:

1. Sender-cell trendlines:
    a. Start 10-17 hour luminescence measurement for pSB1C3 + lux in both HNS and NEB10
    b. Analyze the data with the pDawn + mRFP experiment. Determine the threshold?
    c. 1:100 (at 10:30) pSB1C3 + lux in HNS (in LB + chlorom) to observe the trend in hours 1-6
    d. At 12:30 PM
        i. Separate into 4 different tubes with 3 mL each (1 is an uninduced control)

ii. Add 30 ul of 1 M arabinose to each tube and take OD and luminescence measurements (approximately 1 PM - 7 PM)

2. Housekeeping:
   a. Glycerol stock SY102 and SY104 + add to LIMS
   b. Pick up plates and nalidixic acid from the cell center.
   c. Pour LB + Chlor plates
3. Plan co-tubing experiment:
   a. 1:100 pSB1C3 + lux in HNS_1000 uL to 100 mL; 1:100 pDawn + mRFP in DH5alpha (in the dark)__500 uL to 50 mL
   b. Prepare 6 tubes
      i. Tube 1 (X2)- in dark; IND 10 mL of pSB1C3 + lux; 3 mL of pDawn + mRFP inside
      ii. Tube 2 (X2)- in dark; UNIND 10 mL of pSB1C3 + lux; 3 mL of pDawn + mRFP inside
      iii. Tube 3 (X2)- in light; 3 mL of pDawn + mRFP inside
   c. At 2 hours, check the OD has reached ~0.3-0.4. Add the appropriate volume of the appropriate culture to the tubes after inducing with 1 M arabinose.
   d. At 20 hr take a fluorescence measurement.
4. Replating for split lux (yesterday proved that the cells have not lost the plasmid)
   a. Re-plate SY102 in Chlorom to isolate CDE
   b. Re-plate SY104 in Chlorom to isolate CDE
   c. Re-plate SY102 in Amp to isolate AB
   d. Tomorrow grow up all plates in opposite antibiotic.

8/19/15

Aims for Today:

5. Sender-cell trendlines:
   a. Start 10-17 hour luminescence measurement for pSB1C3 + lux in both HNS and NEB10
   b. Analyze the data with the pDawn + mRFP experiment. Determine the threshold?
6. Housekeeping:
   a. Glycerol stock SY102 and SY104 + add to LIMS
   b. Pick up plates and nalidixic acid from the cell center.
   c. Pour LB + Chlor plates
7. Plan co-tubing experiment:
   a. 1:100 pSB1C3 + lux in HNS_1000 uL to 100 mL; 1:100 pDawn + mRFP in DH5alpha (in the dark)__1000 uL to 100 mL
   b. Prepare 6 tubes
      i. Tube 1 (X2)- in dark; IND 10 mL of pSB1C3 + lux; 3 mL of pDawn + mRFP inside
      ii. Tube 2 (X2)- in dark; UNIND 10 mL of pSB1C3 + lux; 3 mL of pDawn + mRFP inside
      iii. Tube 3 (X2)- in light; 3 mL of pDawn + mRFP inside
   c. At 2 hours, check the OD has reached ~0.3-0.4. Add the appropriate volume of the appropriate culture to the tubes after inducing with 1 M arabinose.
   d. At 20 hr take a fluorescence measurement.
8. Replating for split lux (yesterday proved that the cells have not lost the plasmid)

    a. Re-plate SY102 in Chlorom to isolate CDE
    b. Re-plate SY104 in Chlorom to isolate CDE
    c. Re-plate SY102 in Amp to isolate AB
    d. Tomorrow grow up all plates in opposite antibiotic.
9. For Jane,
    a. Grow up
        i. HNS
        ii. Split lux (both strains)
        iii. Make sure she has enough flasks
        iv. 1:100 at 6 AM to 7 AM ??? so induce at 8 AM - 9AM
        v. Make sure she has 1 M arabinose and appropriate nalidixic acid solution made up
        vi. Split up the timepoints so that she doesn't have to do everything by herself
10. Website and poster layout completed.