6/19/18

- Cian Colgan
    - Re suspended LUX Operon part (BBa_K325909) with 10µL of dH2O. Transformed 1µL into DH10 competent cell (NOTE Rescued cells with 950µL of LB instead of 300µL of SOC medium). Saved the remaining 9µL of LUX Operon in Eppendorf Tube in -20C. Plated Transformed cells onto an LB CBM Plate and cultured overnight
    - Ordered Sequencing Primers for the LUX Operon part from IDT. See Primer spreadsheet for primers ordered.

6/20/18

- Cian Colgan
    - Prepared a 100mg/mL tube of CBM (Measured out 0.556mg of CBM and added .556mL of dH20)
    - Pulled out overnight plate. Had 2 colonies on it. Prepared overnight cultures of 2mL of LB+ 5µL of CBM Mixture above for both colonies.
    - Transformed another 1µL of LUX Operon part into DH10B because overnight plating yield was so low, and I did not perform a visual test of the colonies (The colony should glow blue when in the dark due to the expression of the plasmid part). Incubated with 300µL of SOC medium instead of LB as last time. Plated onto a CBM plate for overnight.

6/21/18

- Cian Colgan
    - Took out overnight plate. No colonies formed. Suspect it is a problem with the toothpick use as a spreader. Will create an L-Spreader out of a Pasteur Pipette as soon as we can find one.
    - Took out overnight liquid cultures. Tested to see if they glowed in the dark. Cultures failed this test. Ran diagnostics
        - Cause: Determined that the wrong part was isolated from the iGEM plates. The LUX Part was located in well 4L, but 1L was accidentally harvested instead. This part does not code for a LUX operon.
        - Resolution: Threw out the originally harvested 8µL of 1L that remained. Discarded Liquid overnight cultures. Resuspepended the LUX operon part in 4L in 10µL of dH2O. Will retransform on a CBM plate
        - Further Actions: Sequencing has been delayed until Saturday. This is fine as the sequencing primers have still not arrived as of this morning.
    - Transformed 1µL of LUX Part into DH10B and plated. Let grow overnight

6/22/18

- Cian Colgan
    - Took out overnight plate. No colonies formed. Suspect it is a problem with the plates as Rachael indicated she may have added too much antibiotic during the plate synthesis.
    - Prepared new CAM plates using antibiotic stock that I had measured out. Did following tests and controls to determine the problem using DH10B cells

        - Rachael's plate
            - Negative control
            - Cell Competency RFP tester control
        - My Plates
            - Negative control
            - Cell competency RFP tester control
            - LUX operon Part

6/23/18

- Cian Colgan
    - Took out overnight plates
        - No colonies formed on either of Rachael's plate indicating too much antibiotic. Threw out plates
        - Colonies grew on all of plates of mine indicating too little antibiotic. Discarded all plates.
    - Prepared LB+CAM plates using a stock concentration of CAM I found in the -20C Ran the following plates in DH5α
        - Negative control
        - Cell competency tester RFP
        - Lux operon part
            - Ran this again to save time, and even if controls came back negative, could still performed streak purification as colonies would also glow blue

6/24/18

- Cian Colgan
    - Took out overnight plates. All plates showed growth indicating not enough antibiotic. Diagnostics
        - Stock concentration of antibiotics had a yellow pigmentation. Sigma Aldrich indicates this means photodegradation has occurred and antibiotic is bad
        - Also determined that our bottle only contained 100mg of antibiotic and lots of additives to make it water soluble. Suspect that this has been causing problems with antibiotic concentrations as CAM plates were a struggle for lats summer as well who used this exact same bottle. Will order CAM that can be dissolved in EtOH and has no additives
    - LUX part had no blue colonies. Suspect that the colonies that do transform are being out competed as Luciferases consume fat stores in the bacteria. Could not perform a streak purification
    - Unfortunately, in a holding pattern until new antibiotic can be ordered so part can be harvested.

6/26/18

- Cian Colgan
    - Received a 33mg/mL CAM stock from Simone. Prepared 20 plates at 25µg/mL. Ran 3 plate controls in DH5α

        - Negative Control
        - Positive Control RFP
        - LUX

6/27/18

- Cian Colgan
    - Took out overnight plates. Negative control had no growth, and positive control had good. LUX has no growth. Determined that the original part that was harvested from plate 1 well 4L was actually the LUX part. Was not growing blue due to the pBAD being arabinose determinant.
    - ATtempted to resuspend LUX part from 2018 distribution kit as original stock had been thrown out. Harvested the 2017 piece as well. Will run diagnostic digest once it is harvested to confirm identity
    - Created overnight plates of both 2018 and 2017 piece stocks.

6/28/18

- Cian Colgan
    - Took out overnight plates. 2018 showed no growth but 2017 did. Prepared 4 overnight cultures with 33µg/mL CAM

6/29/18

- Cian Colgan
    - Took out overnight cultures and mini prepped the LUX plasmid. Yields were good Purities were good.

7/5/18

- Cian Colgan
    - Submitted the LUX operon for Sequencing with all 18 sequencing primers and VF2 and VR

7/9/18

- Cian Colgan
    - Sequencing results came back.
        - All primers except F4 worked. Need to redesign for final gibson sequencing.
        - 4 Silent mutations appeared in sequence. As they did not change any amino acid and were not around any primers, they will be ignored
    - Designed and Ordered final primers for Gibson Assembly of NC64A

7/26/18

- Cian Colgan
    - Received NC64A IDT best effort construct. Do not have the ability to clone due only being given 500ng of product. Need to add overhang to construct. PCR. Need new Alk-Assem 2 primer. Ordered

7/30/18

- Cian Colgan
    - PCR of NC64A IDT
        - 12.6µL dh20

        - 5µL Phusion HF
        - 3µL NC64A
        - 1.3 Alk-1
        - 1.3 Alk-Assem 2
        - 0.5 dNTP
        - 0.3 Phusion
        - Gel Results
            - Sample was horribly smeared. Will need to try again
    - Received Synechococcus sp PCC 7002 from Bryant lab. Placed into Mets incubator with following settings
        - 60µmol light
        - 30.8C
        - 12 hours light
        - Humidity 30%

8/2/18

- Cian Colgan
    - PCR of NC64A IDT
        - 15.6µL dh20
        - 5µL Phusion HF
        - 1µL NC64A
        - 1.3 Alk-1
        - 1.3 Alk-Assem 2
        - 0.5 dNTP
        - 0.3 Phusion
        - Gel Results
            - Sample was smeared and too faint. Will have to use more template and have to do successive PCR to purify

8/3/18

- Cian Colgan
    - PCR of NC64A IDT
        - 14.6µL dh20
        - 5µL Phusion HF
        - 2µL NC64A
        - 1.3 Alk-1
        - 1.3 Alk-Assem 2
        - 0.5 dNTP
        - 0.3 Phusion
        - Gel Results
            - Sample faint and smeared

8/4/18

- Cian Colgan
    - PCR of NC64A IDT
        - 14.6µL dh20

        - 5µL Phusion HF
        - 3µL NC64A
        - 1.3 Alk-1
        - 1.3 Alk-Assem 2
        - 0.5 dNTP
        - 0.3 Phusion
        - Gel Results
            - Sample faint and smeared
    - Restriction Digest of C-LUXA
        - Recipe
            - 5µL Cut Smart
            - 1 µg C-LUX A
            - 0.5µL XbaI
            - 0.5µL SpeI
            - 41 µL dH20
        - Gel Results
            - Sample digested properly but banding is faint
                - Caused by LUX insert: BB being 3:1
                - Will need to digest 4µg to get desired BB
    - Measured IDT construct on nanodrop
        - No DNA present. Think most of it when out in that first PCR
    - Made Stock solutions for the preparation of the A+ Media
    - Made Liquid A+ media and A+ Media plates
        - A+ Plates now denoted by Orange stripe

8/5/18

- Cian Colgan
    - PCR of NC64A IDT
        - Recipe
            - 14.6µL dh20
            - 5µL Phusion HF
            - 3µL NC64A
            - 1.3 Alk-1
            - 1.3 Alk-Assem 2
            - 0.5 dNTP
            - 0.3 Phusion
        - Gel Results
            - Sample faint and smeared
    - Restriction Digest of C-LUXA
        - Recipe
            - 5µL Cut Smart
            - 4 µg C-LUX A (16µL)
            - 0.5µL XbaI
            - 0.5µL SpeI

                - 28 µL dH20
            - Gel Results
                - Sample digested properly and bright
                - Gel Purification failed

8/5/18

- Cian Colgan
    - Created 5mL liquid overnight of 7002 single picked colony from master plate
    - Streaked PCC 7002 onto new A+ plate to test plates growth capabilities
    - Conditions for above growths
        - 30.4C
        - 12:12 of LED light
        - 150rpm

8/9/18

- Cian Colgan
    - Created 5mL liquid overnight of 7002 single picked colony from master plate
    - Streaked PCC 7002 onto new A+ plate to test plates growth capabilities
    - Conditions for above growths
        - 34C
        - 12:12 of LED light
        - 150rpm

8/12/18

- Cian Colgan
    - Added 7µL Vitamin B12 to each overnight. Suspect the B12 was photodegraded when stored
    - Made new A media
        - A+ without the B12
        - Made new EDTA and Tris Stocks
    - 2mL overnight of pSB1C3-RFP with 2.1µL Chloro stock
    - Made 5mL liquid culture of 7002
        - Added 6.0µL of B12 Stock

8/16/18

- Cian Colgan
    - Received NC64A V4 fragment from IDT
    - Restriction Digest of pSb1C3 (RD1)
        - Mix
            - 6µL pSB1C3-RFP
            - 5µL CutSmart
            - 0.5µL XbaI
            - 0.5µL SpeI
            - 38µL dH20
        - Gel Purification 1
            - Run on Gel by RF
                - Only half run

                - Gel purification failed
            - Gel Purification 2
                - Run on gel by CC
                    - Other half run
                - Gel purification worked
                    - Used Lianne's (Glick Lab) Pipettes
                    - Used Zymo kit
                    - Eluted with 8µL
    - Gibson Assembly of NC64A+BB
        - Recipe
            - 1µL of RD1
            - 3µL NC64A fragment
            - 1µL of dH2O
            - 5µL of Gibson Master Mix
        - Run in 50C water bath for 15 min
        - Transformed into JM109 on LB+CAM plate
            - 5µL transformed
    - Transformed pSB1C5 from iGEM Plate 4 Well 4D into JM109

8/17/18

- Cian Colgan
    - NC64A w/BB Came back successful
        - Made 8 overnight cultures
        - 2mL + 1.25µL CAM stock
    - Overnights of pSB1C5
        - 2 2mL with 1.25µL CAM Stock
- Rachael Filzen
    - Measured OD of Overnight cultures
        - 8/6 0.155
        - 8/9 0.015

8/18/18

- Cian Colgan
    - Miniprep of NC64A w/BB
        - All fragments except F came back with less than 200ng/µL
        - Only F was 273.6 ng/µL. Suspect sample that will be correct
    - Diagnostic Digest of NC64A Fragment
        - Test 1
            - 10µL rxn (Master Mix)
                - 7.9µL water
                - 0.1µL SpeI
                - 1µL Plasmid
                - 1µL CutSmart
            - Control was pSB1C3-RFP
            - Results

            - All fragments except F were around 2kb. Consistent with either NC64A alone or digested back bone alone
            - F was around same size as control. Both were around 4kB. NC64A with Backbone is expected to be around this size
    - Test 2
        - 10µL rxn (Master Mix)
            - 7.9µL water
            - 0.1µL SpeI
            - 1µL Plasmid
            - 1µL CutSmart
        - Results
            - All Samples except F linearized around 2Kb. Inconsistent with predicted size
            - pSB1C3-RFP had a drop fragment around 1Kb. Upon examining RFp sequence, found a previously unknown HpaI site
            - F had a drop fragment around 1.5Kb. This is consistent with NC64A As the expected drop fragment was 1.6Kb. Likely confirms F is the NC64A Fragment
    - Overall Results
        - F is only likely NC64A Fragment. Since QC at IDT Did verify the sequence with Mass spect and we only ligated it into RD1, We are proceeding as though F is the target sample. Will sequence confirm with primers later in the week, but to save time, went ahead and proceed with Alk Operon final assembly
- PCR 1 and PCR 2 for Alk-Operon Assembly
    - Recipe for PCR1 (PCR2)
        - 15.6µL dH20
        - 5µL Phusion HF
        - 1.3µL Alk-1 (Alk-3B)
        - 1.2µL Alk-2B (Alk-4)
        - 0.5µL dNTP
        - 0.3µL Phusion Polymerase
    - Run as gradient
        - PCR 1 70.5C
        - PCR 2 67.5
    - Extension Time of 5:05 (5:00 needed for PCR 2)
    - Gel Purification Results
        - PCR1 Original popped open in the machine. Re ran. Same recipe. Ran on Lee Lab machine
            - Extension time 1:30
        - Both PCR1 and PCR2 got bands. Attempted to gel purify
            - Both Succeeded
                - PCR1 Was brighter and worked wonderfully

        - PCR 2 is lighter. Purity was still good but yield was lower
    - Gibson Assembly of Alk-Operon (Gib-2)
        - Recipe
            - 5µL HiFi Master Mix
            - 1.7µL dH20
            - 1µL RD1
            - 2µL PCR2
            - 0.3µL PCR1
        - Transformed 5µL into JM109

8/19/18

- Cian Colgan
    - Transformation of Alk-Final results
        - Plate had growth, but colonies were all clustered in a ring on the plate. There were not enough individual colonies to run the overnights
        - Streak purified a section of the ring on to new LB+CAM Plate
    - Created 4 5mL cultures with 5µL B12 of 7002

8/18/18

- Cian Colgan
    - Restreak results Alk-Final
        - Strong growth along the streak occurred
        - Produced enough isolated colonies for overnights
        - Made 8 overnights with 2µL LB+1.25µL CAM
    - Made DMSO Freezer Stock of 7002 8/6 culture
        - 264µL DMSO added to 5mL cultures for 5% v/v
        - Directly frozen by liquid nitrogen
- Rachael Filzen
    - Measured OD730 of Liquid cultures
        - 8/6 =.806
        - 8/9 = .199
        - 8/12 = 0.026
    - Made A liquid media and A^{+}+CAM Plates
        - 120µL CAM

8/23/18

- Rachael Filzen
    - Ran diagnostic digests of final Alk operon
        - Control sample: RFP located in CC's "magic box" in –20ºC freezer
        - Experimental sample: Alk operon located in CC's "magic box" in –20ºC freezer
            - 1µL of sample/control
            - 7.9µL dH2O
        - 1µL of Cutsmart buffer or 3.1 buffer

        - 0.1 µL of enzyme(s)
    - Digest 1: Msc1 (cutsmart buffer)
    - Digest 2: Msc1 + Bgl1 (3.1 buffer)
    - Digest 3: Msc1 + Spn1 HF (cutsmart buffer)
    - Placed in 37ºC incubator for 1 hour
- Cian Colgan
    - Results of diagnostic digest from Rachael Filzen
        - Sample F was only sample that matched size and drop fragment expected.
    - Transformed Alk-Final F into JM109 and plated on to LB+CAM

8/24/18

- Cian Colgan
    - Alk-Final F plate looked good. Labeled as Digest confirmed
    - Thawed DMSO Stock of Synechococcus sp 7002
        - Placed into 37C water bath for 4:30 min
        - Transferred to 10mL of liquid A media in a 50mL Baffled flask
        - Covered in Tin foil to prevent light from entering
        - Placed into incubator
    - 8/12 Liquid culture was darkly green and opaque
        - Stuck into 4C cold room
- Javi Gaughan
    - Prepared 2 2mL LB+1.25µL CAM Overnights of Alk-Final

8/25/18

- Cian Colgan
    - Mini Prepped Alk-Final Digest Confirmed overnights
    - Sent out Alk-Final Digest and NC64A F for sequencing (30rxn total)
        - Alk-Final
            - S-Alk-F1 through S-Alk-F11
            - S-Alk-R1 through S-Alk-R11
            - VF2
            - VR
        - NC64A
            - S-Alk-F10, S-Alk-F11
            - S-Alk-R10, S-Alk-R11
            - VF2
            - VR
    - Transformation of Alk-Final into Synechococcus sp PCC 7002
        - Put 8/12 liquid culture in incubator for 2 hours to allow to return to 37C
        - Aliquoted the 3mL culture into 3 1mL cultures into culture tubes
        - Added .5µg Alk-final Digest confirmed to each tube
        - Stuck back on shaking incubator
            - Will plate 100µL of each onto A+ +CAM plates on 8/27/18

8/27/18

- Cian Colgan
    - DMSO Stock Reboot
        - Added 11µL of B12 to the stock solution
        - Removed Tin foil
        - Covered with 3 layers of KimWipes on All sides
            - Removed first layer at 2pm
            - WIll remove second layer at 8am tomorrow
            - Will remove final layer at 3pm tomorrow

8/29/18

- Varun Patel
    - Made .5% Agarose gel for Cian's Colony PCRs
        - Added 1ul Loading Dye
        - Gel Setup:
            - Top Row:
                - Well 1: 1kb+ Ladder 5ul
                - Well 2: + Control 6ul
                - Well 3: -Control 6ul
                - Well 4-24: Samples 1-21 6ul
                - Well 25: 1kb+ Ladder 5ul
            - Bottom Row:
                - Well 1: 1kb+ Ladder 5ul
                - Well 2-20: Samples 22-40 6ul
                - Well 21: 1kb+ Ladder 5ul
            - Ran @110V for 30 min
        - Only Sample 9 produced any sort of banding but it was of wrong size. QuickchangePCR must be run

8/30/18

- Varun Patel
    - Set Up QuickChange PCR 2 tubes each with:
        - 1 µL NC64A F (Dilute to 25ng/µL)
        - 1.3µL Alk-qc1
        - 1.3µL Alk-qc2 (DO NOT ADD YET!!!)
        - 0.5µL dNTPs
        - 0.3µL Phusion
        - 15.6µL dH2O
        - Followed Cian's Protocol for QCPCR to run this QPCR
            - Added Alk-qc2 after holding period
    - Removed all Kimwipes from DMSO Stock

9/3/18

- Varun Patel
    - Discarded plate above Master Plate from incubator
    - Refilled water flasks in incubator to ensure hydration

9/5/18

- Varun Patel
    - Transformed Cian's qcPCR results from 9/2/18 onto JM109 and plated transformation
        - Set in incubator before leaving
    - Made Liquid Cultures of DMSO Solution
        - 4.5ml A+ Media made on 8/30 by VP
        - 500ul DMSO Test stock
        - 5ul B12
        - 2 Cultures Made, A & B
        - OD730 Tested:
            - .076- A
            - .120- B
        - Placed DMSO Stock back in incubator

9/7/18-VP

- Varun Patel
    - Restreaked Master Plate on clean A+ Media plate
    - Set up incubator to run to program 92 after changing temperatures to 34 C and saving
    - MiniPrepped NC64A samples A-F
        - Samples B-F produced usable results.
    - Sent Samples of NC64A out for sequencing
        - Sequencing Procedure
          Get out one 8-Strip PCR tube
          Label the first 6 8-Strip tubes A-F. Label the last 2-Strip F10 and R11
          Place 3µL of the corresponding mini prep into its corresponding PCR tube
          Place 15µL of S-Alk-F10 and S-Alk-R11 Aliquots into their respective tubes

9/12/18-VP

- Varun Patel
    - Varun Patel
        - Set Up QuickChange PCR 2 tubes each with:
            - 1 µL NC64A F (Dilute to 25ng/µL)
            - 1.3µL Alk-qc1
            - 1.3µL Alk-qc2 (DO NOT ADD YET!!!)
            - 0.5µL dNTPs
            - 0.3µL Phusion
            - 15.6µL dH2O
            - Followed Cian's Protocol for QCPCR to run this QPCR
                - Set melting temperature to 57C
                - Added Alk-qc2 after holding period

9/13/18

- Varun Patel
    - Transformed Cian's NC64A colony PCR using JM109 E. coli
        - Plated and put in 37 incubator before leaving

5/14/19

- Cian Colgan
    - RD1
        - 5µL Cutsmart
        - 7µL pSB1C3
        - 0.5µL XbaI
        - 0.5µL SpeI
        - 37µL DH2O
        - Allowed to run overnight
    - RD2
        - 5µL Cutsmart
        - 10µL pSB1C3
        - 0.5µL XbaI
        - 0.5µL SpeI
        - 34µL DH2O
        - Allowed to run overnight
    - Transformed pSB1C3 and pSB1C5 into DH5Alpha Cells. Plated on to LB+CAM

5/15/19

- Cian Colgan
    - Dephosphorylated RD1 and RD2 Overnight digests
    - Gel Purification of RD1 and RD2
        - RD1 bands were very faint. Did not proceed with Gel purification
        - RD2 Gel Purification gave low yields (6.1 and 6.2ng/µL), but they were pure (260/230 of 1.77 and 1.8). Have 9µL of each, so we have enough to proceed with Gibson of FASyn fragments
    - Took out pSB1C3 and pSB1C5 plates. Both plates had colonies.
        - Prepared overnight cultures

5/21/19

- Cian Colgan
    - Minprppred pSB1C5 and pSB1C3 overnights to get more plasmid
    - Performed RD1 and RD2 digests as previously described
        - Purification with the promega kit to see if we could get it to work
            - Results
                - Kit has very heavy EtOH contamination, but has ABS 260> 0.1 indicating DNA present
                - Means that Kit can be used if truly desperate but would prefer not to

5/22/19

- Cian Colgan

- Received gBlock fragments for FAS 1-6. Resuspended in 100µL dH2o according to manufacturer instructions.
- Gibson Assembly (performed half reactions to save reagents)
  - 1µL gBlock Fragment
  - 3µL of RD2 (What we had purified)
    - NOTE: Ran out of original stock od RD2 for FAS 6. Used the ones purified with the promega kit
  - 1µL dH20
  - 5µL HiFi 2x Master Mix
- Transformed all of the Gibson Reactions into JM109 and plated on LB+CAM

5/23/19

- Cian Colgan
  - Took out plates. All plates had colonies
    - 6 had more colonies than any plate
    - 2 has the fewest colonies and needed an extra hour in the incubator to see the colonies
  - Made overnights cultures of 4 colonies per plate
    - 2 only made 3 colonies since that is all there were
    - Made 10 colonies of 3 because manufacturer indicated error in part production that requires additional colony screening

5/24/19

- Cian Colgan
  - Mini Prepped all 29 plasmids and ran diagnostic digest with EcoRI (10µL w/ 1µL digestion)
    - Gel results are weird. Several plasmids appear undigested but are not control sized. Will perform PCR with VF2 and VR to get size of insert to determine what to send to sequencing
  - VF2 VR PCR
    - Setup
      - 1µL Plasmid
      - 1µL VF2
      - 1µL VR
      - 0.5µL dNTPs
      - 0.3µL GoTaq
      - 1µL Taq buffer (10x)
    - Gel Results
      - Expected a fragment of 1.2Kb for proper insert. If the plasmid just ligated to itself, then it is 314bp
      - Most constructs returned 314 ish fragments. Suggests that the plasmids ligated to themselves. Size is still a little smaller than expected. 6B and 6D give the banding that is the proper size. Unclear fully what is happening. Will submit a few test cases to sequencing so we can get a better picture.

- Submitted 8 samples for sequencing
    - 1A,2A,3A, 3C, 4A, 5D, 6B, 6D
    - Used VF2 and VR primers
        - 1Kb parts likely to be out of the range of a single primer so need both directions to confirm sequencing

5/29/19

- Cian Colgan
    - Ordered AlkL Fragment from IDT
        - Added 40bp outside of the Prefix and Suffix. Will allow us to digest with non-isoschizomer enzymes and prevent this stupid phosphorylation issue. Will recommend that all Future gBlocks be designed with these overhangs.
        - Expected delivery date 6/3/19

5/30/19

- Cian Colgan
    - Sequencing results
        - All non 6 samples mapped to regions of the pSB1C5 plasmid outside of the prefix and suffix. This indicates that the plasmid ligated onto itself and was unsuccessful.
        - 6D indicated a side product insertion that did not map back to FAS-6. Discarded sample
        - 6B matched all bases to the reference FAS-6 Sequence. Indicates insertion of the FAS-6 fragment (Currently at 1/6)
        - Will need to reset Gibson assemblies for the remaining 5 reactions using the Promega pSB1C5 stock or a new pSB1C3 digest.

5/31/19

- Cian Colgan
    - Re Ran Gibson assembly using the promega stocks same as above. Transformed and plated on to LB+CAM

6/1/19

- Cian Colgan
    - Gibson Results
        - All plates had colonies. Some plates had very storng colony numbers while others did not.
    - Colony PCR
        - Performed COlony PCR on samples to limit the amoutn of Miniprpes. PCRed pSB1C3 RFP control
        - Setup
            - One Taq 5X 2µL
            - VF2 0.5µL
            - VR 0.5µL
            - Template (10µL Colony Solution)
            - dNTPs 0.5µL

                - Polymerase 0.2µL
                - dH2O 1.3µL
                - Setup as a 2x Master mix and added to 5µL of template to the MM
            - Results
                - Majorirty of samples showed lower bands indicating backbone ligation. Suggest using RD3 and devleoping Gibson Preefix and Suffix Adaptors to overcome this issue
                - Sample 1H yielded a postive result of a band of 1Kb. Submitted for miniprep and sequencing
                - Samples 1F, 2B, 3D, 3G, 4B, 5G, and 5I all showed veyr faint bands around the 1kb mark. Possibly due to either unlinearized plasmid or proper amplication of biobrick just faintly. Also minipreppered and overngihted for seuqencing
    - RD3
        - Receipe
            - pSB1C3 10µL
            - CutSmart 10X 5µL
            - EcoRI 0.5µL
            - PstI 0.5µL
            - dH2O 34µL
        - Overnight Digestion followed by gel purifcation
    - RD3
        - Recipe
            - pSB1C3 10µL
            - CutSmart 10X 5µL
            - XbaI 0.5µL
            - SpeI 0.5µL
            - QuickCIP 1µL
                - Done becuase I suspect that the CIP does nto have long enough to work in that dilute of an environment
            - dH2O 33µL
        - Overnight Digestion followed by gel purification

6/2/19

- Cian Colgan
    - Miniprep of all above mentioned samples
    - Gel Results fo RD1 and RD3
        - RD1 yielded two bands larger than expected. Unclear why. Did not purify
        - RD3 yileded single band of correct size (Drop Fragment predicted to be 41bp) Proceeded with Gel purifcation using Zymo kit
            - 26 ish ng/µL with good purity
    - Submitted Minipreppred smapels of sequencing with VF2 and VR

6/4/19

- Cian Colgan

- Recieved AlkL gBlock. Prepared accoridng to manufactuer instructions.
- Sequencing results
  - Mix of constructs mapping back to their respective gBlock and relinearized plasmid. Indicates the dephosphoryaltion only partially effective as suscected 2 days before.
  - 1F and 3G perfectly matched their gBlock fragments. Saved as final constructs
  - 4B showed a short 200bp insert. Likley a side product of the gBlock assembly reaction. Did nto match to the part. Discarded sample
  - Other cosntructs either dinciated deletion (1H) or relinearized backbone. Decided to proceed with Gibson using Adapters and Rd3 for remaining constructs
- Gibson Assembly of AlkL, Fas-2, Fas-4, Fas-5
  - Receipe
    - RD3 1µL
    - Prefix adaptor (100x dilution) 1µL
    - Suffix Adaptor (100x dilution) 1µL
    - gBlock 2µL
    - HiFi Master Mix 5µL
  - Transformed into JM109

6/5/19

- Cian Colgan
  - Decided that it we have several non screened Fas4 and Fas5 colonies. Decided to perform colony PCR with 16 colonies form each plate as described above.
    - Gell Results
  - Gibson Plates
    - Fas-5 Had a lawn of bacteria. Indicates plate does not have sufficient antibiotic and the construct was not selected for. Discarded plate. Hopefully colony PCR is successful

6/17/19

- Jacob Wolf
  - Made 1L of A+ Medium liquid (including autoclave)
  - Made 500mL of LB liquid (including autoclave)
  - Made 3M 500mL glucose solution
  - Initialized PCR reaction, but followed wrong protocol. Solution was discarded.
- Apple Lee
  - Made 1L of A+ Medium liquid (including autoclave)
  - Made 500mL of LB liquid (including autoclave)
  - Made 3M 500mL glucose solution
  - Restriction Digest RD3
    - Recipe
      - 1uL MultiCore Mix 10X Buffer

                - 0.25uL EcoRI
                - 0.25uL PstI
                - 1uL Template (pSB1C5)
                - 7.5uL dH2O
            - Overnight Digestion Incubation at 37C (will perform gel purification tomorrow)

6/18/19

- Apple Lee
    - Made 500mL of LB liquid (including autoclave)
    - Plated LB medium (19 plates LB+CAM and 19 plates LB+AMP)
        - Stored plates at 4 ℃ in cooling room
    - Stored delivery containing new gibson materials at appropriate temperatures (-20C and -80C)
    - Restriction Digest RD1
        - Recipe
            - 5uL CutSmart 10X Buffer
            - 0.5uL Xbal
            - 0.5uL Spel
            - 1uL QuickCIP
            - 7.3uL Template (Alk Final)
            - 14.3uL dH20
        - Overnight Digestion Incubation at 37C (will perform gel purification tomorrow)
    - PCR of Fas 1 (overnight, will perform gel purification tomorrow)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dH20
    - PCR of Fas 3 (overnight)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas3)
        - 1.3uL Fas4
        - 1.3uL Fas5
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dH20
    - PCR of Fas 6 (overnight)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10

        - 1.3uL VR
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dH20
    - Jacob Wolf
        - Remade PCR mixture, but PCR machine was booked all day. Components stored in -20 ℃.
            - Containing all components for a 25uL reaction less 0.5 uL of NC64A solution which will be added when PCR machine is available
        - Made 500mL of LB liquid (including autoclave)
        - Began making 1L SOC Media liquid
            - Not enough yeast extract remaining will finish upon arrival.
        - Programmed main (Environ TC16) photosynthetic incubator to 5am/5pm cycle, chained the program with itself, and ran the program.
            - Will verify temperature functionality tomorrow
        - Began looking over team wiki page and associated requirements
        - Plated LB medium (19 plates LB+CAM and 19 plates LB+AMP)
            - Stored plates at 4 ℃ in cooling room
        - Stored delivery containing new gibson materials at appropriate temperatures

6/19/19

- Apple Lee
    - Transformation of BBa_J04450 on JM109
        - 300uL SOC media
        - Plated 100uL on LB+CAM
    - MegaPrimer PCR of Fas 2, 4, 5
        - Recipe
            - 1uL template (5B)
            - 10uL of respective gBlock fragment
            - 1.5uL of dH20
            - 12.5uL of Q5 2X master mix
            - 1uL DpnI
        - Transformation to JM109
        - Plate 100uL on LB+CAM plate
        - Overnight incubation at 37C
    - Gel purification of PCR samples (Fas1, Fas3, Fas6) and restriction digest RD3
        - PCR failed; no band results for PCR of Fas1, 3, 6
        - Restriction digest RD3 worked- band matched simulation
- Jacob Wolf
    - Began 1st PCR reaction
        - Added NC64A into 25 uL PCR tube, ran in thermocycler for 4 cycles
        - Added 1 uL of DpnI and ran through 75 minute thermocycler process

                - 60 min @37 °C and 15 min @80 °C, followed by a 4 °C hold.
            - Put tube(labelled: JW) in fridge to store until PCR machine is available again
    - Inspected photosynthetic incubator, found issue with fluorescent lighting
        - Intensity of light was half of that which was programmed, programming was changed, and will be reinspected in a few hours...
        - Intensity issue seems to be solved, will check again tomorrow
    - Gathered reactants for 2nd PCR for quick mixing tomorrow
        - Placed in red rack within -20 °C freezer
    - Began typing project description for safety sheet
        - Per Marti's request
    - Began searching for wiki templates from previous iGEM teams
        - Looking for a good structure to copy - found several candidates
    - Unboxed new supply of yeast extract

6/20/19

- Apple Lee
    - PCR of Fas 1
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dh20
    - PCR of Fas 3
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas3)
        - 1.3uL Fas4
        - 1.3uL Fas5
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dh20
    - PCR of Fas 6
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.5uL polymerase
        - 15.6µL dh20
    - Made 1L SOC media liquid (including autoclave)
        - Stored KCl, sterilised MgCl2, 5M NaOH
    - Gel purification of PCR samples (Fas1, Fas3, Fas6) and restriction digest RD1

        - PCR failed; no band results for PCR of Fas1, 3, 6
        - Restriction digest RD3 worked- band matched simulation
            - Gel purification done with Zymo kit but nanodrop results does not look right so samples have been discarded
    - Analysed overnight incubated plating results
        - Fas 2 and Fas 5 both have <8 colonies
        - Fas 4 did not work- there are no colonies present
    - Made liquid overnight cultures for Fas 2 and Fas 5
        - 2mL of LB
        - 2.2uL of CAM
        - Overnight incubation at 37C for 18hr
- Jacob Wolf
    - Continued preparation of SOC media
        - Created all necessary solutions and stored remaining components
        - Sterilized MgCl2 solution and SOB media,
        - Stored 5M NaOH sol'n and 0.25 M KCl sol'n
    - Examined photosynthetic incubator
        - Program is running at proper temperature and brightness
    - Completed first PCR reaction
        - Added Alk-qc2 and ran in thermocycler for 30 cycles
        - Transformed into JM109 cells and plated for overnight incubation
        - Will remove cells before 11:40 am tomorrow
    - Completed preparation of Second PCR reaction
        - Diluted P-Lux to 10 ng/uL in an eppendorf labelled P-Lux
            - Concentration labelled on side of tube
            - Recipe
                - 15.6µL dH20
                - 5µL Phusion HF
                - 1.3µL Alk-3B
                - 1.2µL Alk-4
                - 1µL of P-LUX (Diluted to 10ng/µL)
                - 0.5µL dNTP
                - 0.3µL Phusion Polymerase
                - Annealing Temperature 67.5C
                - Extension Time of 5:05
        - Put in machine for overnight cycling
        - Apple will take product from machine and put into 4C fridge in the morning found in PCR tube labelled LUX

6/21/19

- Apple Lee
    - Restriction Digest RD1 (37C 4hr incubation)
        - Recipe
            - 5uL CutSmart 10X Buffer

                - 0.5uL Xbal
                - 0.5uL SpeI
                - 1uL QuickCIP
                - 7.3uL Template (Alk Final)
                - 35.7uL dH20
            - Gel Purified Samples, Jacob will nanodrop on Monday
        - Restriction Digest RD3 (37C 4 hr incubation)
            - Recipe
                - 1uL MultiCore Mix 10X Buffer
                - 0.25uL EcoRI
                - 0.25uL PstI
                - 1uL Template (pSB1C3)
                - 7.5uL dH2O
            - Gel Purified Samples, Jacob will nanodrop on Monday
        - Made liquid overnight cultures for BBa-J04450
            - 2mL of LB
            - 2.2uL of CAM
            - Overnight incubation at 37C for 18hr
            - Jacob will take it out in the morning and perform miniprep
        - Miniprep Fas 2 liquid overnight culture
            - Followed Promega Protocol
            - Stored in -20C
        - Discarded overnight culture for Fas 5 (no plasmid identified)
        - Made 1L LB medium liquid (including autoclave)
        - Plated LB medium (35 plates of LB+CAM+0.3mMglucose)
            - Stored plates at 4 ℃ in cooling room
        - Woke up DMSO stock
            - 5mL A+ medium
            - 200uL B12
            - 500uL culture
            - Stored in photosynthetic incubator under tin foil (48hrs)
- Jacob Wolf
    - Inspected plate prepared yesterday JM109 with corrected NC64A
        - Many small colonies found -> proceeding with overnight cultures
        - Plate with remaining growths stored in incubator
    - Lux operon fragment stored in fridge labelled Lux on top
    - Prepared 8 overnight cultures labelled NC64A and numbered accordingly
        - Placed in incubator, will miniprep tomorrow
    - Inspected Photosynthetic incubator, temp. and brightness function correctly
    - Completed production of SOC media 1L
        - Stored in cold room
    - Made 1L LB medium liquid (including autoclave)
    - Plated LB medium (39 plates LB+CAM and 35 plates LB+CAM+0.3mMglucose)
        - Stored plates at 4 ℃ in cooling room properly labelled

- Woke up DMSO stock, stored in photosynthetic incubator under tin foil for 48 hrs

6/22/19

- Jacob Wolf
  - Examined overnight cultures
    - Apple's looks murky, my 8 samples still transparent
      - Proceeding with miniprep on Apple's sample
  - Miniprepped BBa_J04450 sample
    - Stored @-20℃ labelled AL in red rack

6/24/19

- Jacob Wolf
  - Examined Photosynthetic Incubator
    - Still correct temp and brightness
  - Found the following samples in -20 °C
    - k1390001 6-15D in whiterack
    - k1390003 6-15H in white rack plated
    - K1390002 6-15F in yellow rack plated
    - K1218011 3-4N in yellow rack plated
    - K1390004 6-15J in methanotrophs box plated
    - I746916 plated
  - **Determined dilution of primers must occur in sterile water with Cian, will reassess during meeting**
  - Discarded plate from previous transformation
  - Transformed K1390001 into competent cells and placed in shaking incubator for 2 hours
    - Plated cells onto LB+CAM plates for overnight incubation
    - Labelled JM109 K1390001
  - Ran Gibson Reaction for two gblock fragments same recipe once for FAS 2 V2 and once for FAS 4 V2
    - Recipe
      - 1 uL RD3
      - 3 uL gblock
      - 1 uL sterile water
      - 5 uL HiFi 2x Master Mix
    - Placed in 50 °C bath for 15 min
    - Transformed into JM109 for incubation and plating
  - Examined DMSO stock flasks from friday, solution appear colorless and of low volume one was discarded, one was rewrapped and put back into incubator
  - Nanodropped RD1.1, RD1.2 and RD3
    - All three samples qualify for disposal according to flow chart values
    - Samples were kept in yellow rack labelled AL and their respective #
  - Replenished singular DMSO stock to 5mL using A+ media and added the appropriate amount of B12
    - 1.9 uL of B12 was added

        - 1.9 mL of A+ media was added
    - DMSO stock was wrapped in 5 layers of kim wipes and returned to photosynthetic incubator at 6:00pm
    - Plated FAS2 V2 and FAS4 V2 onto LB+CAM plates and stored in 37 °C incubator overnight

6/25/19

- Apple Lee
    - PCR of Fas 1
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel results: Sample evaporated
    - PCR of Fas 3
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas3)
        - 1.3uL Fas4
        - 1.3uL Fas5
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel Results: Sample evaporated
    - PCR of Fas 6
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel Results: Sample evaporated
    - Aliquot 1mL sterile water into 1.5mL Eppendorf tubes x10
    - Made overnight liquid cultures (left in shaking 37C incubator for 18hours)
        - K1390004 6-15J- plated
        - k1390001 6-15D- plated
        - k1390003 6-15H- plated
        - K1390002 6-15F- plated
        - K1218011 3-4N- plated
        - I746916- plated
    - Single Diagnostic Digest of Fas 2 (10uL restriction digest)

        - Recipe
            - 1uL Template (Miniprepped Fas 2)
            - 1uL CutSmart 10X Buffer
            - 0.25uL XbaI
            - 7.75uL dH20
            - 37C overnight incubation
            - Gel purification tomorrow
    - Double Diagnostic Digest of Fas 2 (10uL restriction digest)
        - Recipe
            - 1uL Template (Miniprepped Fas 2)
            - 1uL CutSmart 10X Buffer
            - 0.25uL EcoRI
            - 0.25uL ScaI
            - 7.5uL dH20
            - 37C overnight incubation
            - Gel purification tomorrow
    - Restriction Digest RD4 (50uL restriction digest)
        - Recipe
            - 5uL CutSmart 10X Buffer
            - 0.5uL Xbal
            - 0.5uL Spel
            - 1uL QuickCIP
            - 9.1uL Template (pSB1C3)
            - 33.9uL dH20
            - 37C overnight incubation
            - Gel purification tomorrow
- Jacob Wolf
    - Removed K1390001 (6-15D) LB+CAM plate from incubator
        - Strong, well-distributed colonies grew overnight
        - Stored in cold room in stack with other plates from the same part list
    - Removed 2 gblock fragment plates from incubator
        - FAS 2 V2 had very strong growth
        - Punctured gel on FAS 4 V2 limited central area growth, but edges still contain colonies
        - Stored both in cold room
    - Completed first phase of QCPCR protocol
        - Recipe
            - 15.6 uL dH2O
            - 5 uL of Phusion HF 5x
            - 1.3 uL Alk-qc1 diluted
            - 1 uL NC64A diluted to 24.3 ng/uL
            - 0.5 uL dNTPs
            - 0.3 uL Polymerase
        - Run in Thermocycler for 4 cycles followed by 4 °C hold

- Completed Second phase of QCPCR protocol
    - 1 uL DpnI added to PCR mixture
    - 60 min at 37°C then 4 °C hold
    - **Skipped 10-15 min at 80** °C
    - Stored in 4 °C Fridge Labelled QCPCR NC64A
- Prepared Overnight cultures of the FAS 2 V2 and FAS 4 V2
    - 4 colonies were picked from each plate and were labelled A-H
    - Colonies A-D were for FAS 2 V2, E-H for FAS 4 V2
    - All overnight cultures were prepared
- Nanodropped BBa_J04450 sample
    - Marked for storage and stored in -20 °C in red rack
- Removed most of solidified gel from bottle
    - Rachael will assist with remaining portion tomorrow
- Removed 1 kimwipe from DMSO stock in photosynthetic incubator

6/26/19

- Apple Lee
    - Phusion PCR of Fas 1
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Will perform gel purification tomorrow
    - Phusion PCR of Fas 3
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas3)
        - 1.3uL Fas4
        - 1.3uL Fas5
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Will perform gel purification tomorrow
    - Phusion PCR of Fas 6
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Will perform gel purification tomorrow

- Gel purification of diagnostic digest of Fas 2 from miniprep
    - Single digest result: only very faint single band showed up, need to repeat tomorrow
    - Double digest result:only very faint single band showed up, need to repeat tomorrow
- Miniprep overnight cultures of the FAS 2 V2 and FAS 4 V2 (4 samples each)
    - Miniprepped sample stored in -20C
- Miniprep overnight cultures of the following parts
    - K1390004 6-15J- plated
    - k1390001 6-15D- plated
    - k1390003 6-15H- plated
    - K1390002 6-15F- plated
    - K1218011 3-4N- plated
    - I746916- plated
    - Miniprepped samples stored in -20C
- Gel purification of restriction digest 4 (pSB1C3 template)
    - Result: Band results are of uncut plasmids; need to repeat gel purification with pSB1C3 obtained from BBa_J04450
- Single Diagnostic Digest of Fas 2 (10uL restriction digest)
    - Recipe
        - 1uL Template (Miniprepped Fas 2)
        - 1uL CutSmart 10X Buffer
        - 0.25uL XbaI
        - 7.75uL dH20
        - 37C overnight incubation
        - Gel purification tomorrow
- Double Diagnostic Digest of Fas 2 (10uL restriction digest)
    - Recipe
        - 1uL Template (Miniprepped Fas 2)
        - 1uL CutSmart 10X Buffer
        - 0.25uL EcoRI
        - 0.25uL ScaI
        - 7.5uL dH20
        - 37C overnight incubation
        - Gel purification tomorrow

- Jacob Wolf
    - Completed final step of QCPCR protocol
        - Added 1.3 uL of diluted Alk-Qc2
        - Ran in thermocycler for 28 cycles
    - Replated two Psb1C3 colonies (red in color) onto new LB+CAM plates
        - Old culture was extremely dried out, was left in incubator too long
    - Photosynthetic incubator is still operating correctly at 34 C and on the proper light cycle
    - Posted project description from Marti onto team wiki page

    - Transformed QCPCR product into JM109 for overnight incubation
        - Plated cells onto LB+CAM media and labelled NC64A QCPCR JW
    - Removed kimwipe from DMSO stock

6/27/19

- Apple Lee
    - Gel purification of Phusion PCR products of Fas 1, 3, and 6 + single and double diagnostic digest of miniprepped Fas 2
        - PCR gel results
            - Fas 1 had no band results (no primer bands observed- could potentially be a primer issue, primer Fas 1 had weird nanodrop readings)
            - Fas 3 worked well so proceeded onto gel purification and sample stored in -20C
            - Fas 6 only had bands towards the bottom of the ladder observed (primer bands)
        - Single diagnostic digest result: no bands observed; gel discarded
        - Double diagnostic digest result: no bands observed; gel discarded
    - Transformation of BBa_J04450 to JM109
        - Plated 100uL onto LB+CAM
        - Overnight 37C incubation (18hrs)
    - Transformation of BBa_J23119 to JM109
        - Plated 100uL onto LB+CAM
        - Overnight 37C incubation (18hrs)
    - Nanodropped miniprepped samples (registry parts)
- Jacob Wolf
    - Removed plated NC64A QCPCR from incubator
        - Plate shows strong growth, overnight cultures will be grown overnight
    - Sterilized pipette tips
        - 8 racks of tips (all types) to replenish supplies
    - Ran Overnight PCR of Lux fragment
        - Recipe
            - 15.6µL dH20
            - 5µL Phusion HF
            - 1.3µL Alk-3B
            - 1.2µL Alk-4
            - 1µL of P-LUX (Diluted to 10ng/µL)
            - 0.5µL dNTP
            - 0.3 uL Phusion Polymerase
        - Annealing Temperature 67.5C
        - Extension Time of 5:05 (Therefore run overnight)
    - Prepared 8 overnight cultures with LB+CAM media for QCPCR products
        - 8 colonies were streaked and grown overnight
        - Recipe (each tube)

                - 2 mL of LB
                - 2.2 uL of CAM Stock
            - Labelled QCPCR NC64A followed by a letter (A-H)
        - Removed Kimwipe from DMSO stock
        - Completed cleaning of solidified gel

6/28/19

- Apple Lee
    - Phusion PCR of Fas 6 (modification: changed annealing temperature to 61C)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel Results: Phusion PCR worked, upper band cut and gel purified, nanodrop tomorrow
    - Sent Fas 2 V2 and Fas 4 V2 (4 samples each) for sequencing
        - Including primers: VF2 and VR
    - Diluted S-Alk-F10 primer
        - 100uL 1x stock stored in -20C
    - Made overnight liquid cultures for pSB1C3 (BBa_J04450) and BBa_J23119
        - Stored in 37C shaking incubator for 18hours
    - Made LB plates in preparation for competent cells production
    - Made 1L phi broth (including autoclave)
        - 20g tryptone
        - 5g yeast extract
        - 5g anhydrous magnesium sulphate
        - 0.75g potassium chloride
    - Made 500mL TJB1
        - 6g RbCl
        - 4.94g MnCl2.4H20
        - 1.48g potassium acetate
        - 0.72g
        - 75mL glycerol
        - Stored in 4C cold room
        - pH not adjusted to 5.8 yet and not sterilized yet
    - Made 100mL TJB2
        - 0.12g RbCl
        - 1.10g CaCl2.2H20
        - 0.20g MOPS
        - 15mL glycerol
        - Sterilized with bottle filter

        - Stored in 4C cold room
    - Phusion PCR of Fas 1 (modification: Fas 1 primer dilution re-made)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 1
        - 1.3uL VF2
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel purification on Monday
- Jacob Wolf
    - Removed Overnight cultures to find no growth (transparent media)
        - Suspicious of bacterial contamination of PCR products will repeat QCPCR on Monday if necessary
    - Prepared sterile 50% (v/v) glycerol solution
    - Transformed remainder of QCPCR products into JM109 for overnight incubation
        - Apple will remove plate from incubator upon arrival tomorrow
    - Updated Team Wiki page with project inspiration from Cian
    - Prepared LB plates
    - Prepared 300 mL LB solution
    - Prepared 1L phi broth, 500 mL TJB1, and 100 mL TJB2
        - Followed recipes from Making Competent Cells PDF
    - Removed kimwipe from DMSO stock

6/29/19

- Apple Lee
    - Miniprep BBa_J04450 and BBa_J23119 liquid overnight cultures
        - Followed Promega Protocol
        - Nanodrop and stored samples on yellow rack in -20C
    - Nanodrop Fas 6 cut out gel band
        - DNA present but concentration is too low even after re-elution with 6uL of elution buffer; need to repeat phusion PCR of Fas 6 on Monday

7/1/19

- Nathan Sattah
    - Made variable concentration glucose media for future use
        - Followed LB Agar plate protocol to make 500 mL of media
        - Autoclaved 5 250 mL bottles with the media
        - Kept 250 mL bottles in oven to keep warm
        - Added 250 µL CAM and 500 µL AMP
        - Divided media into 5 bottles
        - Added glucose to get 0.3 mM (50 µL), 0.25 mM (41.67 µL), 0.2 mM (33.33 µL), 0.15 mM (25 µL), 0.1 mM (16.67 µL)
        - Poured and later labelled plates
    - Streaked competent cells (JM109)

        - 4 aliquots, 100 µL each, 4 plates on LB only
        - Kept in 37° overnight
    - Prepping for PCR
        - Nanodropped P-AlkL construct: 104.6 ng/µL
        - Diluted a new stock of P-AlkL using 0.48 µL of P-AlkL and 4.52 µL of water
- Jacob Wolf
    - Restarted QCPCR protocol
        - Recipe
            - 15.6 uL dH2O
            - 5 uL of Phusion HF 5x
            - 1.3 uL Alk-qc1 diluted
            - 1 uL NC64A diluted to 24.3 ng/uL
            - 0.5 uL dNTPs
            - 0.3 uL Polymerase
        - Added 1 uL of DpnI for 60 min digest at 37C
            - PCR machine booked until after lab meeting
            - Tube stored at 4C labelled 64A JW
        - Added 1.3 uL of Alk-qc2 diluted
            - Ran in thermocycler for 28 cycles followed by 4C hold
    - Reviewed Progress from previous weeks and prepared notes for lab meeting
    - Read paper from Cian about FAS pathway for math model
    - Prepared overnight culture of PSB1C3 RFP part
        - Run in shaking incubator overnight in falcon tube
    - Added blank LB+CAM plate to incubator to test for contamination
    - Filter Sterilized TJB1 broth
    - Transformed and plated NC64A QCPCR products for overnight incubation
        - Labelled QCPCR NC64A in 37C incubator
    - Began conceptualizing the model for the synechococcus FAS pathway
- Apple Lee
    - RD4
        - Recipe
            - CutSmart 10x buffer 5µL
            - Template (pSB1C3) 9.5µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - QuickCIP 1µL
            - dH2O 33.5uL
        - Digest for 4 hours at 37C incubator
        - Gel purification tomorrow
    - Phusion PCR of Fas 6 (modification: changed annealing temperature to 61C)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10

        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - PCR ran overnight, gel purification tomorrow
    - Streaked 4 aliquots of JM109 on LB only plates
        - Kept in 37C incubator overnight (18hrs)
    - Re-suspended arrived primers with sterile water and stored primers in -20C
- Marti Gendel
    - Made 500mL of TJB1
        - Added 6g RbCl
        - 4.94g $MnCl_2$ $4H_2O$
        - 1.48 g potassium acetate
        - 0.72g $CaCl_2$
        - 75mL glycerol
        - Distilled H_2O until volume at 500 mL
    - Genomic DNA Isolation Synechococcus sp PCC 7002 following protocol using the Wizard SV Genomic DNA purification System
        - Steps from the “Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria” procedure was followed for steps 1-8
            - Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.
            - Centrifuged at 13,000–16,000 × g for 2 minutes to pellet the cells and removed the supernatant
            - Add 600µl of Nuclei Lysis Solution
            - Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature
            - 3µl of RNase Solution to the cell lysate
            - Incubate at 37°C for 15–60 minutes. Cool the sample to room temperature
        - Then the procedure for the Wizard SV Genomic DNA Purification System was picked up in section 3.C. step 1
            - Wizard. SV Minicolumn assembly containing the sample lysate into a microcentrifuge and spin at high speed for 3 minutes
            - 315mL ethanol added to the Column Wash Solution (CWA)
            - 650µl of Column Wash Solution (CWA) to each Wizard. SV
            - Centrifuge at 13,000 Å~ g for 1 minute and discard liquid x4
            - Add 250µl of room temperature Nuclease-Free Water to the Wizard. SV Minicolumn and place the Wizard. SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 for 1 minute x2
            - NanoDrop Results: 2.8ng/uL
            - The elution tube containing the purified genomic DNA and stored at –20 C

7/2/19

- Jacob Wolf
    - Removed plates from 37C incubator
        - Blank plate was blank as expected, plates likely aren't contamination source
        - QCPCR products showed similar contaminated appearance to previously failed attempt; however, one colony was present
    - Autoclaved tips
    - Restarted entire QuickChange protocol
        - Extra precautions taken to keep sample sterile including
            - New stock dilutions in fresh eppendorf tubes
            - Use of freshly autoclaved tips
        - Recipe - Run for 4 cycles
            - 15.6 uL dH2O
            - 5 uL of Phusion HF 5x
            - 1.3 uL Alk-qc1 diluted
            - 1 uL NC64A diluted to 24.3 ng/uL
            - 0.5 uL dNTPs
            - 0.3 uL Polymerase
        - DpnI digest
            - 1 uL of DpnI added
            - 60 min @37C followed by 4C hold
        - Will complete remaining Portion of the protocol tomorrow (7/3)
    - Created Overnight cultures from the two colonies on yesterday's (7/1/19) plate
        - Two tubes labelled "7/1 plate" to indicate date of plate from which they were picked
    - Grew 5 uL of primer stocks in 100 uL of SOC media in shaking incubator for 2 hours to test for contamination
        - Alk-qc1 and Alk-qc2 are being tested
        - Plated on LB+CAM plates for overnight incubation
- Apple Lee
    - Phusion PCR of Fas 1 (modification: re-dilution of Fas 1 primer)
        - 5µL Phusion HF
        - 1.3uL Fas 1
        - 1.3uL VF2
        - 1uL Diluted Template (Fas1)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Gel results: No band results; PCR did not work
    - Gel purification of Phusion PCR of Fas 6
        - Results: Some bands present but too faint to gel purify
    - Gel purification of RD4 (pSB1C3 as template)

        - Results: Band best matching simulation cut out and gel purified; sample stored in -20C and will be nanodropped tomorrow
    - Phusion PCR of Fas 5 Reaction 1 (modification: changed primer combinations)
        - 5µL Phusion HF
        - 1.3uL Fas 8
        - 1.3uL Fas 5A
        - 1uL Diluted Template (Fas5)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - PCR will run tomorrow
    - Phusion PCR of Fas 5 Reaction 2 (modification: changed primer combinations)
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - PCR will run tomorrow
    - Phusion PCR of Fas 2C (modification: changed template)
        - 5µL Phusion HF
        - 1.3uL Fas 2
        - 1.3uL Fas 3
        - 1uL Diluted Template (Fas2C)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 58C
        - PCR will run tomorrow
    - Phusion PCR of Fas 4E (modification: changed template)
        - 5µL Phusion HF
        - 1.3uL Fas 6
        - 1.3uL Fas 7
        - 1uL Diluted Template (Fas4E)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 59C
        - PCR will run tomorrow
- Nathan Sattah

    - Made liquid overnight cultures for JM109 competent cells, stored in 37° shaker at 3:30
    - Streaked and replated one plate of JM109 competent cells
    - Made A+ + CAM plates for future use
        - Followed A+ Media protocol for 1 liter
        - Added 0.1 mL of CAM per 500 mL A+ media
        - Autoclaved for 45 minutes in two 1 liter bottles, 500 mL each
        - Poured and labelled plates
    - RD-4 Prep
        - CutSmart 10x buffer 5µL
        - Template (pSB1C3) 9.5µL
        - XbaI 0.5µL
        - PstI 0.5µL
        - QuickCIP 1µL
        - dH2O 33.5uL
        - Left in 37° overnight, will run gel tomorrow
    - Prepped PCR-3
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 15.6µL sterile water
    - Prepped PCR-4
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 15.6µL sterile water
    - Made an overnight culture for BBa_J04450, need more of this part for RD-1
- Marti Gendel
    - pSBAI promoter (Use Primers Alk-1 and parB-A and FAS-1 as a template (10ng))
        - 5µL Phusion HF
        - 1.3uLAlk-1
        - 1.3uL parB-A
        - 1uL FAS-1
        - 0.5uL dNTP
        - 15.6µL sterile water
    - Genomic DNA isolation Synechococcus sp PCC 7002 following protocol using the Wizard SV Genomic DNA purification System
        - Wash cells with A+ Media
        - 100uL of Wizard SV Lysis Buffer
        - Wash with 650uL Column Wash Solution x4

        - 250uL Nuclease-Free Water
        - Yielded insufficient concentration of DNA
    - Genomic DNA isolation Synechococcus sp PCC 7002 following protocol using the Wizard SV Genomic DNA purification System
        - Wash cells with A+ Media
        - Resuspend pellet in 1x TE and heated in 70C for 10 minutes and proceeded using the supernatant for DNA purification
        - 100uL of Wizard SV Lysis Buffer
        - Wash with 650uL Column Wash Solution x4
        - 250uL Nuclease-Free Water
        - Yielded insufficient concentration of DNA

7/3/19

- Nathan Sattah
    - PCR-3 (Phusion): VR and AlkL-FLAG-1 (57°) [56.7°]
        - PCR was prepped yesterday, just added enzyme
        - PCR did not work, nothing present
        - Checked primers on Nanodrop, looked fine
        - Need to redo PCR
    - PCR-4 (Phusion): VF2 and AlkL-FLAG-2 (55°)
        - PCR was prepped yesterday just added enzyme
        - PCR worked, vivid bands present
    - Ran gels for the two PCRs and RD3
    - Gel purification
        - PCR-4, did not work, need to redo PCR and gel purification
        - RD-3.1, did not work
        - RD-3.2, worked, keep product for future use
    - BBa_J04450 Miniprep (eventually for RD1)
        - Accidentally discarded supernatant, will have to redo overnight culture
        - Made overnight culture, placed in 37° shaker
    - Examined Apple's nanodrop samples and followed flowchart, kept all examined samples
    - Made LB+CAM+AMP plates for future use
- Apple Lee
    - Ran Phusion PCR of FAS 2C (58.4C), FAS 4E (60.5C), and 2 reactions for Fas 5 (60.5C & 64C) prepared yesterday
    - Nanodrop gel purified products of restriction digest 4 (pSB1C3 template) and discarded sample; need to re-do restriction digest 4 on Friday
    - Gel purification of PCR products of FAS 2C, FAS 4E, FAS 5
        - Results
            - FAS 2C: bands present and matched with simulation (PCR worked); gel extracted and sample nanodropped and stored in -20C

                - FAS 4E: bands present and matched with simulation(PCR worked); gel extracted and sample nanodropped and stored in -20C
                - FAS 5.1: bands present and matched with simulation(PCR worked); gel extracted and sample nanodropped and stored in -20C
                - FAS 5.2: bands present and matched with simulation(PCR worked); gel extracted and sample nanodropped but DNA concentration was not high enough so sample discarded
                    - Need to re-run PCR on Friday
        - Phusion PCR of Fas 6 (modification: changed annealing temperature to 61C)
            - 5µL Phusion HF
            - 1uL Diluted Template (Fas6)
            - 1.3uL Fas 10
            - 1.3uL VR
            - 0.5uL dNTP
            - 0.3uL polymerase
            - 15.6µL sterile water
            - Sample evaporated; need to re-run PCR on Friday
        - Phusion PCR of Fas 1 (modification: re-dilution of Fas 1 primer)
            - 5µL Phusion HF
            - 3.9uL Fas 1
            - 1.3uL VF2
            - 1uL Diluted Template (Fas1)
            - 0.5uL dNTP
            - 0.3uL polymerase (add on Friday)
            - 13µL sterile water
            - PCR will run on Friday
- Jacob Wolf
    - Removed Alk-qc1 and Alk-qc2 contamination test plates
        - Plates did not show any signs of growth, primers likely aren't the source of contamination
    - Removed overnight cultures from two colonies plated on 7/1/19 and noticed a significant amount of bacterial growth
    - Miniprepped Two QuickChange Overnight Cultures for Sequencing
        - Submitted for Sequencing alongside F10 primer
    - Completed QC Protocol on new sample (initiated on 7/2/19)
        - Added 1.3 uL of Alk-Qc2 and ran in thermocycler for 28 cycles
    - Transformed PCR products into JM109 for overnight incubation
        - Two Liquid cultures were prepared
            - One to be plated onto LB+CAM
            - One plated onto LB+CAM+0.3 mM Glucose
    - Continued developing an understanding of the FAS pathway and considering what a model will need to encompass

    - Reviewed Code syntax in R for systems of ODEs
- Marti Gendel
    - Made LB+CAM+AMP plates
    - Examined Apple's nanodrop samples and followed flowchart
    - Did research on companies working in the biofuels sector to potentially reach out to and collaborate with

7/5/19

- Nathan Sattah
    - PCR-3 (Phusion): VR and AlkL-FLAG-1 (57°) [56.7°]
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 0.3 uL Phusion enzyme
        - 15.6µL sterile water
        - Tube evaporated
    - PCR-4 (Phusion): VF2 and AlkL-FLAG-2 (55°)
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 0.3 uL Phusion enzyme
        - 15.6µL sterile water
        - PCR worked, vivid bands present
    - Gel purification for PCR-4
        - Nanodrop: 42.5 ng/µL
        - Looks very good, kept sample
    - Miniprepped pSB1C3 (BBa_J04450) from overnight culture
        - According to protocol
        - Nanodrop: 140.6 ng/µL
    - RD-1
        - CutSmart 10x buffer 5µL
        - Template (pSB1C3) 7.1µL
        - XbaI 0.5µL
        - SpeI 0.5µL
        - QuickCIP 1µL
        - dH2O 35.9uL
        - Removed from 37°C after 4 hours and placed in 4°C
    - RD-3
        - CutSmart 10x buffer 5µL
        - Template (pSB1C3) 7.1µL

        - EcoRI 0.5µL
        - PstI 0.5µL
        - QuickCIP 1µL
        - dH2O 35.9uL
        - Removed from 37°C after 4 hours and placed in 4°C
    - pSB1A2: Transformation
        - Added 10 µL of water to sample from plate 3, 16D
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of pSB1A2 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+AMP plate
        - Stored in 37°C overnight and moved to the cold room
    - Ran PCR-3 again overnight, tube was moved to 4°C fridge
- Jacob Wolf
    - Removed two plates from incubator
        - 7 colonies on LB+CAM plate
            - Will pick 6 for overnight cultures to be miniprepped tomorrow
        - No colonies on LB+CAM+0.3mM Glucose plate
    - Updated Index of Items spreadsheet with information from reactions run during this and the previous week
    - Aided Marti in the preparation of next week's stock of cyanobacteria
    - Prepared 6 overnight cultures of QuickChange Colonies
        - Samples will be miniprepped tomorrow by Marti
    - Wrote down chemical reactions in FAS pathway
        - Preparation to program the backbone of the code until further data is available
- Marti Gendel
    - PCR-6 (Phusion): Alk-1 and ParB-A (56°)
        - PCR was prepped on tuesday just added 0.3 uL Polymerase
        - Assisted in the miniprep of pSB1C3
    - Separate cyanobacteria stock into 2 new waffle flasks and one culture tube
        - Waffle Flask One: 1mL of stock + 9mL of A+ media + 9uL B12
        - Waffle Flask Two: 500uL of stock + 4.5mL of A+ media + 4.5uL B12
        - Culture Tube: 1mL of stock + 9mL of A+ media + 9uL B12
    - Gel purification for PCR-6
        - Nanodrop: 36.9ng/uL
        - Looks good, kept sample
- Apple Lee
    - Phusion PCR of Fas 6 (modification: changed annealing temperature to 61C)
        - 5µL Phusion HF

        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - Gel results: Band results present and matched simulation; gel extracted but nanodropped reading does not look good; sample discarded
    - Phusion PCR of Fas 5 Reaction 2 (modification: changed primer combinations)
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Gel results: Band results present and matched simulation; gel extracted and sample stored in -20C
    - Ran Phusion PCR of FAS 1 (57C) prepared on Wednesday
    - Gel purification of FAS 1 PCR products
        - Result: No band results present; PCR did not work; need to re-run PCR with different reagents on Monday
    - RD4
        - Recipe
            - CutSmart 10x buffer 5µL
            - Template (pSB1C3) 7.1µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - QuickCIP 1µL
            - dH2O 35.9uL
        - Digest for 4 hours at 37C incubator
        - Sample stored in 4C cold room
        - Gel purification on Monday
    - Designed FLAG primers for each gene on FAS operon with Q5 method and sent them to Cian

7/6/19

- Moved Nathans plate from 37C to cold room
- Took out Nathan's pcr-3 and placed in the 4C
- Miniprepped Jacob's 6 overnight cultures of QuickChange Colonies
    - According to protocol

7/8/19

- Nathan Sattah
    - PCR-3 tube evaporated slightly again, only a little bit of liquid left
    - Ran PCR-3 again
        - PCR worked
    - Gel purified RD-1, RD-3, PCR-3
        - Kept PCR-3: 92.2 ng/µL
        - Kept RD-1.1: 62.3 ng/µL
        - Kept RD-3.1: 56.1 ng/µL
        - Kept RD-3.2: 96.8 ng/µL
        - Kept RD-3.3: 87.7 ng/µL
    - pSB1A2:
        - Made overnight culture from plate
        - 2 mL LB + 2 µL AMP
        - Stored in 37°C shaker at 5:00 pm
- Apple Lee
    - Phusion PCR of Fas 6 (modification: changed annealing temperature to 61C)
        - 12.5µL Q5 2X Master Mix
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 10
        - 1.3uL VR
        - 8.9µL sterile water
        - Annealing temperature: 64C
        - Gel results: Band results are too faint; PCR needs to be re-run
    - Gel purification of restriction digest 4
        - Result: Band results showed that the plasmid was uncut; gel discarded
    - Designed primers for each FASyn gene and sent to Cian
    - Phusion PCR of Fas 6 (modification: changed total volume to 50µL)
        - 10µL Phusion HF
        - 2uL Diluted Template (Fas6)
        - 2.6uL Fas 10
        - 2.6uL VR
        - 1uL dNTP
        - 0.6uL polymerase
        - 31.2µL sterile water
        - Annealing temperature: 61C
        - Sample evaporated
    - Autoclaved tips
- Jacob Wolf
    - Received 6 miniprepped samples from Marti
        - Discarded solutions
    - Verified proper function of photosynthetic incubator
        - Synechococcus cultures appear quite opaque and green

        - Temperature and brightness cycles still operating correctly
    - Prepared 4 tubes overnight cultures of PsB1C3 in LB+CAM media
        - Picked four colonies and grew in falcon tube overnight in shaking incubator for 16-18 hours
    - Prepared PCR 2 (Lux operon Fragment)
        - Recipe
            - 15.6µL dH20
            - 5µL Phusion HF
            - 1.3µL Alk-3B
            - 1.2µL Alk-4
            - 1µL of P-LUX (Diluted to 10ng/µL)
            - 0.5µL dNTP
            - 0.3µL Phusion Polymerase (to be added)
        - Annealing Temperature 67.5C
        - Extension Time of 5:05 (Therefore run overnight)
    - Acquired liquid Nitrogen
        - Passed off to Marti
    - Prepared 7 cultures of NC64A QCPCR products repicked from same 7/3/19 plate
        - Previously untouched colony is now colony “G”
    - Placed picked QCPCR plate in 37C incubator to continue growing overnight in case of failed growth of overnight cultures
- Marti Gendel
    - Nano dropped Jacob’s Mini prep samples
    - Removed 2ml of s.7002 stock from 10mL waffle flask placed into a new waffle flask and added 18mL of A+ media and 18uL of B12 and returned to photosynthetic incubator
    - Removed 1.5mL of s.7002 from the waffle flask and performed new genomic DNA isolation procedure
        - Transfer 1.5 ml of fresh, dense liquid culture of the cell into a microcentrifuge tube. Pellet cells by centrifugation at 20,000 × g for 5 minutes.
        - Add 200 µl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0 OR 0.2 M Lithium acetate, 1% SDS solution).
        - Freeze the cells either using liquid nitrogen or freezer. Boil at 95˚C for 2 min. Repeat; vortex 30 seconds.
        - Add 200 µl of chloroform; vortex 2 minutes.
        - Centrifuge 3 minutes, room temperature, 20,000 × g.
        - Transfer the upper aqueous phase to a microcentrifuge tube containing 400 µl ice-cold 100% ethanol. Mix by inversion or gentle vortexing.
        - Incubate at room temperature, 5 minutes. Alternatively, precipitate at -20°C to increase yield.
        - Centrifuge 5 minutes, room temperature, 20,000 × g. Remove supernatant.

        - Wash the pellet with 0.5 ml 70% ethanol, spin down as described in step 8 above. Remove supernatant.
        - Air-dry the pellets at room temperature or for 5 minutes.
        - Resuspend in 25–50 µl TE [10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0)] or water.

7/9/19

- Nathan Sattah
    - Gib-9: PCR-4, PCR-3, and RD-3.2
        - PCR-4: 1.4 µL
        - PCR-3: 0.7 µL
        - RD-3.2: 0.4 µL
        - NEB HiFi 2X Master Mix: 2.5 µL
        - Incubated in thermocycler for 20 min at 50°C
    - Transformation of Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of Gib-9 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Moved to the cold room until 3:00 pm
        - Plated 100 µL on an LB+CAM plate
        - Stored in 37°C overnight
    - pSB1A2
        - Miniprepped overnight culture
        - Nanodrop looked very good: 51.4 ng/µL
    - RD-5
        - pSB1A2 19.5 µL
        - MultiCore Mix 10X buffer 5µL
        - EcoRI 0.5µL
        - PstI 0.5µL
        - Sterile water 24.5 µL
        - Incubated in 37°C for 4 hours then moved to the cold room
    - Making more pSB1A2
        - Made overnight culture, 3 tubes: 2 mL LB + 2 µL AMP each
        - 2 for miniprep and 1 for glycerol stock
        - Stored in 37°C shaker overnight
    - Diluted AlkL-qc-1 and AlkL-qc-2 primers
        - 90 µL of water + 10 µL of primer stock
- Apple Lee
    - Phusion PCR of Fas 6 (modification: changed total volume to 50µL)
        - 10µL Phusion HF
        - 2uL Diluted Template (Fas6)

        - 2.6uL Fas 10
        - 2.6uL VR
        - 1uL dNTP
        - 0.6uL polymerase
        - 31.2µL sterile water
        - Annealing temperature: 61C
        - Gel results: fragments were too light; bands did not match simulation so gel discarded; PCR needs to be re-run tomorrow
    - Made 500mL liquid LB (autoclaved)
    - Made overnight cultures for JM109 competent cells (18hr 37C shaking incubation)
        - 25mL LB medium
        - 1 colony selected from replated JM109 LB only plate
    - Phusion PCR of Fas 6 (modification: changed total volume to 50µL and increased annealing temperature)
        - 10µL Phusion HF
        - 2uL Diluted Template (Fas6)
        - 2.6uL Fas 10
        - 2.6uL VR
        - 1uL dNTP
        - 0.6uL polymerase (adding tomorrow)
        - 31.2µL sterile water
        - Annealing temperature: 63C
        - PCR running tomorrow
    - Phusion PCR of Fas 6 (modification: changed total volume to 50µL and increased annealing temperature)
        - 10µL Phusion HF
        - 2uL Diluted Template (Fas6)
        - 2.6uL Fas 10
        - 2.6uL VR
        - 1uL dNTP
        - 0.6uL polymerase (adding tomorrow)
        - 31.2µL sterile water
        - Annealing temperature: 65.8C
        - PCR running tomorrow
    - Phusion PCR of Fas 1
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 57C

        - PCR running tomorrow
- Jacob Wolf
    - Received overnight culture tubes and regrown NC64A plate
    - Miniprepped 7 QCPCR overnight cultures
        - Sample G was the only non-repick
    - Miniprepped 4 Psb1C3 overnight cultures
        - Colony D did not produce a red tint before miniprep and was discarded
    - Nanodropped all samples
        - None were marked for disposal
    - Initialized Another round of QCPCR products
        - Recipe
            - 31.2 uL dH2O
            - 10 uL Phusion HF
            - 2.6 uL Alk-qc1diluted
            - 2.0 uL NC64A diluted to 24.6 ng/uL
            - 1.0 uL dNTPs
            - 0.6 uL polymerase
        - Run in thermocycler for 4 cycles followed by 4C hold overnight
    - Prepared 6 QCPCR samples for sequencing
    - Removed Nathan's restriction digest to 4C
- Marti Gendel
    - Ordered p1000 and p2 tips
    - Organized meeting with Chris to get involved in volunteer work at MSI
    - Sent out emails to various corporations in the biofuel sectors
    - Redid DNA isolation protocol with Haneul
        - Removed 1.5mL of s.7002 from the waffle flask
        - Pellet cells by centrifugation at 20,000 × g for 5 minutes.
        - Add 200 µl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0 OR 0.2 M Lithium acetate, 1% SDS solution).
        - Freeze the cells either using liquid nitrogen or freezer. Boil at 95˚C for 2 min. Repeat; vortex 30 seconds.
        - Add 200 µl of chloroform; vortex 2 minutes.
        - Centrifuge 3 minutes, room temperature, 20,000 × g.
        - Transfer the upper aqueous phase to a microcentrifuge tube containing 400 µl ice-cold 100% ethanol. Mix by inversion or gentle vortexing
            - Note that at this step there was still a green tint to the solution
            - The green could have partitioned into the aqueous phase because the other phases had been saturated (potential fix add more chloroform)
        - Centrifuge 5 minutes, room temperature, 20,000 × g. Remove supernatant.
        - Wash the pellet with 0.5 ml 70% ethanol, spin down as described in step 8 above. Remove supernatant.

        - Air-dry the pellets at room temperature or for 5 minutes.
        - Resuspend in 25–50 µl or water
            - Note that at this step part of the pellet was still green and not all of the pellet (DNA) dissolved in the water indicating potentially other cell material was still present

7/10/19

- Nathan Sattah
    - Making more pSB1A2
        - Miniprepped two of the overnight cultures
        - pSB1A2-1: 68 ng/µL
        - pSB1A2-2: 67.2 ng/µL
        - Both Nanodrop graphs looked very good
    - Glycerol stock of pSB1A2
        - Added 750 µL of overnight culture to 750 µL of 50% glycerol in a 2 mL cryovial
        - Stored in -80°C
    - RD-5
        - Ran gel
        - Looked good
        - Gel purified
        - RD-5.1: heavily ethanol contaminated
        - RD-5.2: not good either
    - Gib-9
        - Plate looks good, tons of colonies
        - Made 6 overnight cultures: 3 mL LB + 3 µL CAM each
        - Accidentally took 4 pink colonies, so redid 4 overnight cultures
    - Assisted with competent cells
- Apple Lee
    - Ran Phusion PCR of FAS 1 and 2 reactions of FAS 6 prepared yesterday
    - Gel purification of FAS 1 and FAS 6
        - Results
            - FAS 1: band results matched simulation; gel purified and sample stored in -20C
            - FAS 6 (63C annealing temperature): bands too faint; gel discarded
            - FAS 6 (65.8C annealing temperature): bands too faint; gel discarded
    - Made competent cells of JM109 by following the protocol
        - 200mL phi broth x5
        - 5mL culture x5
        - 15mL ice-cold TJB1 for each tube
        - 2mL ice-cold TJB2 for each tube
        - 100uL aliquots stored in -80C fridge

- Jacob Wolf
    - Updated Team Wiki with modified project description
    - Continued QCPCR Protocol
        - Added 1 uL of DpnI
        - Held at 37C for 60 min
        - Added 2.6 uL Alk-qc2 diluted
        - Run in thermocycler for 28 cycles
    - Prepared Competent cells
        - Acquired liquid Nitrogen
        - Centrifuged and resuspended pellets
        - Flash-freezed eppendorf aliquots
    - Transformed QCPCR product (entire volume) into JM109
        - Plated onto LB+CAM for overnight incubation
    - Transformed Psb1C3 (5 uL of 167.4 ng/uL) into freshly prepared JM109 cells
        - *USE 10 ng OF TEMPLATE NEXT TIME*
        - Plated onto LB+CAM for overnight incubation
    - Checked Photosynthetic Incubator
        - Temperature and Brightness still correct
- Marti Gendel
    - Assisted in the competent cells of JM109
        - 200mL phi broth x5
        - 5mL culture x5
        - 15mL ice-cold TJB1 for each tube
        - 2mL ice-cold TJB2 for each tube
        - 100uL aliquots stored in -80C fridge
    - Edited project description for website
    - Went back to Haneuls lab and redid Genomic DNA isolation protocol
        - Removed 1.5mL of s.7002 from the waffle flask
        - Pellet cells by centrifugation at 20,000 × g for 5 minutes.
        - Add 200 µl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0 OR 0.2 M Lithium acetate, 1% SDS solution).
        - Freeze the cells either using liquid nitrogen. Boil at 95˚C for 2 min. Repeat; vortex 30 seconds.
        - Add 200 µl of phenol: chloroform (1:1 mixture); vortex 2 minutes.
        - Centrifuge 3 minutes, room temperature, 20,000 × g.
        - Transfer the upper aqueous phase to a microcentrifuge tube containing 400 µl ice-cold 100% ethanol. Mix by inversion or gentle vortexing
            - The green did not partition into the aqueous phase
        - Centrifuge 5 minutes, room temperature, 20,000 × g. Remove supernatant.
        - Wash the pellet with 0.5 ml 70% ethanol, spin down as described in step 8 above. Remove supernatant.
        - Air-dry the pellets at room temperature or for 5 minutes.

        - Resuspend in 25–50 µl or water
            - Note that at this step part of the pellet was white
        - Nano-Drop Results:
            - 260/280: 1.98
            - 260/230: 1.41
            - 140ng/uL concentration
            - DNA good to use for PCR reactions
    - Set up PCR for parB gene
        - Add 1.78uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O

7/11/19

- Nathan Sattah
    - RD-5
        - pSB1A2 14.7 µL
        - MultiCore Mix 10X buffer 5µL
        - EcoRI 0.5µL
        - PstI 0.5µL
        - Sterile water 29.3 µL
        - Incubated in 37°C for 4 hours
    - Ran gel for RD-5
        - Gel looked good
        - Gel purification did not go well
    - Gib-9
        - Miniprepped 6 overnight cultures according to protocol
        - Gib-9.1: 63.4 ng/µL
        - Gib-9.2: 68.6 ng/µL
        - Gib-9.3: 67.5 ng/µL
        - Gib-9.4: 67.2 ng/µL
        - Gib-9.5: 95.8 ng/µL
        - Gib-9.6: 64.5 ng/µL
        - All graphs looked very good
    - Submitted Gib-9 samples for sequencing
        - 8-tube strip
        - 3 µL of sample in the first 6, labelled L-A through L-F
        - 15 µL of VF2 in the 7th tube
        - 15 µL of VR in the 8th tube
        - Labelled first tube A1 and last tube H1
    - Transformed Apple's Gibson sample into JM109
        - Took JM109 from -80°C and thawed for 15 minutes

        - Added all 5 µL of sample to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
    - Re-made RD-5
        - Same recipe
        - Stored in 37°C overnight
- Apple Lee
    - Phusion PCR of Fas 6 (modification: changed primer FAS 10 to FAS 9)
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 9
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - Gel results: Band results matched simulation; gel purified and sample stored in -20C
    - Gibson Assembly-10 (pSB1C3 backbone and all FASyn fragments)
        - Recipe (5uL)
            - 0.76uL RD4 (pSB1C3)
            - 0.15uL FAS 1
            - 0.15uL FAS 2
            - 0.53uL FAS 3
            - 0.38uL FAS 4
            - 0.30 uL FAS 5 (5.1)
            - 0.23uL FAS 6
            - 2.5uL HiFi 2x Mastermix
            - 50C 1 hr incubation
        - Nathan will complete transformation onto JM109
        - Jacob will plate 100uL onto LB+CAM plate
        - Plate doesn't seem to have any colonies (did not add FAS 5.2)
- Marti Gendel
    - Added .3uL polymerase to PCR-18
    - Put PCR-18 in machine at annealing temperature of 58C
    - Gel of PCR-18 band not visible but two primers are there - discarded gel
    - Redid PCR-18 sample
        - Set up PCR for parB gene
            - Add 1.78uL of DNA
            - 5uL of Phusion HF
            - 1.3uL of parB-F primer
            - 1.3uL of parB-R primer

                - .5uL of dNTPS
                - 14.8uL of H_2O
                - .3uL of polymerase
                - Annealing temp. 58C
        - Ran PCR-18 sample in gel
            - Band was clear and in right spot
            - Gel purified according to procedure on box, nano drop results were not good - discarded sample
- Jacob Wolf
    - Removed plates from photosynthetic incubator
        - QuickChange samples did not produce colonies
            - Will repeat procedure until sequencing confirms a successful sample
        - Competent cells produced a scattered colony pattern
            - Comp cells were successfully Flash-Freezed
    - Prepared another round of PCR5 (QuickChange)
        - Recipe
            - 31.2 uL of dH2O
            - 10 uL of Phusion HF
            - 2.6 uL of Alk-qc1 diluted
            - 2.0 uL of NC64A at 24.6 ng/uL
            - 1.0 uL of dNTPs
            - Polymerase to be added: 0.6 uL
    - Unpackaged and sterilized p1000 tips
    - Continued working on model skeletal code
    - Plated Apple's Transformed Gib-10 onto LB+CAM for overnight incubation
    - Prepared Diagnostic PCR x2 stored @ -20C to be run tomorrow
        - Recipe (QC2 Test)
            - 17.975 uL dH2O
            - 5 uL 5X OneTaq Buffer
            - 0.5 uL dNTPs
            - 0.5 uL VF2
            - 0.5 uL Alk-QC2
            - 0.4 uL NC64A @ 24.6 ng/ uL (approx 10 ng of template)
            - 0.125 uL of Taq Polymerase (to be added)
        - Recipe (QC1 Test)
            - 17.975 uL dH2O
            - 5 uL 5X OneTaq Buffer
            - 0.5 uL dNTPs
            - 0.5 uL VR
            - 0.5 uL Alk-QC1
            - 0.4 uL NC64A @ 24.6 ng/ uL (approx 10 ng of template)
            - 0.125 uL of Taq Polymerase (to be added)

7/12/19

- Nathan Sattah
    - RD-5
        - Ran gel and purified
        - RD-5.1: 32.3 ng/µL
        - RD-5.2: 18.2 ng/µL (kept this one)
    - Synechococcus Stock
        - Made a new 20 mL culture
            - Took 2 mL from 7/8/19 20 mL culture
            - Added 18 mL A+ media
            - Added 18 µL B12
        - Made a new 7 mL culture
            - Took 700 µL from 7/18/19 20 mL culture
            - Added 6.3 mL A+ media
            - Added 6.3 µL B12
    - Designed primers for SigA Promoter and GFP
- Jacob Wolf
    - Ran Diagnostic PCRs (x2) for Alk-qc1 and Alk-qc2 primers
        - Gel: Samples did not produce bands
    - Nanodropped Quickchange primer stocks
        - Alk-qc1
        - Alk-qc2
    - Designed Flag primers for NC64A
    - Pulled html files from 2018 wiki
        - Will begin to change content starting next monday
- Apple Lee
    - Added 0.125uL taq polymerase to Jacob's PCR tubes and put in thermocycler
    - Gibson Assembly-10 (pSB1C3 backbone and all FASyn fragments)
        - Recipe (5uL)
            - 0.5uL RD4 (pSB1C3)
            - 0.1uL FAS 1
            - 0.1uL FAS 2
            - 0.25uL FAS 3
            - 0.3uL FAS 4
            - 0.18 uL FAS 5.1
            - 1.02uL FAS 5.2
            - 0.15uL FAS 6
            - 2.5uL HiFi 2x Mastermix
            - 50C 1 hr incubation
        - Transformation onto JM109
            - Took JM109 from -80°C and thawed for 15 minutes
            - Added all 5 µL of sample to JM109 tube, thawed on ice for 30 minutes

                - Heat shocked in 42°C bath for 45 seconds
                - Ice thaw for 2 minutes
                - Added 300 µL of SOC media
                - Put into 37°C shaker for an hour
            - Plate 100uL on LB+CAM plate and placed in 37C incubator for 18hrs; Nathan will take plate out tomorrow and store in 4C; remaining sample stored in 4C cold room
        - RD6
            - Recipe
                - CutSmart 10x buffer 5µL
                - Template (BBa_J23119) 12.4µL
                - SpeI 0.5µL
                - PstI 0.5µL
                - dH2O 31.6uL
            - Digest for 4 hours at 37C incubator
            - Gel results: band results matched simulation; gel purified; sample stored in -20C
        - RD7
            - Recipe
                - CutSmart 10x buffer 5µL
                - Template (BBa_I746916) 10.4µL
                - XbaI 0.5µL
                - PstI 0.5µL
                - dH2O 33.6uL
            - Digest for 4 hours at 37C incubator
            - Gel results: band results did not match simulation; gel discarded
- Marti Gendel
    - Set up PCR for parB gene
        - Add 1.78uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - .3uL Polymerase
        - 14.8uL of H_2O
    - Ran gel for PCR-18 x2 and no band appeared either time although the two primers were present - discarded gels both times the procedure was repeated

7/13/19

- Nathan Sattah
    - Moved Apple's Gibson plate to the cold room

7/15/19

- Nathan Sattah
    - Redid Gib-9

        - PCR-4: 0.84 µL
        - PCR-3: 0.39 µL
        - RD-3: 1.27 µL
        - NEB HiFi 2X Master Mix: 2.5 µL
        - Incubated in thermocycler for 20 min at 50°C
    - Transformation of Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 5 µL of Gib-9 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Moved to cold room until 3:00 pm
        - Stored in 37°C overnight
    - Synechococcus Stock
        - Made a new 10 mL culture
            - Took 1 mL from 7/12/19 20 mL culture
            - Added 9 mL A+ media
            - Added 9 µL B12
    - Cleared out leftover overnight tubes from cold room
    - Completed iGEM Budget for SGFC form
    - RD-3
        - CutSmart 10x buffer 5µL
        - pSB1C3 16.8µL
        - EcoRI 0.5µL
        - PstI 0.5µL
        - Sterile Water 27.2µL
    - RD-4
        - CutSmart 10x buffer 5µL
        - pSB1C3 6.5µL
        - XbaI 0.5µL
        - PstI 0.5µL
        - Sterile Water 37.5uL
    - Ran gel for digests
        - Lane 1: 1 kb Plus Ladder
        - Lane 2: RD-3
        - Lane 3: Template (300 ng = 5.1 µL, 4.9 µL water, 2 µL dye)
        - Lane 4: RD-4
        - Lane 5: Template (300 ng = 1.94 µL, 8.06 µL water, 2 µL dye)
        - Gels didn’t work
    - Made overnight cultures for GFP part (BBa_I746916)
- Marti Gendel
    - Redid PCR-18 (25uL)

        - Add 1.62uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - .3uL Polymerase
        - 14.8uL of H_2O
        - Annealing Temp: 62
    - Made 500mL of LB liquid (including autoclave)
    - Ran PCR-18 in gel- got solid band
    - Gel purified sample (.16 grams) according to protocol and nano drop results were not good - discarded sample
    - Redid PCR-18 (50uL)
        - Add 3.24 uL of DNA
        - 10uL of Phusion HF
        - 2.6uL of parB-F primer
        - 2.6 uL of parB-R primer
        - 1 uL of dNTPS
        - .6 uL Polymerase
        - 29.6 uL of H_2O
        - Annealing Temp: 62
- Jacob Wolf
    - Continued working on FAS model
    - Pulled and began updating webpages from 2018 uchicago igem wiki
- Apple Lee
    - RD6
        - Recipe
            - CutSmart 10x buffer 5µL
            - Template (BBa_J23119) 12.4µL
            - SpeI 0.5µL
            - PstI 0.5µL
            - dH2O 31.6uL
        - Digest for 4 hours at 37C incubator
        - Gel results: Band results showed extra bands; gel discarded
    - RD7
        - Recipe
            - CutSmart 10x buffer 5µL
            - Template (BBa_I746916) 10.4µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 33.6uL
        - Digest for 4 hours at 37C incubator
        - Gel results: Band results showed extra bands; gel discarded
    - RD3

        - Recipe
            - CutSmart 10x buffer 5µL
            - pSB1C3 12.6µL
            - EcoRI 0.5µL
            - PstI 0.5µL
            - Sterile Water 31.4µL
            - Overnight digest at 37C incubator
    - RD4
        - Recipe
            - CutSmart 10x buffer 5µL
            - pSB1C3 6.5µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - Sterile Water 37.5uL
            - Overnight digest at 37C incubator
    - Gibson Assembly-10 (pSB1C3 backbone and all FASyn fragments); modification: use RD3 instead of RD4 as template backbone
        - Recipe (5uL)
            - 0.46uL RD3 (pSB1C3)
            - 0.1uL FAS 1
            - 0.12uL FAS 2
            - 0.28uL FAS 3
            - 0.22uL FAS 4
            - 0.19 uL FAS 5.1
            - 0.97uL FAS 5.2
            - 0.15uL FAS 6
            - 2.5uL HiFi 2x Mastermix
            - 50C 1 hr incubation
        - Transformation onto JM109
            - Took JM109 from -80°C and thawed for 15 minutes
            - Added all 5 µL of sample to JM109 tube, thawed on ice for 30 minutes
            - Heat shocked in 42°C bath for 45 seconds
            - Ice thaw for 2 minutes
            - Added 300 µL of SOC media
            - Put into 37°C shaker for an hour
        - Plate 100uL on LB+CAM plate and placed in 37C incubator for 18hrs

7/16/19

- Nathan Sattah
    - Ran gel for RD3 and RD4
        - Discarded gel
    - Miniprepped overnights for GFP part
        - Nanodrops looked very good, kept both samples

        - GFP-1: 111.3 ng/µL
        - GFP-2: 88.5 ng/µL
    - Made sterile water
    - Made overnight cultures for Gib-9
        - Left on windowsill for one hour to confirm the absence of red colonies
        - Red colonies present, but did not select those colonies
        - Made 6 overnight cultures, 3 mL LB + 3 µL CAM each
        - Stored in 37°C shaker
    - RD3 overnight
        - Buffer H 5 µL
        - pSB1C3 6.5 µL
        - EcoRI 0.5 µL
        - PstI 0.5 µL
        - Sterile water 37.5 µL
        - Stored in 37°C
    - RD4 overnight
        - Buffer H 5 µL
        - pSB1C3 6 µL
        - XbaI 0.5 µL
        - PstI 0.5 µL
        - Sterile water 38 µL
        - Stored in 37°C
    - Primer resuspension
        - Sig-A2: added 421.9 µL water
        - GFP-1: added 312.4 µL water
        - GFP-2: added 312.7 µL water
        - Made dilutions of 90 µL water and 10 µL of primer stock
- Marti Gendel
    - Ran 50mL PCR-18 in gel but PCR got contaminated by nucleases
    - Set up PCR for parB gene
        - Add 1.62uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O
        - .3uL of polymerase
        - Annealing temp. 62C
    - Ran gel but band not visible it seems like there are primer dimers forming such that the PCR protocol should adjusted will test TD-PCR protocol
- Apple Lee
    - Examined Gibson-10 plate: lack of colonies present so replated using same cells and left in 37C incubator for 18hrs (2 plates; Nathan plated one too)
    - Miniprep 3 ON cultures of BBa_J23119

        - Concentrations were good and nanodrop graphs looked good so samples are stored in -20C
    - Phusion PCR of Fas 5 Reaction 1 (modification: changed template from 5B to 5D)
        - 5µL Phusion HF
        - 1.3uL Fas 8
        - 1.3uL Fas 5A
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - Gel results: only primer band present; gel discarded
    - Phusion PCR of Fas 5 Reaction 2 (modification: changed template from 5B to 5D)
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Gel results: band matched simulation; gel purified and sample stored in -20C
    - RD6
        - Recipe
            - Buffer H 5µL
            - Template (BBa_J23119) 9.7µL
            - SpeI 0.5µL
            - PstI 0.5µL
            - dH2O 34.3uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
    - RD7
        - Recipe
            - Buffer H 5µL
            - Template (BBa_I746916) 9µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 35uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
    - Resuspended new FAS primers with sterile water

        - FAS-uni: 337.3uL
        - FAS-25: 333.4uL
        - FAS-26: 376.7uL
        - FAS-27: 273.2uL
        - FAS-28: 373.7uL
        - FAS-29: 268.7uL
- Jacob Wolf
    - Continued updating igem wiki pages from the previous year
    - Autoclaved Tips
    - Resuspended new quick-change primers from well plate
        - 25.5 nmol of alk-qc1b (A4) resuspended in 255 uL sterile water
        - 28.58 nmole of alk-qc2b (A5) resuspended in 285.8 uL sterile water
    - Prepared Quick Change PCR reactions to be run on 7/17

7/17/19

- Nathan Sattah
    - Gib-9
        - Miniprepped 6 overnight cultures according to protocol
        - Gib-9.1: 96.0 ng/µL
        - Gib-9.2: 95.2 ng/µL
        - Gib-9.3: 123.1 ng/µL
        - Gib-9.4: 97.3 ng/µL
        - Gib-9.5: 187.7 ng/µL
        - Gib-9.6: 72.2 ng/µL
        - All graphs looked very good
    - Submitted Gib-9 samples for sequencing
        - 8-tube strip
        - 3 µL of sample in the first 6, labelled L-A through L-F
        - 15 µL of VF2 in the 7th tube
        - 15 µL of VR in the 8th tube
        - Labelled first tube A1 and last tube H1
    - Ran gel for RD3 and RD4 from overnight
        - Digest did not work
    - PCR-21: VF2 and Sig-A2 (56°)
        - 5 µL Phusion HF Buffer
        - 1.3 µL VF2
        - 1.3 µL Sig-A2
        - 1 µL Diluted Template (P-AlkL)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - PCR-22: GFP-1 and GFP-2 (58°)
        - 5 µL Phusion HF Buffer
        - 1.3 µL GFP-1

        - 1.3 µL GFP-2
        - 1 µL Diluted Template (BBa_I746916)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - Ran gel for PCR-21 and PCR-22
        - Gel looked correct
        - Gel purified
        - PCR-21: 45.5 ng/µL
        - PCR-22: 127.9 ng/µL
    - Updated primer index with sequences
- Apple Lee
    - Gel purification of RD6 and RD7
        - RD6 result: Bright band matched simulation; gel purified; sample stored in -20C
        - RD7 result: Lower band matched simulation; gel purified; sample discarded
    - Resuspended new FAS primers with sterile water
        - FAS-30: 299.8uL
        - FAS-31: 274.5uL
        - FAS-32: 268.2uL
        - FAS-33: 332.2uL
        - FAS-34: 335.9uL
        - FAS-35: 271.9uL
        - FAS-36: 303.3uL
        - FAS-37: 351.5uL
        - FAS-38: 263.9uL
    - Phusion PCR of Fas 5 Reaction 1 (modification: changed annealing temperature from 61C to 58C)
        - 5µL Phusion HF
        - 1.3uL Fas 8
        - 1.3uL Fas 5A
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 58C
        - Gel results: band matched simulation; gel purified; sample stored in -20C
    - Phusion PCR of Fas 5 Reaction 1 (modification: changed total volume to 50uL)
        - 10µL Phusion HF
        - 2.6uL Fas 8
        - 2.6uL Fas 5A
        - 2uL Diluted Template (Fas5D)
        - 1uL dNTP

        - 0.6uL polymerase
        - 31.2µL sterile water
        - Annealing temperature: 58C
        - Gel purification tomorrow
- Jacob Wolf
    - Assembled reactants for quick change pcr (pcr-20)
        - Tube 1 Recipe
            - 12.5 uL Q5 2X master mix
            - 9.05 uL dH2O
            - 1.25 uL dilute alk qc 1b
            - 2.20 uL NC64A 22.9 ng/uL
        - Tube 2 Recipe
            - 12.5 uL Q5 2X master mix
            - 9.05 uL dH2O
            - 1.25 uL dilute alk qc 2b
            - 2.20 uL NC64A 22.9 ng/uL
        - Annealing Temp 72C
        - Ran in thermocycler for 35 cycles
    - Completed quick change protocol
        - Added 1 uL of DpnI and put on thermocycler
        - Allowed to cool at room temperature before placing back in the -20C
    - Transformed 10 uL of QCPCR product into JM109 for overnight incubation
        - Plated onto LB+CAM plate
    - Plated Marti's Gibson Transform
- Marti Gendel
    - Prepared PCR18 again
        - Add 1.62uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O
        - .3uL of polymerase
    - Ran TDPCR
        - Starting at 72 and decreasing by 1C each cycle for 10 cycles until 62C reached → Phase 2 was at 62 for 25C
        - Gel results solid clear band at appropriate spot
    - Gel purified according to protocol
        - Nano drop results were off concentration of 49.5ng/uL but peak was at 240 and had a bowdown at 260
    - Set up 15min Gibson (Gib11)
        - 1.06uL RD3
        - .83 PCR6
        - .61 PCR18

        - 2.5uL HiFi
    - Added Gib11 to JM109
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 5 µL of Gib-11 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Moved to cold room until 3:00 pm
        - Stored in 37°C overnight

7/18/19

- Nathan Sattah
    - Gib-12
        - RD-3 0.71 µL
        - PCR-21 0.4 µL
        - PCR-22 0.14 µL
        - NEB HiFi 2X Master Mix 1.25 µL
        - Incubated in thermocycler at 50°C for 15 min
        - Stored in -20°C
    - Transformation of Gib-12
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 2.5 µL of Gib-12 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Centrifuged at max speed for 1 minute
        - Siphoned off 250 µL of supernatant
        - Resuspended cells and plated remaining volume onto an LB+CAM plate
        - Stored in 37°C overnight
    - Made more pSB1C3
        - 4 overnight cultures, 2 mL LB + 2 µL CAM each
    - Made overnight culture for glycerol stock
        - 2 mL LB + 2 µL CAM
    - Took OD of 7 mL synechococcus culture at 730: 1.018
    - Made a new 7 mL culture (8X)
        - 6.125 mL A+
        - 6.125 µL B12
        - 875 µL culture from previous 7 mL culture
- Apple Lee
    - Gel purification of PCR product-FAS 5.1

        - Result: band matched simulation; gel purified; sample discarded due to ethanol contamination
    - Phusion PCR of Fas 5 Reaction 1 (modification: changed total volume to 50uL)
        - 10µL Phusion HF
        - 2.6uL Fas 8
        - 2.6uL Fas 5A
        - 2uL Diluted Template (Fas5D)
        - 1uL dNTP
        - 0.6uL polymerase
        - 31.2µL sterile water
        - Annealing temperature: 58C
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - RD7 (modification: changed template concentration to 2ng)
        - Recipe
            - Buffer H 5µL
            - Template (BBa_I746916) 18µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 26uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
    - Phusion PCR of FabTesA (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas 37
        - 1.3uL Fas 38
        - 1uL Diluted Template (Fas6)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 59C
        - PCR will run tomorrow
- Jacob Wolf
    - Prepared 8 overnight cultures of quick change pcr products in LB+CAM
        - Will have samples to submit for sequencing by friday 7/19
    - Assembled PCR reactions to follow Gibson Promoter exchange
        - PCR 23
            - 32.4 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk qc 1b dilute
            - 2.6 uL Alk 10 dilute
            - 1.0 uL dNTPs
            - 0.8 uL PsB1C3 @ 77 ng/uL
            - 0.6 uL Polymerase
        - PCR 24

                - 32.4 uL dH2O
                - 10 uL Phusion HF 5X
                - 2.6 uL Alk qc 2b dilute
                - 2.6 uL Alk 9 dilute
                - 1.0 uL dNTPs
                - 0.8 uL PsB1C3 @ 77 ng/uL
                - 0.6 uL Polymerase
            - Ran in thermocycler for 35 cycles
            - Gel: neither PCR reaction produced bands, a review of primers indicated the incorrect annealing temperature was used, will adjust tomorrow
- Marti Gendel
    - Inspected Gib-11 plate saw a few small colonies
    - Prepared 4 overnight cultures of Gib-11 products in LB+CAM
        - Will have samples to submit for sequencing by friday 7/19
    - RD3 overnight
        - Buffer H 5 µL
        - pSB1C3 12.6 µL
        - EcoRI 0.5 µL
        - PstI 0.5 µL
        - Sterile water 31.4 µL
        - Stored in 37°C

7/19/19

- Nathan Sattah
    - Submitted samples for sequencing
        - PCR-3
        - PCR-4
        - P-AlkL
        - VF2
        - VR
    - Autoclaved P1000, P200, and P2 tips
    - PCR-QC-29 overnight
        - Tube 1 (51°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL AlkL-qc-1
            - 0.5 µL P-AlkL (104.6 ng/µL)
            - 10.75 µL Sterile Water
        - Tube 2 (51°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL AlkL-qc-2
            - 0.5 µL P-AlkL (104.6 ng/µL)
            - 10.75 µL Sterile Water
    - Made overnight cultures for Gib-12
        - 4 cultures of 2 mL LB + 2 µL CAM

    - Miniprepped pSB1C3 cultures (4 samples)
- Jacob Wolf
    - Prepared 20 ml cyanostock
        - Took 2mL from 7/12/19 Cyano stock
        - Added 18 mL A+
        - Added 18 uL B12
    - Miniprepped quick change samples for sequencing
- Apple Lee
    - Gibson Assembly-10
        - Recipe (5uL)
            - 0.7uL RD3 (pSB1C3)
            - 0.13uL FAS 1
            - 0.17uL FAS 2
            - 0.41uL FAS 3
            - 0.32uL FAS 4
            - 0.14uL FAS 5.1
            - 0.42uL FAS 5.2
            - 0.21uL FAS 6
            - 2.5uL HiFi 2x Mastermix
            - 50C 1 hr incubation
        - Transformation onto JM109
            - Took JM109 from -80°C and thawed for 15 minutes
            - Added all 5 µL of sample to JM109 tube, thawed on ice for 30 minutes
            - Heat shocked in 42°C bath for 45 seconds
            - Ice thaw for 2 minutes
            - Added 300 µL of SOC media
            - Put into 37°C shaker for an hour
        - Plate 100uL on LB+CAM plate and placed in 37C incubator for 18hrs
            - Jacob will take plate out tomorrow morning and store in 4C
    - Run PCR FabTesA
        - Gel result: band result matched simulation; gel purified; sample stored in -20C
    - Gel purification of RD7
        - Result: band result did not match simulation; gel discarded
- Marti gendel
    - Took OD of cyano stock created on 7/18 and recorded value of .247A
    - Moved Gib-11 and JW overnight cultures from shaking incubator
    - Miniprep 4 overnight cultures from gib-11
    - Set up samples (3uL each) with primers VF2 and VR on sample racks to submit for sequencing
    - Ran gel of RD3
        - Gel results were not going showing three distinct separate brands

7/20/19

- Jacob Wolf
    - Moved QCPCR samples from thermocycler to 4C fridge
    - Moved Gib 10 plate from incubator to cold room
    - Miniprepped Gib-12 (4 samples)
        - All samples had relatively low concentrations labelled and stored in -20C

7/22/19

- Nathan Sattah
    - Submitted samples of Gib-12 for sequencing
        - Gib12 A
        - Gib12 B
        - Gib12 C
        - Gib 12 D
        - VF2
        - VR
    - Nanodropped pSB1C3 samples
        - 117.5 ng/µL
        - 82.8 ng/µL
        - 115.7 ng/µL
        - 64.4 ng/µL
    - Part 2 of PCR-QC-29
        - Combined tubes 1 and 2
        - Added 1 µL DnpI
    - Made glycerol stock of pSB1C3
        - Took 500 µL from overnight culture and 500 µL 50% glycerol
        - Made 2 vials and stored in -80°C
    - Transformation of PCR-QC-29
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10 µL of PCR-QC-29 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
- Apple Lee
    - Phusion PCR of FabTesA (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas 37
        - 1.3uL Fas 38
        - 1uL Diluted Template (Fas6)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)

        - 15.6µL sterile water
        - Annealing temperature: 59C
        - Gel result: band matching simulation is too faint; primer dimer present; gel discarded
    - Prepared overnight cultures of GIbson-10
        - 7 cultures of 2mL LB + 2uL CAM
        - 18hrs 37C shaking incubator
    - Prepared overnight cultures of gfp (BBa_I746916)
        - 3 cultures of 2mL LB + 2uL CAM
        - 18hrs 37C shaking incubator
    - RD7
        - Recipe
            - Buffer H 5µL
            - Template (BBa_I746916) 11.3µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 32.7uL
        - 4hr digest at 37C incubator
        - Gel result: band did not match simulation; presence of uncut plasmids; gel discarded
    - Universal Promoter PCR (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas-Uni
        - 1.3uL Fas 25
        - 1uL Diluted Template (Fas1)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 57C
        - PCR will run tomorrow
- Jacob Wolf
    - PCR 23
        - Recipe
            - 31.2 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk qc 1b dilute
            - 2.6 uL Alk 10 dilute
            - 1.0 uL dNTPs
            - 2.0 uL PsB1C3 @ 11.7 ng/uL
            - 0.6 uL Polymerase
        - Anneal Temp 64C
    - PCR 24
        - Recipe
            - 31.2 uL dH2O

                - 10 uL Phusion HF 5X
                - 2.6 uL Alk qc 2b dilute
                - 2.6 uL Alk 9 dilute
                - 1.0 uL dNTPs
                - 2.0 uL PsB1C3 @ 11.7 ng/uL
                - 0.6 uL Polymerase
            - Anneal Temp 62C
        - Gel Purification of PCR 23 and PCR 24
            - Result: Gel did not produce distinct bands. Gel appeared granular on the surface indicating an incomplete dissolution of the agarose.
        - Prepared 10 mL Cyanostock
            - Added 9 mL A+ media
            - 9 uL B12
            - 1 mL from the 7/19/19 20 mL culture
- Marti Gendel
    - Assisted in making of glycerol stock of pSB1C3 and retrieving liquid nitrogen
        - Took 500 µL from overnight culture and 500 µL 50% glycerol
        - Made 2 vials and stored in -80°C
    - Sterilized H_2O and made five 1mL aliquot to store in -20C
    - Created folder and digitized all gel images named Gel Pictures in Summer 2019 folder

7/23/19

- Nathan Sattah
    - PCR-QC-29 plate has only 2 colonies, and tube containing PCR product was lost when ice bucket was dumped
        - Plan to make overnights and miniprep the 2 colonies and sequence them alongside a new quick change PCR
    - Ran PCR-QC-29 again
        - Tube 1 (51°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL AlkL-qc-1
            - 0.5 µL P-AlkL (104.6 ng/µL)
            - 10.75 µL Sterile Water
        - Tube 2 (51°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL AlkL-qc-2
            - 0.5 µL P-AlkL (104.6 ng/µL)
            - 10.75 µL Sterile Water
- Apple Lee
    - Run Universal Promoter PCR
        - Gel result: band that matches simulation was a dark band; primer band was very bright; gel discarded
    - Miniprep 3 ON cultures of BBa_I746916

        - Concentrations were good and nanodrop graphs looked good so samples are stored in -20C
    - Miniprep 7 ON cultures of Gibson-10
        - Concentrations were good and nanodrop graphs looked good so samples are stored in -20C
    - Single Diagnostic Digest of Gibson-10 (10uL restriction digest)
        - Recipe
            - 1uL Template (GibA-GibG)
            - 1uL CutSmart 10X Buffer
            - 0.25uL EcoRI
            - 7.75uL dH20
            - 37C overnight incubation
            - Gel purification tomorrow
- Jacob Wolf
    - Prepared PCR 2, 23, 24 without adding polymerase
        - PCR 2
            - Recipe
                - 31.2 uL dH2O
                - 10 uL Phusion HF 5X
                - 2.6 uL Alk 3b dilute
                - 2.6 uL Alk 4 dilute
                - 1.0 uL dNTPs
                - 2.0 uL p-lux @ 10 ng/uL
                - 0.6 uL Polymerase
            - Anneal Temp 67.5C
            - Extension Time 5:05
        - PCR 23
            - Recipe
                - 31.2 uL dH2O
                - 10 uL Phusion HF 5X
                - 2.6 uL Alk qc 1b dilute
                - 2.6 uL Alk 10 dilute
                - 1.0 uL dNTPs
                - 2.0 uL PsB1C3 @ 11.7 ng/uL
                - 0.6 uL Polymerase
            - Anneal Temp 64C
        - PCR 24
            - Recipe
                - 31.2 uL dH2O
                - 10 uL Phusion HF 5X
                - 2.6 uL Alk qc 2b dilute
                - 2.6 uL Alk 9 dilute
                - 1.0 uL dNTPs
                - 2.0 uL PsB1C3 @ 11.7 ng/uL

        - 0.6 uL Polymerase
    - Anneal Temp 62C
- Began creating a bullet list of all content needed for the team wiki page
- Resubmitted QuickChange Samples for sequencing

7/24/19

- Nathan Sattah
    - PCR-QC-29 Part 2
        - Mixed 2 tubes and added 1 µL DpnI
    - Transformation of PCR-QC-29
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10 µL of PCR-QC-29 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
    - CPEC for Gib-9 (53°C) overnight
        - 4 µL HF Buffer
        - 1.6 µL dNTP's
        - 0.2 µL Phusion
        - 2 µL RD3 (54.4 ng/µL)
        - 2.17 µL PCR-3
        - 4.71 µL PCR-4
        - 5.32 µL Water
    - CPEC for Gib-12
        - Prepped tube, but realized I need RD8 instead of RD3
    - Prepped PCR-3
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 15.6µL sterile water
    - Prepped PCR-4
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 15.6µL sterile water
- Apple Lee

- Gel purification of Gibson single digest products:
  - Gib10A: single band present; did not match simulation; fragments are too light
  - Gib10B: 2 bands present
  - Gib10C: single band present; did not match simulation; fragments are too light
  - Gib10D: single band present; did not match simulation; fragments are too light
  - Gib10E: single band present; did not match simulation; fragments are too light
  - Gib10F: single band present; did not match simulation; fragments are too light
  - Gib10G: single band present; did not match simulation; fragments are too light
- Double Diagnostic Digest of Gibson-10 (10uL restriction digest)
  - Recipe
    - 1uL Template (GibA-GibG)
    - 1uL CutSmart 10X Buffer
    - 0.25uL SacI
    - 0.25uL KpnI
    - 7.5uL dH20
    - 37C overnight incubation
    - Gel result: bands present did not match simulation; fragments are too light
- Phusion PCR of FabTesA (25uL)
  - 5µL Phusion HF
  - 1.3uL Fas 37
  - 1.3uL Fas 38
  - 1uL Diluted Template (Fas6)
  - 0.5uL dNTP
  - 0.3uL polymerase
  - 15.6µL sterile water
  - Annealing temperature: 64C
  - Gel purification tomorrow
- Universal Promoter PCR (25uL)
  - 5µL Phusion HF
  - 1.3uL Fas-Uni
  - 1.3uL Fas 25
  - 1uL Diluted Template (Fas1)
  - 0.5uL dNTP
  - 0.3uL polymerase
  - 15.6µL sterile water
  - Annealing temperature: 61C
  - Gel purification tomorrow

    - Autoclaved tips
    - CPEC PCR
        - 4µL Phusion HF
        - 0.68uL FAS 1
        - 0.85uL FAS 2
        - 2uL FAS 3
        - 1.6uL FAS 4
        - 0.7uL FAS 5.1
        - 2.1uL FAS 5.2
        - 1.03uL FAS 6
        - 2uL RD3
        - 1.6uL dNTP
        - 0.2uL polymerase
        - 3.24uL dH20
        - PCR run overnight
        - Gel purification tomorrow
    - RD3 x2
        - Recipe
            - Buffer H 5µL
            - Template (BBa_I746916) 8.6µL
            - EcoRI 0.5µL
            - PstI 0.5µL
            - dH2O 35.4uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
- Marti Gendel
    - Loaded RD3 into gel: good result with big band in the right spot
    - Performed gel purification protocol with top big band cut
    - Nano drop results for RD3 good: 54ng/uL
    - Prepared PCR18 again
        - Add 1.62uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O
    - Prepared PCR6B
        - Add 1uL of FAs1 Template
        - 5uL of Phusion HF
        - 1.3uL of parB-Aprimer
        - 1.3uL of VF2 primer
        - .5uL of dNTPS
        - 15.6uL of H_2O
    - Made RD8

        - Cut Smart: 5uL
        - PSB1C3: 12.072uL
        - .5uL ECORI
        - .5 uL SPEI
        - 32uL H_2O
- Jacob Wolf
    - Ran PCR 23 and PCR 24 for 35 cycles
        - Gel: Bands for both reaction matched the simulation proceeding with gel purification
    - Gel purified PCR 23 and PCR 24
        - Samples both had quite small concentrations and heavy ethanol contamination. Samples were discarded.

7/25/19

- Nathan Sattah
    - PCR-21
        - 5 µL Phusion HF Buffer
        - 1.3 µL VF2
        - 1.3 µL Sig-A2
        - 1 µL Diluted Template (P-AlkL)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - PCR-22
        - 5 µL Phusion HF Buffer
        - 1.3 µL GFP-1
        - 1.3 µL GFP-2
        - 1 µL Diluted Template (BBa_I746916)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - Also ran PCR-3 and PCR-4 with PCR-21 and PCR-22
    - Ran CPEC for Gib-9 on a gel
        - Took 3 µL of CPEC sample and added 0.6 µL loading dye
        - Ran alongside 3 µL of Gib-9 that did not work as negative control (also added 0.6 µL loading dye)
        - Faint band seen at 2.8 kb
    - Transformation of CPEC for Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10 µL of CPEC to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour

        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
    - Gel purified PCR-3, PCR-4, PCR-21, and PCR-22
        - PCR-3: 76.4 ng/µL, kept sample
        - PCR-4: 37.3 ng/µL, ethanol contaminated with no peak at 260, discarded
        - PCR-21: 49.7 ng/µL, ethanol contaminated with slight peak at 260, kept
        - PCR-22: 50.5 ng/µL, ethanol contaminated with slight bow at 260, kept
    - Made overnight cultures for PCR-QC-29
        - Two samples from 7/22 labelled 1A and 1B
        - One sample from 7/24 labelled 2
    - Prepped PCR-4
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 15.6µL sterile water
- Apple Lee
    - Gel purification of PCR products of TesA and Universal Promoter
        - TesA result: bands matched simulation; gel purified; sample stored in -20C
        - Universal promoter result: bands did not match simulation; gel discarded
    - Ran gel for CPEC Gibson-10
        - Result: bands did not match simulation; gel discarded
    - Gel purification of RD3
        - Result 1: bands matched simulation; gel purified; sample stored in -20C
        - Result 2: bands matched simulation; gel purified; sample stored in -20C
    - Resuspended new FAS primers with sterile water
        - S-FAS-F1: 461uL
        - S-FAS-F2: 460uL
        - S-FAS-F3: 502uL
        - S-FAS-F4: 403uL
        - S-FAS-F5: 499uL
        - S-FAS-R1: 434uL
        - S-FAS-R2: 336uL
        - S-FAS-R3: 441uL
        - S-FAS-R4: 273uL
        - S-FAS-R5: 367uL
        - S-FAS-R6: 362uL
    - Gibson Assembly-10
        - Recipe (5uL)
            - 0.75uL RD3 (pSB1C3)
            - 0.13uL FAS 1
            - 0.16uL FAS 2

                - 0.4uL FAS 3
                - 0.32uL FAS 4
                - 0.13uL FAS 5.1
                - 0.41uL FAS 5.2
                - 0.2uL FAS 6
                - 2.5uL HiFi 2x Mastermix
                - 50C 1 hr incubation
            - Transformation onto JM109
                - Took JM109 from -80°C and thawed for 15 minutes
                - Added all 5 µL of sample to JM109 tube, thawed on ice for 30 minutes
                - Heat shocked in 42°C bath for 45 seconds
                - Ice thaw for 2 minutes
                - Added 300 µL of SOC media
                - Put into 37°C shaker for an hour
                - Plated 100uL onto LB+CAM
                - 37C incubation (18hrs)
        - Transformed 1uL of RD3.1 and 1uL of RD3.2 onto JM109 and plated 100uL each onto LB+CAM
- Marti Gendel
    - Put PCR6B in thermocycler - 56C
    - Ran RD8 and two RD3 samples in gel - results good cut all three and prepped for gel purification
    - RD8 bad nano drop results discarded sample
    - Re made PCR-6B
        - Add 1uL of FAs1 Template
        - 5uL of Phusion HF
        - 1.3uL of parB-Aprimer
        - 1.3uL of VF2 primer
        - .5uL of dNTPS
        - 15.6uL of H_2O
    - Remade RD8 and stored in 37C
        - Cut Smart: 5uL
        - PSB1C3: 15.52uL
        - .5uL ECORI
        - .5 uL SPEI
        - 28.5uL H_2O
- Jacob Wolf
    - Setup and Programmed black thermocycler
    - Ran PCR 23 and PCR 24 in black thermocycler
        - Recipe PCR 23
            - 31.2 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk qc 1b dilute

                - 2.6 uL Alk 10 dilute
                - 1.0 uL dNTPs
                - 2.0 uL PsB1C3 @ 11.7 ng/uL
                - 0.6 uL Polymerase
                - Anneal Temp 64C
            - Recipe PCR 24
                - 31.2 uL dH2O
                - 10 uL Phusion HF 5X
                - 2.6 uL Alk qc 1b dilute
                - 2.6 uL Alk 10 dilute
                - 1.0 uL dNTPs
                - 2.0 uL PsB1C3 @ 11.7 ng/uL
                - 0.6 uL Polymerase
                - Anneal Temp 62C
    - Gel Results
        - Similar gel bands to yesterday were produced, moving to gel purification
    - Gel purified PCR 23 and PCR 24
        - PCR 23
            - Nanodrop showed heavy contamination and low DNA yield. Sample was discarded.
        - PCR 24
            - Nanodrop showed yields which may be useable, stored as PCR-24b in -20C.

7/26/19

- Nathan Sattah
    - Ran PCR-4
    - Miniprepped PCR-QC-29 overnights
        - 1A: 156.8 ng/µL
        - 1B: 159.7 ng/µL
        - 2: 56.8 ng/µL
    - Colony PCR for CPEC for Gib-9
        - Labelled 9 colonies on the plate
        - Picked and dissolved each colony into 20 µL of water
        - Heated at 80°C for 10 minutes
        - Prepared MasterMix (multiplied numbers by 9)
            - 138.6 µL water
            - 45 µL HF Buffer 5x
            - 4.5 µL dNTP’s
            - 4.5 µL VF2
            - 4.5 µL VR
            - 0.9 µL Taq
        - Added 22 µL of MasterMix to each tube of bacteria
        - Put into thermocycler according to protocol

    - Submitted PCR-QC-29 samples for sequencing
        - 1A
        - 1B
        - 2
        - VF2
    - Ran PCR-4 and Colony PCR's on a large gel
        - Gel Recipe
            - 120 mL Buffer
            - 0.96 g Agarose
        - Negative Control
            - Took 1 µL of negative control for Gib-9 and added 2 µL of water and 0.6 µL loading dye
        - Only colony 9 looked correct
    - Made overnight cultures for pSB1C3 and Colony 9 for CPEC for Gib-9
        - 1 tube for CPEC for Gib-9
        - 3 tubes for pSB1C3
        - 2 mL LB + 2.2 µL CAM each
        - Used glycerol stock for pSB1C3
        - Used remaining 17 µL of colony 9 for overnight culture
    - Gel purified PCR-4
        - 21.4 ng/µL, but graph looks usable
- Apple Lee
    - Prepared 20 ml cyanostock
        - Took 2mL from 7/22/19 Cyano stock
        - Added 18 mL A+
        - Added 18 uL B12
    - Autoclaved tips
    - Universal Promoter PCR (50uL)
        - 10µL Phusion HF
        - 2.6uL Fas-Uni
        - 2.6uL Fas 25
        - 2uL Diluted Template (Fas1)
        - 1uL dNTP
        - 0.6uL polymerase
        - 31.2µL sterile water
        - Annealing temperature: 64C
        - Gel result: band did not match simulation; gel discarded
    - Universal Promoter PCR (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas-Uni
        - 1.3uL VF2
        - 1uL Diluted Template (Fas1)
        - 0.5uL dNTP
        - 0.3uL polymerase

        - 15.6µL sterile water
        - Annealing temperature: 57C
        - Gel purification on Monday
- Marti Gendel
    - Took out PCR6B from overnight PCR and RD8 from 37C
    - Ran PCR6B gel and purified 61.6ng/uL
    - RD8 gel purification concentration was not sufficient
    - Remade RD8 using
        - Made RD8
            - Cut Smart: 5uL
            - PSB1C3: 18.072uL
            - .5uL ECORI
            - .5 uL SPEI
            - 26uL H_2O
- Jacob Wolf
    - Ran PCR 23
        - 31.2 uL dH2O
        - 10 uL Phusion HF 5X
        - 2.6 uL Alk qc 1b dilute
        - 2.6 uL Alk 10 dilute
        - 1.0 uL dNTPs
        - 2.0 uL NC64A @ 22.9 ng/uL
        - 0.6 uL Polymerase
        - Anneal Temp 64C
        - Gel: Band was once again of appropriate size will proceed with gel purification.
    - Ran PCR 2
        - Recipe
            - 31.2 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk 3b dilute
            - 2.6 uL Alk 4 dilute
            - 1.0 uL dNTPs
            - 2.0 uL p-lux @ 10 ng/uL
            - 0.6 uL Polymerase
        - Anneal Temp 67.5C
        - Extension Time 5:05
        - Gel: Band was hazy and non-distinct. Gel was discarded.
    - Gel purification of PCR 23
        - Result: Sample showed heavy contamination. Remaining sample was discarded.

7/27/19

- Apple Lee

- Miniprepped CPEC Gib-9
  - Result: concentration and graph looks good; sample stored in -20C
- Miniprepped 3 samples of pSB1C3
  - Result: concentration and graph looks good; sample stored in -20C
- RD8 taken out of 37C incubator and stored in -20C
- Submit samples for sequencing
  - CPEC-9 with VR and VF2
  - Qcb-c and qcb-d with Alk-1

7/29/19

- Nathan Sattah
  - CPEC for Gib-9 was submitted for sequencing on Saturday
  - Poured more LB+CAM plates
  - Transformation of pSB1C3 into S7002
    - OD730: 1.884
    - Made a 5 mL culture, 3x dilution from 7 mL culture from 7/12/19
    - Took 1665 µL of culture + 3335 µL A+ + 3.3 µL B12
  - Gib-12
    - RD-8 (13.3 ng/µL) 1.82 µL
    - PCR-21 0.49 µL
    - PCR-22 0.19 µL
    - NEB HiFi 2X Master Mix 2.5 µL
    - Incubated in thermocycler at 50°C for 20 min
    - Stored in -20°C
  - Loaded and gel purified one of Jacob's samples
  - RD-8 overnight
    - Cutsmart 10x 5 µL
    - pSB1C3 (1 µg) 5.42 µL
    - EcoRI-HF 0.5 µL
    - SpeI-HF 0.5 µL
    - Sterile water 38.58 µL
- Marti Gendel
  - Prepared RD8 with 2ug PSB1C3 template
    - Cut Smart: 5uL
    - PSB1C3: 11.8 2uL
    - .5uL ECORI
    - .5 uL SPEI
    - 32.2uL H_2O
  - Ran gel with other RD8
  - Gel purified RD8 sample and got low concentration of 13.3 ng/uL on nano drop
  - Ran TDPCR
    - Starting at 72 and decreasing by 1C each cycle for 10 cycles until 62C reached → Phase 2 was at 62 for 25C
    - Gel results had black spot and no band → discarded gel

- Apple Lee
    - Prepared 10 mL Cyanostock
        - Added 9 mL A+ media
        - 9 uL B12
        - 1 mL from the 7/26/19 20 mL culture
    - Gel purification of Universal Promoter PCR product
        - Result: band matched simulation; gel purified; sample stored in -20C
    - Ran 1uL of gel purified universal promoter PCR product
        - Result: band matched simulation; sample stored in -20C
    - Finalised overlap PCR protocol
- Jacob Wolf
    - Ran PCR 2
        - Recipe
            - 15.6 uL dH2O
            - 5 uL Phusion HF 5X
            - 1.3 uL Alk 3b dilute
            - 1.3 uL Alk 4 dilute
            - 0.5 uL dNTPs
            - 1.0 uL p-lux @ 10 ng/uL
            - 0.3 uL Polymerase
        - Anneal Temp 67.5C
        - Ext Time 5:05
        - Gel: Yielded no band in a gel, will switch to q5 instead of phusion for next run.
    - Ran 2 tubes of PCR 23 at 50 uL
        - Recipe
            - 31.2 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk qc 1b dilute
            - 2.6 uL Alk 10 dilute
            - 1.0 uL dNTPs
            - 2.0 uL NC64A @ 22.9 ng/uL
            - 0.6 uL Polymerase
        - Anneal Temp 64C
        - Gel: Extraction yielded two useable tubes of PCR 23.
    - Transformed into E Coli and Plated both sequence verified quick change products onto LB+CAM for overnight incubation
    - Took OD730 of Transformation dilution : 1.244 A at 5:40 pm

7/30/19

- Nathan Sattah
    - Took OD730 of s7002 from 7/29/19
        - OD730: 1.316
    - Transformation of pSB1C3 into s7002

        - Sample 1: Added 0.55 µL (0.1 µg) pSB1C3 to 9.45 µL of water, and added all 10 µL to 1 mL of culture (A++CAM)
        - Sample 2: Added 10 µL sterile water to 1 mL of culture (A+ only)
        - Sample 3: Added 10 µL sterile water to 1 mL of culture (A++CAM)
        - Moved existing cultures to incubator in Mets lab
        - Incubated transformation samples at 30°C, 150 rpm, 60 µmol photons for 24 hours
    - Transformation of PCR-QC-29 into JM109 for glycerol stock
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of PCR-QC-29 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
    - Gel purified RD8
        - Result: 20.2 ng/µL
    - Made 250 mL of A+ media and poured A+ only plates
    - Prepped CPEC for Gib-12
        - 4 µL HF Buffer
        - 1.6 µL dNTP's
        - 0.2 µL Phusion
        - 5 µL RD8 (20.2 ng/µL)
        - 4 µL PCR-21
        - 1.56 µL PCR-22
        - 3.64 µL Water
- Apple Lee
    - PCR-36 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2
        - 1.3uL FAS3
        - 1uL Diluted Template (FAS1 & FAS2)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 57C
        - Gel result: bands do not match simulation; primer dimers present
    - PCR-37 (25uL)
        - 5µL Phusion HF
        - 1.3uL FAS4
        - 1.3uL FAS7
        - 1uL Diluted Template (FAS3 & FAS4)
        - 0.5uL dNTP

        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 59C
        - Gel result: bands do not match simulation; primer dimers present
    - PCR-38 (25uL)
        - 5µL Phusion HF
        - 1.3uL FAS8
        - 1.3uL FAS5C
        - 1uL Diluted Template (FAS5.1 & FAS 5.2)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 54C
        - Gel result: bands do not match simulation; primer dimers present
    - RD4
        - Recipe
            - Buffer H 5µL
            - Template (pSB1C3) 5.4µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 38.6uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
    - Autoclaved tips
    - Prepared PCR 36-38 again but ran 10 cycles without addition of primers at overlap annealing temperatures
        - PCR 36: 71C
        - PCR 37: 72C
        - PCR 38: 54C
        - Products stored in 4C
        - Adding respective primers and running remaining 30 cycles tomorrow
- Marti Gendel
    - Ran RD8 in gel → two clear cut bands in right spot
    - Purified RD8 in gel → nano drop results 26.8ng/uL
    - Set up Gib-13
        - 1.26uL RD8
        - .69uL PCR-18
        - .55uL PCR-6B
        - 2.5 uL HiFi MM
    - Put Gib-13 for 20 minutes 50C
    - Set up Cpec for Gib13 without Polymerase (stored in-20C)
        - 4uL HF buffer
        - 1.6uL dNTP
        - 3.72uL RD8

        - 4.04uL PCR-18
        - 3.24uL PCR-6B
        - 3.2uL Water
    - Transformation of Gib-13 into JM109 for glycerol stock
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 5 µL of Gib-13 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
- Jacob Wolf
    - Reprogrammed Green Photosynthetic incubator to 24 hours of light at 30C
        - Transferred synechococcus stocks to Mets backup incubator which was programmed to a 12 hours on/12 hours off light cycle at 34C
        - Both machines set to 150 rpm
    - Prepared RD4
        - Recipe
            - 5 uL Buffer H
            - 0.5 uL PstI
            - 0.5 uL XbaI
            - 5.9 uL Psb1C3 @ 168.5 ng/uL
            - 38.1uL dH2O
        - Digest Overnight @ 37C
    - Prepared overnight cultures from both plates of NC64A with the single bp insertion

7/31/19

- Nathan Sattah
    - PCR-3
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 0.3µL Phusion
        - 15.6µL sterile water
    - PCR-4
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP

        - 0.3µL Phusion
        - 15.6µL sterile water
    - Ran CPEC for Gib-12 with Touch-Up PCR protocol
    - Transformation of pSB1C3 into s7002
        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated sample 1 onto A++CAM, sample 2 onto A+ only, and sample 3 onto A++CAM
        - Sealed plates with tape and stored in photosynthetic incubator
    - Ran CPEC for Gib-12 on a gel with PCR-3 and PCR-4
        - Took 3 µL of CPEC sample and added 0.6 µL loading dye
        - CPEC lane had nothing
        - PCR-3 and PCR-4 looked very bright
    - Gel purified PCR-3 and PCR-4
        - PCR-3: 46.5 ng/µL
        - PCR-4: 67.2 ng/µL
        - Both graphs looked good
    - Made an overnight culture of PCR-QC-29 for glycerol stock
        - 2 mL LB + 2.2 µL CAM
    - Prepped PCR-34
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (PCR-QC-29)
        - 0.5uL dNTP
        - 15.6µL sterile water
    - Prepped PCR-35
        - 5µL Phusion HF Buffer
        - 1.3uL VF2
        - 1.3uL AlkL-FLAG-2
        - 1uL Diluted Template (PCR-QC-29)
        - 0.5uL dNTP
        - 15.6µL sterile water
- Marti Gendel
    - Checked plate of Gibson-13: no colonies
    - Ran TouchUp PCR with the prepared Gibson-13 cpec tube
    - Transferred 3uL of Gibson-13 Cpec Tube to gel
        - Gel result: no visible band
    - Prepared PCR18 again
        - Add 1.62uL of DNA
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer

        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O
- Apple Lee
    - Added respective primers to PCR 36-38 and ran remaining 30 cycles at annealing temperatures:
        - PCR 36: 57C
        - PCR 37: 59C
        - PCR 38: 61C
        - Gel results: bands did not match simulation; fragments are too light
    - Gel purification of RD4
        - Results: bands matched simulation; gel purified; sample stored in -20C
    - Prepared PCR 36 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2 (adding after 10 cycles)
        - 1.3uL FAS3 (adding after 10 cycles)
        - 1uL Diluted Template (FAS1 & FAS2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 71C (10 cycles)
        - PCR running tomorrow
    - Prepared PCR 37
        - 5µL Phusion HF
        - 1.3uL FAS4 (adding after 10 cycles)
        - 1.3uL FAS7 (adding after 10 cycles)
        - 1uL Diluted Template (FAS3 & FAS4)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - PCR running tomorrow
    - Prepared PCR 38
        - 5µL Phusion HF
        - 1.3uL FAS8 (adding after 10 cycles)
        - 1.3uL FAS5C (adding after 10 cycles)
        - 1uL Diluted Template (FAS5.1 & FAS 5.2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 54C (10 cycles)
        - PCR running tomorrow
- Jacob Wolf
    - PCR 39

        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 1.3 uL Alk-9 Diluted
            - 1.3 uL Alk-10 Diluted
            - 1.0 uL pNC64A @ 12.3 ng/uL(corrected)
            - 8.9 uL dH2O
        - Anneal Temp 64C
        - Gel: Band yielded correct size of approximately 2kb and was quite bright. Proceeding with gel purification.
    - Gel Purification of RD4
        - Good yield at 36.2 ng/uL will proceed with Gibson assembly.
    - Gel purification of PCR-39
        - Yielded 153.6 ng/uL. Stored in -20C
    - Gib-14
        - Recipe
            - 2.5 uL 2X HiFi Master Mix
            - 0.88 uL RD4 @36.2 ng/uL (Vector)
            - 0.99 uL PCR-23 @ 41.0 ng/uL
            - 0.53 uL PCR-24 @ 21.4 ng/uL
            - 0.05 uL Alk-8 @ 4.5 uM
            - 0.05 uL Suffix Adaptor @ 4.5 uM
        - Placed 5 uL volume in 50C water bath for 15 min

8/1/19

- Nathan Sattah
    - Ran PCR-34 and PCR-35
    - Made glycerol stock of PCR-QC-29
        - 750 µL of overnight culture + 750 µL of 50% glycerol
    - Transformed Gib-12 and Cian's Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 5 µL of Gib-12 and Cian's Gib-9 to JM109 tubes, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
    - Gel purified PCR-34 and PCR-35
        - PCR-34: 54.9 ng/µL
        - PCR-35: 77.7 ng/µL
    - Miniprepped remaining culture of PCR-QC-29
        - Result: 70.3 ng/µL
    - Re-designed Gib-9 and Gib-12 primers with Simone

- Jacob Wolf
    - Prepared Glycerol Stocks of corrected pNC64A and stored in -80C
    - Miniprepped remaining ON cultures of pNC64A and stored in -20C.
        - Sample A had 135.7 ng/uL
        - Sample B had 136.7 ng/uL
    - Transformed Gib-14 and plated onto LB+CAM for overnight incubation
    - Ran PCR-2 overnight
        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 1.0 uL pLux @ 10 ng/uL
            - 1.3 uL Alk-3b Diluted
            - 1.3 uL Alk-4 Diluted
            - 8.9 uL dH2O
        - Annealing Temp 67.5C
        - Ext Time 5:05
- Marti Gendel
    - Added .3uL polymerase to PCR-18 and ran Touchdown protocol with 10x starting at 72 and down one degree each cycle- then 25 cycles at 62C with 1:15 extension time
    - Ran PCR-18 in gel and gel purified: Nano-drop results 49.5ng/uL
    - Saw colonies on Gib-13 plate, selected 5 for Colony PCR
        - Picked and dissolved each colony into 20 µL of water
        - Heated at 80°C for 10 minutes
        - To each tube
            - 3uL of dissolved colony
            - 15.4 µL water
            - 5 µL HF Buffer 5x
            - .5 µL dNTP’s
            - .5 µL VF2
            - .5 µL VR
            - 0.1 µL Taq Polymerase
        - Ran Colony PCR for Gib-13 in Thermo-cycler according to protocol
        - Put Colony PCR Gib-13 in a gel - results bad no bands discarded gel
    - Re-ran colony PCR Gib-13 overnight
        - To each tube
            - 3uL of dissolved colony
            - 15.4 µL water
            - 5 µL HF Buffer 5x
            - .5 µL dNTP’s
            - .5 µL VF2
            - .5 µL VR
            - 0.1 µL Taq Polymerase
        - Ran Colony PCR for Gib-13 in Thermo-cycler according to protocol

- Apple Lee
    - Ran PCR 36, PCR 37, PCR 38 prepared yesterday for 10 cycles
    - Added respective primers to PCR 36-38 and ran remaining 26 cycles at annealing temperatures with new protocol:
        - PCR 36: 57C
        - PCR 37: 59C
        - PCR 38: 61C
        - Gel results: bands matched simulation; gel purified; sample stored in -20C but concentrations are low and there are ethanol contamination
    - Finalised overlap PCR protocol for PCR40 and PCR 41

8/2/19

- Nathan Sattah
    - PCR-3
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 0.3µL Phusion
        - 15.6µL sterile water
    - PCR-4
        - 5µL Phusion HF Buffer
        - 1.3uL AlkL-FLAG-2
        - 1.3uL VF2
        - 1uL Diluted Template (P-AlkL)
        - 0.5uL dNTP
        - 0.3µL Phusion
        - 15.6µL sterile water
    - PCR-21
        - 5 µL Phusion HF Buffer
        - 1.3 µL VF2
        - 1.3 µL Sig-A2
        - 1 µL Diluted Template (P-AlkL)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - PCR-22
        - 5 µL Phusion HF Buffer
        - 1.3 µL GFP-1
        - 1.3 µL GFP-2
        - 1 µL Diluted Template (BBa_I746916)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme

        - 15.6 µL sterile water
    - PCR-34
        - 5µL Phusion HF Buffer
        - 1.3uL VR
        - 1.3uL AlkL-FLAG-1
        - 1uL Diluted Template (PCR-QC-29)
        - 0.5uL dNTP
        - 0.2µL Phusion
        - 15.6µL sterile water
    - PCR-35
        - 5µL Phusion HF Buffer
        - 1.3uL VF2
        - 1.3uL AlkL-FLAG-2
        - 1uL Diluted Template (PCR-QC-29)
        - 0.5uL dNTP
        - 0.2µL Phusion
        - 15.6µL sterile water
    - Made new 250 mL A+ Media
    - Gel purified PCR-3, PCR-4, PCR-22, and PCR-34
        - PCR-21 and PCR-35 bands were too faint
        - PCR-3: 71.1 ng/µL
        - PCR-4: 48.2 ng/µL
        - PCR-22: 44.3 ng/µL
        - PCR-34: 64.1 ng/µL
    - Synechococcus Stock
        - Made a new 20 mL culture
            - Took 2 mL from 7/29/19 culture
            - Added 18 mL A+ media
            - Added 18 µL B12
- Marti Gendel
    - Ran gel of GIbson-13 Colony PCR no visible bands
    - Remade colony PCR samples for Gib-13
    - To each tube
        - 3uL of dissolved colony
        - 15.4 µL water
        - 5 µL Taq Buffer
        - .5 µL dNTP's
        - .5 µL VF2
        - .5 µL VR
        - 0.1 µL Taq Polymerase
    - Ran Colony PCR for Gib-13 in Thermo-cycler according to protocol
- Apple Lee
    - Ran gel for PCR 2 (Jacob's lux operon)
        - Result: band matched simulation; gel purified; sample stored in -20C

- Ran Jacob's PCR 39
  - 12.5 uL Q5 2X Master Mix
  - 1.3 uL Alk-9
  - 1.3 uL Alk-10
  - 1.0 uL template (NC64A)
  - 8.9 uL dH2O
  - Annealing temperature: 64C
  - Gel result: no band results present; only primer bands shown
- PCR 40 (25uL)
  - 5µL Phusion HF
  - 1.3uL VF2 (adding after 10 cycles)
  - 1.3uL FAS7 (adding after 10 cycles)
  - 1uL Diluted Template (PCR36 & PCR37)
  - 0.5uL dNTP
  - 0.3uL polymerase
  - 15.6µL sterile water
  - Overlap temperature: 72C (10 cycles)
  - Annealing temperature: 57C (30 cycles)
  - Gel purification on Monday
- PCR 41 (25uL)
  - 5µL Phusion HF
  - 1.3uL FAS8 (adding after 10 cycles)
  - 1.3uL VR (adding after 10 cycles)
  - 1uL Diluted Template (PCR38 & FAS6)
  - 0.5uL dNTP
  - 0.3uL polymerase (adding tomorrow)
  - 15.6µL sterile water
  - Overlap temperature: 57C (10 cycles)
  - Annealing temperature: 57C (30 cycles)
  - Gel purification on Monday
- Autoclaved tips

8/3/19

- Marti Gendel
  - Ran gel of GIbson-13 Colony PCR no visible bands, but band of negative control of failed gibson was visible
  - Remade PCR-6B (Temp at 56C)
    - Add 1uL of FAs1 Template
    - 5uL of Phusion HF
    - 1.3uL of parB-Aprimer
    - 1.3uL of VF2 primer
    - .5uL of dNTPS
    - 15.6uL of H_2O

8/5/19

- Nathan Sattah
    - PCR-21
        - 5 µL Phusion HF Buffer
        - 1.3 µL VF2
        - 1.3 µL Sig-A2
        - 1 µL Diluted Template (P-AlkL)
        - 0.5 µL dNTP
        - 0.3 µL Phusion enzyme
        - 15.6 µL sterile water
    - PCR-35
        - 5µL Phusion HF Buffer
        - 1.3uL VF2
        - 1.3uL AlkL-FLAG-2
        - 1uL Diluted Template (PCR-QC-29)
        - 0.5uL dNTP
        - 0.3µL Phusion
        - 15.6µL sterile water
    - Gel purified PCR-21 and PCR-35
        - PCR-21: 71.9 ng/µL
        - PCR-35: 86.5 ng/µL
        - Both had a peak at 240, but A260 was good enough to keep
    - Synechococcus Stock
        - Made a new 10 mL culture
            - Took 1 mL from 8/2/19 culture
            - Added 9 mL A+ media
            - Added 9 µL B12
    - Transformation of pSB1C3 into s7002
        - OD730: 0.787
        - Made a 2 mL culture, 3x dilution from 7/26/19
        - Took 666 µL of culture + 1334 µL A+ + 1.33 µL B12
        - Digested pSB1C3 overnight
            - 0.86 µL pSB1C3 (0.1 µg)
            - 1 µL CutSmart
            - 0.1 µL EcoRI-HF
            - 8.04 µL sterile water
- Apple Lee
    - Gel purification of PCR 40 and PCR 41
        - Result: no bands present; gel discarded
    - PCR 42 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2 (adding after 10 cycles)
        - 1.3uL FAS7 (adding after 10 cycles)
        - 2uL Diluted Template (PCR36 & PCR37)
        - 0.5uL dNTP

        - 0.3uL polymerase
        - 14.6µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 61C (30 cycles)
        - Gel purification tomorrow
    - PCR 43 (25uL)
        - 5µL Phusion HF
        - 1.3uL FAS8 (adding after 10 cycles)
        - 1.3uL VR (adding after 10 cycles)
        - 2uL Diluted Template (PCR38 & FAS6)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 14.6µL sterile water
        - Overlap temperature: 61C (10 cycles)
        - Annealing temperature: 60C (30 cycles)
        - Gel purification tomorrow
    - RD7
        - Recipe
            - Buffer H 5µL
            - Template (BBa_I746916) µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow
    - Phusion PCR of FabTesA (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas 37
        - 1.3uL Fas 38
        - 1uL Diluted Template (Fas6)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Gel purification tomorrow
    - Universal Promoter PCR (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas-Uni
        - 1.3uL VF2
        - 1uL Diluted Template (Fas1)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 57C

        - Gel purification tomorrow
    - Prepared PCR 36 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2 (adding after 10 cycles)
        - 1.3uL FAS3 (adding after 10 cycles)
        - 1uL Diluted Template (FAS1 & FAS2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 71C (10 cycles)
        - PCR running tomorrow
    - Prepared PCR 37
        - 5µL Phusion HF
        - 1.3uL FAS4 (adding after 10 cycles)
        - 1.3uL FAS7 (adding after 10 cycles)
        - 1uL Diluted Template (FAS3 & FAS4)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - PCR running tomorrow
    - Prepared Phusion PCR of Fas 5 Reaction 2
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - PCR running tomorrow
- Marti Gendel
    - Ran PCR-6B in gel, good clear band → gel purified
        - Nanodrop results of PCR-6B: 116.5ng/uL
    - Made overnights of the dissolved colony from Gibson-13 colonies labeled 1 and 4
        - 2mL LB+2.2µL CAM Overnights
- Jacob Wolf
    - PCR 39
        - Recipe
            - 25 uL Q5 2X Master Mix
            - 2.6 uL Alk-9 Diluted
            - 2.6 uL Alk-10 Diluted
            - 2.0 uL pNC64A @ 12.3 ng/uL(corrected)

            - 17.8 uL dH2O
        - Anneal Temp 64C
        - Gel: Bright band present @ 2kb, proceeding with gel purification.
    - Gel purification of PCR-39
        - Result yielded 125 ng/uL of sample and a good looking nanodrop. Capped and stored remaining 9 uL in -20C.
    - Ran PCR 44 Overnight
        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 1.3 uL Alk-qc-2b
            - 1.3 uL Prefix Adaptor
            - 1.0 uL pNC64A correct @12.3 ng/uL
            - 8.9 uL dH2O
        - Anneal Temp 65C
        - Ext Time 1:00

8/6/19

- Nathan Sattah
    - Boiled digest of pSB1C3 at 80°C in thermocycler for 10 minutes to denature enzymes
    - Transformation of pSB1C3 into s7002
        - Made a new 3 mL culture, 3x dilution from 7/26/19
        - Took 1 mL of culture + 2 mL of A+ + 1 µL B12
        - Stored in photosynthetic incubator overnight
    - Redid Gibson calculations
    - Prepped Gib-9 using new numbers
        - 0.44 µL RD-3
        - 1.6 µL PCR-3
        - 2.36 µL PCR-4
- Jacob Wolf
    - Ran Gel for PCR 44
        - Result: Bright band present @ 0.8 kb.
    - Gel purification of PCR 44
        - Nanodrop had excellent shape and ratios. 74.6 ng/uL was the yield
    - Transformed Gib-13 into JM109 for Marti
- Marti Gendel
    - Checked overnights- no growth
    - Add 1.62uL of DNA
    - Remade PCR18
        - 5uL of Phusion HF
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R primer
        - .5uL of dNTPS
        - 14.8uL of H_2O

        - .3uL of polymerase
    - Set up a new Gib-13
        - .62uL RD8
        - .67uL PCR18
        - .28uL PCR6B
        - 1.56uL Hifi Buffer
        - Put in 50C for 20 minutes
    - Transformed Gib-13 into JM109
        - Added to JM109 sit for 30 minutes on ice
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
- Apple Lee
    - Ran PCR 36 and PCR 37 prepared yesterday for 10 cycles
    - Added respective primers to PCR 36 and PCR 37 and ran remaining 26 cycles at annealing temperatures with new protocol:
        - PCR 36: 57C
        - PCR 37: 59C
        - Gel results: bands do not match simulation; gel discarded
    - Ran gel for RD7
        - Result: bands do not match simulation; gel discarded
    - Ran gel for Universal Promoter PCR, tesA PCR product, and PCRs 42-43
        - Universal Promoter PCR: band matched simulation; gel purified; sample discarded due to low concentration
        - tesA: band matched simulation; gel purified; sample stored in -20C
        - PCR 42: no bands present; gel discarded
        - PCR 43: band does not match simulation; gel discarded
    - Ran PCR FAS5.2 prepared yesterday
        - Gel result: only primer band present; gel discarded
    - Prepared PCR 36 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2 (adding after 10 cycles)
        - 1.3uL FAS3 (adding after 10 cycles)
        - 1uL Diluted Template (FAS1 & FAS2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 71C (10 cycles)
        - Annealing temperature: 57C (26 cycles)
        - Overnight PCR
        - Gel purification tomorrow

    - Prepared PCR 37
        - 5µL Phusion HF
        - 1.3uL FAS4 (adding after 10 cycles)
        - 1.3uL FAS7 (adding after 10 cycles)
        - 1uL Diluted Template (FAS3 & FAS4)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 59C (26 cycles)
        - Overnight PCR
        - Gel purification tomorrow
    - Prepared Phusion PCR of Fas 5 Reaction 2
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Overnight PCR
        - Gel purification tomorrow
    - Phusion PCR of Fas 6
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 9
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - Overnight PCR
        - Gel purification tomorrow

8/7/19

- Nathan Sattah
    - Gib-9
        - Added 4.4 µL of 2x HiFi Assembly MasterMix
        - Incubated in 50°C bath for 20 minutes
    - Transformation of Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 8.8 µL of Gib-9 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds

        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in cold room until 3:00 pm
        - Stored in 37°C overnight
    - Transformation of pSB1C3 into s7002
        - Took OD of 7/26 culture: 0.926
        - Took 1 mL of 7/26 culture and added digested pSB1C3
        - Stored in photosynthetic incubator for 24 hours
        - Made another digest overnight
            - 0.86 µL pSB1C3 (0.1 µg)
            - 1 µL CutSmart
            - 0.1 µL EcoRI-HF
            - 8.04 µL sterile water
- Apple Lee
    - Ran gel for PCR 36 and PCR 37
        - Results: bands matching simulations present, though there are additional bands present
        - Gel purified with Rachael to obtain 30uL final yield; low concentrations present on nanodrop; sample discarded
    - Ran gel for PCR products of FAS 5.2 and FAS 6
        - Results: bands matched simulations
        - Gel purified with Rachael to obtain 30uL final yield; low concentrations present on nanodrop; sample discarded
    - Phusion PCR of FabTesA (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas 37
        - 1.3uL Fas 38
        - 1uL Diluted Template (Fas6)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - Universal Promoter PCR (25uL)
        - 5µL Phusion HF
        - 1.3uL Fas-Uni
        - 1.3uL VF2
        - 1uL Diluted Template (Fas1)
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 57C

        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - Phusion PCR of Fas 1
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas1)
        - 1.3uL VF2
        - 1.3uL Fas1
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 57C
        - Overnight PCR
        - Gel purification tomorrow
    - Phusion PCR of Fas 2C (modification: changed template)
        - 5µL Phusion HF
        - 1.3uL Fas 2
        - 1.3uL Fas 3
        - 1uL Diluted Template (Fas2C)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 58C
        - Overnight PCR
        - Gel purification tomorrow
    - Phusion PCR of Fas 3
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas3)
        - 1.3uL Fas4
        - 1.3uL Fas5
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 57C
        - Overnight PCR
        - Gel purification tomorrow
    - Phusion PCR of Fas 4E (modification: changed template)
        - 5µL Phusion HF
        - 1.3uL Fas 6
        - 1.3uL Fas 7
        - 1uL Diluted Template (Fas4E)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 59C
        - Overnight PCR

        - Gel purification tomorrow
    - Prepared Phusion PCR of Fas 5 Reaction 2
        - 5µL Phusion HF
        - 1.3uL Fas 5B
        - 1.3uL Fas 5C
        - 1uL Diluted Template (Fas5D)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Annealing temperature: 64C
        - Overnight PCR
        - Gel purification tomorrow
    - Phusion PCR of Fas 6
        - 5µL Phusion HF
        - 1uL Diluted Template (Fas6)
        - 1.3uL Fas 9
        - 1.3uL VR
        - 0.5uL dNTP
        - 0.3uL polymerase
        - 15.6µL sterile water
        - Annealing temperature: 61C
        - Overnight PCR
        - Gel purification tomorrow
- Marti Gendel
    - Made 4 colony PCR samples for Gib-13
    - To each tube
        - 3uL of dissolved colony
        - 15.4 µL water
        - 5 µL Taq Buffer
        - .5 µL dNTP's
        - .5 µL VF2
        - .5 µL VR
        - 0.1 µL Taq Polymerase
        - Ran according to protocol with annealing temp at 60C
    - Ran colony PCR tubes in gel, sample 1 and 3 look to produced good bands
    - Made overnights with remaining 17uL of colony dissolved in water
        - 2mL and 2.2 uL Cam
- Jacob Wolf
    - PCR 23
        - Recipe
            - 31.2 uL dH2O
            - 10 uL Phusion HF 5X
            - 2.6 uL Alk qc 1b dilute
            - 2.6 uL Alk 10 dilute

                - 1.0 uL dNTPs
                - 2.0 uL NC64A @ 12.4 ng/uL
                - 0.6 uL Polymerase
            - Anneal Temp 64C
            - Gel: Faint band present @ 0.9kb. Proceeding with gel purification.
    - Replenished Synechococcus DMSO stock
        - 5 tubes prepared at 1.8 mL
        - Recipe
            - 90 uL DMSO 100%
            - 1.7 mL Synechococcus AL 7/26/19 culture
        - Flash-freezed in liquid nitrogen and stored in -80C
    - Gel purification of PCR 23
        - Result: purification had heavy ethanol contamination and a yield of 24 ng/uL. Sample was stored, but reaction will be rerun tomorrow.

8/8/19

- Nathan Sattah
    - Autoclaved tips
    - Colony PCR for Gib-9
        - Labelled 4 colonies on the plate
        - Picked and dissolved each colony into 20 µL of water
        - Heated 3 µL of each sample at 80°C for 10 minutes
        - Prepped controls
            - Negative: 1 µL of failed Gib-9 + 2 µL water
            - Positive: 1 µL pSB1C3 + 2 µL water
        - Added reagents to all 6 tubes
            - 15.4 µL water
            - 4 µL Dream Taq Buffer
            - 0.5 µL dNTP’s
            - 0.5 µL VF2
            - 0.5 µL VR
            - 0.1 µL Taq
        - Put into thermocycler according to protocol (Tm = 60°C)
    - Transformation of pSB1C3 into s7002
        - Transferred 1 mL culture into microfuge tube and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated sample onto A++CAM plate
        - Sealed plate with tape and stored in photosynthetic incubator
    - Ran large gel for Colony PCR
        - Did not work
- Jacob Wolf
    - PCR 23

        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 8.9 uL dH2O
            - 1.3 uL Alk qc 1b dilute
            - 1.3 uL Alk 10 dilute
            - 1.0 uL NC64A @ 12.4 ng/uL
        - Anneal Temp 64C
        - Gel: Bright, thick band present near 0.9-1 kb, proceeding with gel purification.
    - Gel purification of PCR 23
        - Result: Good nanodrop with a concentration of 46.2 ng/uL.
    - RD4
        - Recipe
            - 5 uL Buffer H
            - 0.5 uL PstI
            - 0.5 uL XbaI
            - 6.0 uL Psb1C3 @ 167.4 ng/uL
            - 38.0uL dH2O
        - Digest Overnight @ 37C
    - Prepared 8 overnight cultures of Psb1C3 from glycerol stocks in LB+CAM
- Marti Gendel
    - Checked Overnights: Gib-13 colony 1 overnight was red
    - Miniprepped Gib-13 colony 3 as the pellet was white
        - Note additional volume liquid as some of the wash solution was still on the column (?)
    - Make more RD8 (2ug)
        - 5uL CutSmart
        - 12.75uL PSB1C3
        - .5uL EcorI
        - .5uL SPEI
        - 31.3 uL dH2O
- Apple Lee
    - Ran gel for PCR products of FAS1-6
        - Results: FAS1, FAS2, and FAS4 bands matched simulations; gel purified; samples stored in -20C; FAS 3, 5.2 and 6 bands did not match simulations; gel discarded
    - Phusion PCR of FAS3
        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 8.9 uL dH2O
            - 1.3 uL FAS4
            - 1.3 uL FAS5
            - 1.0 uL template (FAS3)
        - Anneal Temp 57C

        - Gel result: Band matched simulation; gel purified; sample stored in -20C
    - Phusion PCR of FAS5.2
        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 8.9 uL dH2O
            - 1.3 uL FAS5B
            - 1.3 uL FAS5C
            - 1.0 uL template (FAS5D)
        - Anneal Temp 64C
        - Gel result: Band matched simulation; gel purified; sample stored in -20C
    - Phusion PCR of FAS6
        - Recipe
            - 12.5 uL Q5 2X Master Mix
            - 8.9 uL dH2O
            - 1.3 uL FAS9
            - 1.3 uL VR
            - 1.0 uL template (FAS6)
        - Anneal Temp 61C
        - Overnight PCR
        - Gel purification tomorrow

8/9/19

- Nathan Sattah
    - Ran Cian's RD-3 on a gel
        - Gel showed 3 bands, so did not cut
    - Transformation of pSB1C3 into s7002
        - Plate showed growth, so transformation worked!
        - Boiled digest at 80°C for 10 minutes in thermocycler
    - RD-3
        - 5 µL Buffer H
        - 7.5 µL pSB1C3 (2 µg)
        - 0.5 µL EcoRI
        - 0.5 µL PstI
        - 36.5 µL sterile water
        - Stored in 37°C for 4 hours
    - Gel purified Marti's PCR-6B
        - Result: 71.9 ng/µL
    - Resuspended and diluted Cut1, Cut2, Cut3, and SigA primers
    - Filled out Purchase Request for iGEM
- Jacob Wolf
    - Ran gel for RD4
        - Result: 3 distinct, bright bands were produced, sample was discarded.
    - Miniprepped 8 tubes of Psb1C3
    - Prepared 20 mL Synechococcus culture from 8/2/19

        - Recipe
            - 2 mL 8/2/19 Culture
            - 18 mL A+ Media
            - 18 uL B12
    - Autoclaved Tips
    - RD4 (2 ug of template this time)
        - Recipe
            - 8.2 uL Psb1C3 @243.7 ng/uL
            - 5 uL Buffer H
            - 0.5 uL PstI
            - 0.5 uL XbaI
            - 35.8 uL dH2O
        - Gel: Once again 3 distinct bands were produced. Gel discarded.
    - Plated Splice-16 and Gib-16 onto LB+CAM for overnight incubation in 37C
    - Replated Synechococcus transformation trial 2 onto A++CAM plate and placed in 34C
- Marti Gendel
    - Ran RD8 in a gel and two clear bands gel purified the top band: nano-drop 31.7ng/uL
    - Prepared more 6B
        - Add 1uL of FAs1 Template
        - 5uL of Phusion HF
        - 1.3uL of parB-Aprimer
        - 1.3uL of VF2 primer
        - 12.5uL Q5 Master Mix
    - Aliquot dH20
- Apple Lee
    - Prepared PCR 36 (25uL)
        - 5µL Phusion HF
        - 1.3uL VF2 (adding after 10 cycles)
        - 1.3uL FAS3 (adding after 10 cycles)
        - 1uL Diluted Template (FAS1 & FAS2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 71C (10 cycles)
        - Annealing temperature: 57C (26 cycles)
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - Prepared PCR 37
        - 5µL Phusion HF
        - 1.3uL FAS4 (adding after 10 cycles)
        - 1.3uL FAS7 (adding after 10 cycles)
        - 1uL Diluted Template (FAS3 & FAS4)
        - 0.5uL dNTP

        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 59C (26 cycles)
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - Prepared PCR 38 (25uL)
        - 5µL Phusion HF
        - 1.3uL FAS8 (adding after 10 cycles)
        - 1.3uL FAS5C (adding after 10 cycles)
        - 1uL Diluted Template (FAS5.1 & FAS 5.2)
        - 0.5uL dNTP
        - 0.3uL polymerase (adding tomorrow)
        - 15.6µL sterile water
        - Overlap temperature: 54C (10 cycles)
        - Annealing temperature: 61C
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - Gel purified RD7 with bands matching simulation and sample stored in -20C
    - Ran gel for FAS 6
        - Result: band matched simulation; gel purified; sample stored in -20C
    - Gibson 16
        - 2.1uL RD8
        - 0.9uL PCR36
        - 0.8uL PCR37
        - 0.7uL PCR38
        - 0.5uL FAS6
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 1 hr
    - Transformation of Gib-16
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10µL of Gib-16 to JM109 tube, thawed on ice for 20 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Jacob plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight
    - Transformation of Gib-16 (Slice)
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10µL of Gib-16(Slice) to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Jacob plated 100 µL onto LB+CAM plate

        - Stored in 37°C overnight

8/10/19

- Jacob Wolf
    - Removed gibson and splice plates from incubator
        - Colonies showed strong numbers with a couple dozen present on each plate, only one non-red colony spotted
    - Examined photosynthetic incubator
        - Synechococcus cultures and colonies showed slow growth

8/12/19

- Nathan Sattah
    - Threw out pSB1C3 glycerol stocks
    - PCR-47
        - 12.5 µL Q5
        - 1.3 µL VF2
        - 1.3 µL SigA
        - 1 µL Diluted Template (P-AlkL)
        - 8.9 µL sterile water
    - PCR-35
        - 12.5 µL Q5
        - 1.3 µL VF2
        - 1.3 µL AlkL-FLAG-2
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - PCR-45
        - 12.5 µL Q5
        - 1.3 µL Cut1
        - 1.3 µL Cut3
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - PCR-46
        - 12.5 µL Q5
        - 1.3 µL Cut1
        - 1.3 µL Cut2
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - Digest for s7002 Transformation
        - P-AlkL
            - 0.96 µL P-AlkL (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 7.94 µL sterile water
        - Failed Gibson
            - 1.17 µL Failed Gibson (0.1 µg)

                - 1 µL CutSmart
                - 0.2 µL EcoRI-HF
                - 7.73 µL sterile water
            - Put in 37°C overnight
        - Ran gel for PCR products
            - All bands looked good
            - Jacob will purify
- Jacob Wolf
    - Discarded remaining Psb1C3 tubes and glycerol stocks
    - Prepared 8 overnights from pAlk-L glycerol stocks
    - RD10 (tube labelled RD4-2)
        - Recipe
            - 5 uL Cutsmart
            - 0.5 uL PstI
            - 0.5 uL XbaI
            - 14.2 uL p-AlkL-PS @ 70.4 ng/uL
            - 29.8 uL dH2O
        - Digested overnight @ 37C
    - RD9 (tube labelled RD4-3)
        - Recipe
            - 5 uL cutsmart
            - 0.5 uL xbaI
            - 0.5 uL pstI
            - 0.5 uL hpaI
            - 17.6 uL QC-29 @56.8 ng/uL
            - 25.9 uL dH2O
        - Digested Overnight @37C
    - Created 10 mL Cyanobacteria dilution
    - Gel purified 5 samples
        - RD8
        - PCR 35
        - PCR 45
        - PCR 46
        - PCR 47
- Apple Lee
    - Colony PCR of Gib-16 (S)
        - 5uL 5X HF Buffer
        - 0.5uL VF2
        - 0.5uL VR
        - 0.5uL dNTP
        - 3uL colony of Gib-16 (S) boiled at 80C for 10 mins
        - 0.1uL Taq polymerase
        - 15.4uL dH20
        - Positive control using another rfp colony set up with same recipe

        - Negative control using previously failed Gibson set up with same recipe
    - Ran colony PCR on gel
        - Result: bands match simulation; overnights made
    - RD8
        - 5uL CutSmart
        - 14.2uL PCR-QC-29
        - 0.5uL EcoRII
        - 0.5uL SpeI
        - 29.8uL dH2O
        - Overnight digest at 37C incubator

8/13/19

- Nathan Sattah
    - Results from Jacob's gel purification
        - PCR-35: 69.4 ng/µL
        - PCR-45: 94.4 ng/µL
        - PCR-46: 83.1 ng/µL
        - PCR-47: 88.7 ng/µL
    - Gib-9
        - 0.5 µL PCR-45
        - 1.5 µL PCR-3
        - 3 µL PCR-4
        - 5 µL 2x HiFi Assembly MasterMix
    - Gib-12
        - 1 µL PCR-46
        - 2 µL PCR-47
        - 2 µL PCR-22
        - 5 µL 2x HiFi Assembly MasterMix
    - Gib-15
        - 0.5 µL PCR-45
        - 2.5 µL PCR-34
        - 2 µL PCR-35
        - 5 µL 2x HiFi Assembly MasterMix
    - Incubated Gibsons in 50°C water bath for 20 minutes
    - Transformation of Gib-9, Gib-12, and Gib-15
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10 µL of Gib-9, Gib-12, and Gib-15 to JM109 tubes, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Moved to the cold room until 3:00 pm

        - Stored in 37°C overnight
    - s7002 Transformation
        - Boiled digests at 80°C for 10 minutes
        - Made two 1 mL cultures from 7/19/19 stock (OD730: 1.339)
        - Added digested P-AlkL to one, and Failed Gibson to the other
        - Stored in photosynthetic incubator for 24 hours (30°C, 150 rpm, 60 µmol photons)
    - Ran Jacob's RD-9 and RD-10 on a gel and purified
        - RD-9: 36.3 ng/µL
        - RD-10: 48.9 ng/µL
    - PCR-4 overnight
        - 12.5 µL Q5
        - 1.3 µL VF2
        - 1.3 µL AlkL-FLAG-2
        - 1 µL Diluted Template (P-AlkL)
        - 8.9 µL sterile water
- Jacob Wolf
    - Ran gel for RD9 and RD10
        - Result: Both bands were of correct size (approx 2kb).
    - Gel purification of RD9 and RD10
        - RD9 - low yield of 17 ng/uL, discarded.
        - RD10 - low yield of 6.6 ng/uL, discarded.
    - Reprepared RD9
        - Recipe
            - 5 uL Cutsmart
            - 0.5 uL XbaI
            - 0.5 uL PstI
            - 0.5 uL HpaI
            - 14.7 uL NC64A corrected @135.7 ng/uL
            - 28.8 uL dH2O
    - Reprepared RD10
        - Recipe
            - 5 uL Cutsmart
            - 0.5 uL XbaI
            - 0.5 uL PstI
            - 16.7 uL pAlkLPS @119.9 ng/uL
            - 27.3 uL dH2O
    - Prepared 4 ON cultures of pNC64A
    - Plated Gib-17 for AL
- Apple Lee
    - Miniprep 8 samples of qc29 (alkL promoter swap)
        - Concentrations and nanodrop graphs look good; samples stored in -20C
    - Miniprep Gib-16 (pSB1C3 backbone)
        - Concentrations and nanodrop graphs look good; samples stored in -20C

- RD8
  - 5uL CutSmart
  - 6.5uL PCR-QC-29
  - 0.5uL EcoRI
  - 0.5uL SpeI
  - 37.5uL dH2O
  - 3hr digest at 37C incubator
  - Gel result: bands matched simulation; gel purified; sample stored in -20C
- Single diagnostic digest
  - 1uL CutSmart
  - 1uL plasmid (Gib-16 v.s. qc29)
  - 0.1uL EcoRI
  - 7.9uL dH20
  - 1 hr digest at 37C incubator
  - Gel result: bands did not match simulation; gel discarded
- Double diagnostic digest
  - 1uL CutSmart
  - 1uL plasmid (Gib-16 v.s. qc29)
  - 0.1uL SacI
  - 0.1uL KpnI
  - 7.8uL dH20
  - 1 hr digest at 37C incubator
  - Gel result: bands did not match simulation; gel discarded
- Gibson 17
  - 2.2uL RD11
  - 0.86uL PCR36
  - 0.77uL PCR37
  - 0.67uL PCR38
  - 0.5uL FAS6
  - 5uL 2x HiFi Master Mix
  - 50C incubator for 1 hr
- Transformation of Gib-17
  - Took JM109 from -80°C and thawed for 15 minutes
  - Added 10µL of Gib-17 to JM109 tube, thawed on ice for 10 minutes
  - Heat shocked in 42°C bath for 45 seconds
  - Put on ice for 2 minutes
  - Added 300 µL of SOC media
  - Put into 37°C shaker for an hour
  - Jacob plated 100 µL onto LB+CAM plate
  - Stored in 37°C overnight
- Autoclaved tips
- RD3
  - 5uL CutSmart
  - 6.5uL PCR-QC-29

        - 0.5uL EcoRI
        - 0.5uL PstI
        - 37.5uL dH2O
        - Overnight digest at 37C incubator
        - Gel purification tomorrow

8/14/19

- Nathan Sattah
    - Colony PCR for Gibsons
        - 5 Gib-9 colonies
        - 20 Gib-12 colonies
        - 1 Gib-15 colony
        - Three lanes in a big gel so 1 Gib-9 and 2 Gib-12 negative controls
        - 29 samples total
        - Picked and dissolved each colony into 20 µL of water
        - Heated 3 µL of each sample at 80°C for 10 minutes
        - Prepped controls
            - Negative for Gib-9: 1 µL failed Gib-9 + 2 µL water
            - Negative for Gib-12: 1 µL failed Gib-12 + 2 µL water
            - Boiled controls this time as well
        - MasterMix
            - 446.6 µL sterile water
            - 145 µL Taq Buffer
            - 14.5 µL dNTP's
            - 14.5 µL VF2
            - 14.5 µL VR
            - 2.9 µL Taq polymerase
        - Ran in thermocycler
    - s7002 Transformation
        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated samples onto A++CAM
        - Sealed plates with tape and stored in photosynthetic incubator
    - Ran Colony PCR samples on two big gels
        - Gib-12 H and Gib-15 A worked
    - Made overnights of one sample of Gib-12 H and one sample of Gib-15 A
        - 2 µL LB + 2.2 µL CAM + 17 µL bacteria
    - Autoclaved tips
    - Colony PCR for Gib-9 again, using same bacteria
        - Heated another 3 µL of the 5 samples at 80°C for 10 min
        - MasterMix
            - 92.4 µL sterile water

                - 30 µL Taq Buffer
                - 3 µL dNTP’s
                - 3 µL VF2
                - 3 µL VR
                - 0.6 µL Taq polymerase
            - Ran in thermocycler overnight with Jacob
- Jacob Wolf
    - Gib-18
        - Recipe
            - 5 uL 2X HiFi Assembly Mix
            - 1.4 uL RD10 @48.9 ng/uL
            - 1.8 uL PCR-44 @74.6 ng/uL
            - 1.4 uL PCR-23 @112.5 ng/uL
            - 0.4 uL Suffix Adaptor @1.125 uM
        - Spun down for 15 seconds
        - Run @50C in thermocycler for 15 min
        - Immediately transformed into JM109 and plated onto LB+CAM
    - Miniprepped NC64A x4 tubes
    - Resuspended ALK-FLAG-1 from primer plate
    - RD11
        - Recipe
            - 5 uL Cutsmart
            - 0.5 uL EcoRI
            - 0.5 uL SpeI
            - 20.5 uL P-ALKL-PS @97.6 ng/uL
            - 23.5 uL dH2O
    - PCR 54 Run Overnight
        - Recipe
            - 12.5 uL Q5 2x Master mix
            - 1.0 uL NC64A @12.4 ng/uL
            - 1.3 uL Alk-10
            - 1.3 uL Alk-FLAG-1
            - 8.9 uL dH2O
        - Anneal Temp 57C
        - Ext time 1:15
    - PCR 56 Run Overnight
        - Recipe
            - 12.5 uL Q5 2x Master mix
            - 1.0 uL NC64A @12.4 ng/uL
            - 1.3 uL Alk-2b
            - 1.3 uL Alk-FLAG-1
            - 8.9 uL dH2O
        - Anneal Temp 63C
        - Ext time 1:15

    - Added 22 uL Master Mix to NS's 5 PCR and ran at 59.4C anneal temp overnight
    - Imaged AL's gel
    - Replaced Gibson plates 9,12, 15 and 17 into 37C incubator
- Apple Lee
    - Ran PCR 48
        - 1.3uL FAS 25
        - 1.3uL FAS 26
        - 1uL Diluted Template (PCR36)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Annealing temperature: 57C
        - Gel result: bands matched simulation; gel purified; sample stored in -20C
    - Ran PCR 49
        - 1.3uL FAS 33
        - 1.3uL FAS 34
        - 1uL Diluted Template (PCR37)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Annealing temperature: 57C
        - Gel result: bands matched simulation; gel purified; sample stored in -20C
    - Ran PCR 50
        - 1.3uL FAS 29
        - 1.3uL FAS 30
        - 1uL Diluted Template (PCR38)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Annealing temperature: 57C
        - Gel result: bands matched simulation; gel purified; sample stored in -20C
    - Ran PCR 51
        - 1.3uL FAS 11
        - 1.3uL FAS 12
        - 1uL Diluted Template (PCR36)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Annealing temperature: 61C
        - Gel result: bands did not match simulation; gel discarded
    - Ran PCR 52
        - 1.3uL FAS 19
        - 1.3uL FAS 20
        - 1uL Diluted Template (PCR37)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Annealing temperature: 61C
        - Gel result: bands did not match simulation; gel discarded

- Ran PCR 53
    - 1.3uL FAS 15
    - 1.3uL FAS 16
    - 1uL Diluted Template (PCR38)
    - 12.5 Q5 Master Mix
    - 8.9µL sterile water
    - Annealing temperature: 61C
    - Gel result: bands did not match simulation; gel discarded
- Ran gel for RD3
    - Result: bands matched simulation; gel purified; sample stored in -20C
- Colony PCR of Gib-17 (20 colonies A-T)
    - 5uL 5X HF Buffer
    - 0.5uL VF2
    - 0.5uL VR
    - 0.5uL dNTP
    - 3uL colony of Gib-17 boiled at 80C for 10 mins
    - 0.1uL Taq polymerase
    - 15.4uL dH20
    - Negative control using previously failed Gibson set up with same recipe
    - Gel result: bands did not match simulation; gel discarded
- Autoclaved tips

8/15/19

- Nathan Sattah
    - Picked up Gibson MasterMix from Gordon storeroom
    - Colony PCR for Gib-12 again, using same bacteria
        - Boiled 19 remaining samples of Gib-12 (one was used for overnights) at 80°C for 10 min
        - Made four MasterMixes (three 6x and one 5x)
        - Added 22 µL of the MasterMix to each tube using multichannel pipette
        - Ran in thermocycler
    - Miniprepped Gib-12 H and Gib-15 A from 8/14/19
    - Submitted Gib-12 and Gib-15 samples for sequencing with VR and VF2
    - Ran Colony PCR for Gib-12 and Gib-9 on a gel
        - Bands present were not the right size
    - Autoclaved tips
    - Reran Gib-9
        - 1 µL PCR-45
        - 2 µL PCR-3
        - 2 µL PCR-4
        - 5 µL 2x HiFi Assembly MasterMix
        - Incubated in 50°C water bath for 20 minutes
    - Transformation of Gib-9
        - Took JM109 from -80°C and thawed for 15 minutes

        - Added 10 µL of Gib-9 to JM109 tubes, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Stored in 37°C overnight
- Jacob Wolf
    - Moved Regrown plates back to cold room
    - Autoclaved p1000 tips
    - Ran gel for RD11, PCR 54 & PCR 56 and Gel purified
        - RD11: correct band size, yield: 42.7ng/uL
        - PCR 54: correct band size, yield: 38.4 ng/uL
        - PCR 56: correct band size, yield: 47.2 ng/uL
    - Gib-18 had approximately 2 dozen colonies present
        - Numbered 20 colonies for colony PCR
    - Colony PCR (20 colonies)
        - Prepared Master Mix
            - Recipe
        - Boiled 3 uL of resuspended cells @80C for 10 min
        - Run in Thermocycler with 60C anneal temp and 2:05 ext time
        - Run 25 cycles
    - Ran gel for Colony PCR (20 samples)
        - Result: Limited banding was observed around 1.2 kb in samples 13 and 14, sample 15 seemed to have a faint band just above 2 kb.
    - Prepared Overnight Cultures of samples 13,14 and 15
        - 15 was determined to be correct size
- Apple Lee
    - Gibson 19
        - 0.6uL PCR45
        - 1.3uL PCR36
        - 1.2uL PCR37
        - 1uL PCR38
        - 0.9uL FAS6
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 1 hr
    - Transformation of Gib-19
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-19 to JM109 tube, thawed on ice for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate

        - Stored in 37°C overnight (18hrs)
    - Gfp Promoter Ligation
        - 1uL T4 ligase buffer
        - 1uL RD6 (Promoter BBa_J23119)
        - 3.9uL RD7 (gfp BBa_I746916)
        - 0.5uL T4 ligase
        - 3.6uL dH20
        - 1hr room temperature incubation
        - 10 mins 65C water bath incubation
    - Transformation of gfp promoter ligation
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of gfp promoter ligation to JM109 tube, thawed on ice for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - RD7
        - Recipe
            - CutSmart 10x buffer 5µL
            - Template (BBa_I746916) 7.4µL
            - XbaI 0.5µL
            - PstI 0.5µL
            - dH2O 36.6uL
        - Overnight digest at 37C incubator
        - Gel purification tomorrow

8/16/19

- Nathan Sattah
    - Picked up tips from Gordon storeroom
    - Miniprepped Jacob's Gib-18, sample 15
        - Graph looked perfect
        - Result: 78.5 ng/µL
    - Colony PCR for Gib-9 again
        - 20 colonies with negative control
        - Same MasterMix recipe
        - Ran in thermocycler
    - Ran Colony PCR for Gib-9 on a big gel
    - Made 5 mL liquid cultures of P-AlkL and Failed Gib-9 s7002 transformations
        - 5 mL A+ + 5 µL B12
        - Picked colony with pipette tips and dropped into waffled flasks
    - Streak purified P-AlkL and Failed Gib-9 plates onto new A++CAM plates

    - Looked at 8/15/19 gel again and decided that Gib-9 A worked
    - Made overnight culture for Gib-9 A
    - Reran Colony PCR for Gib-9 again overnight
- Apple Lee
    - Colony PCR of Gib-19 (2 colonies A-B)
        - 5uL Taq Buffer
        - 0.5uL VF2
        - 0.5uL VR
        - 0.5uL dNTP
        - 3uL colony of Gib-19 boiled at 80C for 10 mins
        - 0.1uL Taq polymerase
        - 15.4uL dH20
        - Negative control using previously failed Gibson set up with same recipe
        - Positive control using qc-29 set up with same recipe
        - Gel result: bands did not match simulation; gel discarded
    - Ran RD7 on gel
        - Result: bands matched simulation; gel purified
    - Prepared 20 mL Synechococcus culture from 8/12/19
        - Recipe
            - 2 mL 8/12/19 Culture
            - 18 mL A+ Media
            - 18 uL B12
    - Made overnight liquid cultures for gfp promoter ligation
        - 2.5mL LB
        - 2.5uL CAM
        - Colony from plate
        - 18hrs 37C shaking incubator
        - Miniprep tomorrow
    - Prepared PCR 42
        - 1.3uL VF2 (added after 10 cycles)
        - 1.3uL FAS 7 (added after 10 cycles)
        - 1uL Diluted Template (PCR36 & PCR37)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 61C (30 cycles)
        - PCR running tomorrow
        - Gel purification on Monday
    - Prepared PCR 43
        - 1.3uL FAS 8
        - 1.3uL VR
        - 1uL Diluted Template (PCR38 & FAS6)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water

        - Overlap temperature: 61C (10 cycles)
        - Annealing temperature: 60C (30 cycles)
        - PCR running tomorrow
        - Gel purification on Monday
- Jacob Wolf
    - Prepared and submitted Gib-18 for sequencing with VR, VF2, Alk-R11 and Alk-F10
    - Resuspended primer cut-4 and stored in methanotrophs box
    - PCR-57
        - Recipe
            - 12.5 uL Q5 2X MM
            - 8.9 uL dH2O
            - 1.0 uL QC-29 (p-AlkLPS) @9.5 ng/uL
            - 1.3 uL Cut2 dilute
            - 1.3 uL Cut4 dilute
        - Anneal Temp 64C
        - Ext time 1:15
        - Gel: Expected band @2kb, NO band present @2kb. Smeared, faint band around 600 bp. Potential contamination: re-diluted cut-4 and sterile water. Old aliquots of water and primer dilution discarded. Gel discarded.
    - Prepared PCR-55 for AL to run on 8/17
        - Recipe
            - 12.5 uL Q5 2X MM (to be added tomorrow by AL)
            - 8.9 uL dH2O
            - 1.0 uL NC64A @14.5 ng/uL
            - 1.3 uL VF2 dilute
            - 1.3 uL Alk-FLAG-2 dilute
        - Anneal Temp: 60C
        - Ext Time 0:30
        - Gel: Expected band @219 bp
    - Prepared PCR-57
        - Recipe
            - 12.5 uL Q5 2X MM (to be added tomorrow by AL)
            - 8.9 uL dH2O
            - 1.0 uL QC-29 (p-AlkLPS) @9.5 ng/uL
            - 1.3 uL Cut2 dilute
            - 1.3 uL Cut4 dilute
        - Anneal Temp 67C (use NEB for anneal temp)
        - Ext time 1:15
        - PCR running tomorrow by AL
        - Gel: Expected band @2kb,

8/17/19

- Apple Lee

- Miniprep gfp promoter ON culture
  - Concentration and nanodrop graph looks good; sample stored in -20C
- Miniprep Gib-9 ON culture
  - Concentration and nanodrop graph looks good; sample stored in -20C
- Submitted Gib-9 A for sequencing
- Autoclaved tips
- Single diagnostic digest
  - 1uL CutSmart
  - 1uL plasmid (Gfp promoter ligation)
  - 0.1uL EcoRI
  - 7.9uL dH20
  - 1 hr digest at 37C incubator
  - Gel result:
- Double diagnostic digest
  - 1uL CutSmart
  - 1uL plasmid (Gfp promoter ligation)
  - 0.1uL EcoRV
  - 0.1uL KpnI
  - 7.8uL dH20
  - 1 hr digest at 37C incubator
  - Gel result:
- Ran PCR 42, 43, 55, and 57
  - Gel purification tomorrow by Cian
- Colony PCR of Gib-17 (20 colonies A-T)
  - 5uL 5X HF Buffer
  - 0.5uL VF2
  - 0.5uL VR
  - 0.5uL dNTP
  - 3uL colony of Gib-17 boiled at 80C for 10 mins
  - 0.1uL Taq polymerase
  - 15.4uL dH20
  - Negative control using previously failed Gibson set up with same recipe
  - Positive control using qc 29 set up with same recipe
  - Gel result: bands did not match simulation; gel discarded

- Cian Colgan
  - Gib-20
    - HiFi 2X Master Mix 3.5µL
    - PCR 45 0.5µL
    - PCR 18 2µL
    - PCR 6B 1µL
    - Ran for 15 minutes. Transformed into commercial JM109 and plated on LB+CAM
  - Ran Nathan's Colony PCR products

        - All samples except C produced bands that resembled the expected product but not the negative control. Made 2.5mL overnights of A, B, E, F, P, and Q
    - Ran Gel of Apple's PCR 42, 43, 55, and 57
        - 42 and 43 produced smears that did not give discernible bands in the expected 4Kb overlap
        - 55 was in the right size but faint
        - 57 was bright and in the right place
        - Gel Purification 55 and 57
            - Both samples gave good purity but PCR 55 yield low (15.6ng/µL). Suspect due to faint band
    - Gib-22
        - PCR 54 1µL
        - PCR 55 1µL
        - PCR 57 0.5µL
        - NEB HiFi 2X Master Mix 2.5µL
        - Run for 15min, transformed into commercial JM109 and plated on LB+CAm
    - Gib-21
        - PCR 55 2µL
        - PCR 56 2µL
        - PCR 57 1µL
        - PCR 2 2µL
        - NEB HiFi 2X Master Mix 7µL
        - Run for 30min, transformed into commercial JM109 and Plated on LB+CAM

8/18/19

- Nathan Sattah
    - Colony PCR for second round of Gib-9 had 18 correct samples
    - Miniprepped Cian's overnights for samples A, B, E, F, P, Q
        - Gib-9A: 188.9 ng/µL
        - Gib-9B: 161.2 ng/µL
        - Gib-9E: 175.1 ng/µL
        - Gib-9F: 228.8 ng/µL
        - Gib-9P: 188 ng/µL
        - Gib-9Q: 190.9 ng/µL
    - Sent 6 samples of Gib-9 for sequencing with VR and VF2
- Cian Colgan
    - Plate results
        - Gib-20 plate had no colonies. Suspect used DNA purified before switching to 365.
        - Gib-21 and Gib-22 had lots of colonies. Performed colony PCR
            - Recipe (Prepared as 35X Master Mix)

        - dNTPS 0.5µL
        - VF2 0.5µL
        - VR 0.5µL
        - Boiled DNA 3µL
        - OneTaq Standard Buffer 5µL
        - Taq Poly 0.1µL
        - dH20 15.4µL
        - Total volume 24µL
    - Gel Results
        - Both plates had bands around 300bp. Suggests that PCR57 ligated back to itself rather than insert
            - Ran Prefix and Suffix Sequence using Multiple Primer Analyzer. Appears that they can Ligate to each other and chops off the last 5 bases of the Prefix. Explains the self ligating results we are seeing. Unsure about how to proceed next. Will bring up at lab meeting
- Ran Apple's colony PCR results on a gel
    - No bands were produced in most colonies.

8/19/19

- Nathan Sattah
    - Made new A++CAM plates (500 mL)
    - Autoclaved tips
    - Restreak and liquid culture for s7002 showed no growth
    - Original plates grew more, so remade restreaks and liquid cultures
        - Remade two 5 mL cultures
    - Transformation of Gib-12 into s7002
        - Digested Gib-12 overnight
            - 0.76 µL Gib-12 (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.04 µL sterile water
    - Transformation of Gib-15 for Western Blot
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of Gib-15 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Stored in 37°C overnight
    - PCR-61
        - 12.5 µL Q5

        - 1.3 µL Cut3
        - 1.3 µL Cut4
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - Made overnight cultures for the one Gib-20 colony, and Jacob's Gib-22 Colony PCR (sample J and Q)
        - 2 mL LB + 2.2 µL CAM + 17 µL bacteria for Jacob's
- Apple Lee
    - Synechococcus Stock
        - Made a new 10 mL culture
            - Took 1 mL from 8/16/19 culture
            - Added 9 mL A+ media
            - Added 9 µL B12
    - Colony PCR of Gib-22 (20 colonies A-T)
        - 5uL 5X HF Buffer
        - 0.5uL VF2
        - 0.5uL VR
        - 0.5uL dNTP
        - 3uL colony of Gib-17 boiled at 80C for 10 mins
        - 0.1uL Taq polymerase
        - 15.4uL dH20
        - Gel result: colonies J and Q bands matched simulation; overnights made
    - PCR 42
        - 1.3uL FAS 7 (added after 10 cycles)
        - 1.3uL VF2 (added after 10 cycles)
        - 1uL Diluted Template (PCR36 & PCR37)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 56C (26 cycles)
        - Gel purification tomorrow
    - PCR 43
        - 1.3uL FAS 8 (added after 10 cycles)
        - 1.3uL VR (added after 10 cycles)
        - 1uL Diluted Template (PCR38 & FAS6)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 61C (10 cycles)
        - Annealing temperature: 60C (26 cycles)
        - Gel purification tomorrow
    - PCR 60
        - 1.3uL FAS 9 (added after 10 cycles)
        - 1.3uL Cut1 (added after 10 cycles)
        - 1uL Diluted Template (QC-29 & FAS6)

        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 59C (10 cycles)
        - Annealing temperature: 65C (26 cycles)
        - Gel purification tomorrow
    - Finalised 3 overlap PCR protocols (PCR58 - PCR60)
    - PCR 36
        - 2.6uL FAS 3 (adding after 10 cycles tomorrow)
        - 2.6uL VF2 (adding after 10 cycles tomorrow)
        - 2uL Diluted Template (FAS1 & FAS2)
        - 25 Q5 Master Mix
        - 17.8µL sterile water
        - Overlap temperature: 71C (10 cycles)
        - Annealing temperature: 57C (26 cycles)
        - Gel purification tomorrow
    - PCR 37
        - 1.3uL FAS 4 (adding after 10 cycles tomorrow)
        - 1.3uL FAS7 (adding after 10 cycles tomorrow)
        - 1uL Diluted Template (FAS3 & FAS4)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 59C (26 cycles)
        - Gel purification tomorrow
    - PCR 38
        - 1.3uL FAS 8 (adding after 10 cycles tomorrow)
        - 1.3uL FAS 5C (adding after 10 cycles tomorrow)
        - 1uL Diluted Template (FAS5.1 & FAS5.2)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 54C (10 cycles)
        - Annealing temperature: 61C (26 cycles)
        - Gel purification tomorrow

8/20/19

- Nathan Sattah
    - Miniprepped Jacob's Gib-22 overnights, Marti's Gib-20 overnight had no growth
        - Gib-22J: 217.8 ng/µL
        - Gib22Q: 189.5 ng/µL
    - Submitted Gib-22J and Gib-22Q for sequencing with VR and VF2
    - s7002 Transformations
        - Boiled digested Gib-12 at 80°C for 10 min
        - Digested Gib-22J for 1 hour
            - 0.46 µL Gib-22J (0.1 µg)

                - 1 µL CutSmart
                - 0.2 µL EcoRI-HF
                - 8.34 µL sterile water
            - Digested Gib-22Q for 1 hour
                - 0.53 µL Gib-22Q (0.1 µg)
                - 1 µL CutSmart
                - 0.2 µL EcoRI-HF
                - 8.27 µL sterile water
            - Took OD730 of 8/2/19 culture: 1.073
            - Added 1 mL of culture to 3 tubes + 10 µL Gib-12/Gib-22J/Gib-22Q
            - Put in photosynthetic incubator for 24 hours
        - Moved Gib-15 plate (for WB) to cold room
        - Made a 3 mL seed overnight culture of Gib-15 for WB
- Apple Lee
    - Ran PCR 42, 43, 60, and 61 on gel
        - Result 1: PCR 42 band did not match simulation; gel discarded
        - Result 2: PCR 43, 60, and 61 bands matched simulation; gel purified; sample stored in -20C
    - Completed remaining 26 cycles for PCR 36-38
    - Ran PCR 36-38 on gel
        - Result: bands matched simulation; gel purified; samples stored in -20C
    - PCR 43 (modification: changed reaction volume to 50uL)
        - 2.6uL FAS 8 (added after 10 cycles)
        - 2.6uL VR (added after 10 cycles)
        - 2uL Diluted Template (PCR38 & FAS6)
        - 25 Q5 Master Mix
        - 17.8µL sterile water
        - Overlap temperature: 61C (10 cycles)
        - Annealing temperature: 60C (26 cycles)
        - Gel purification tomorrow
    - PCR 60 (modification: changed reaction volume to 50uL)
        - 2.6uL FAS 9 (added after 10 cycles)
        - 2.6uL Cut1 (added after 10 cycles)
        - 2uL Diluted Template (QC-29 & FAS6)
        - 25 Q5 Master Mix
        - 17.8µL sterile water
        - Overlap temperature: 59C (10 cycles)
        - Annealing temperature: 65C (26 cycles)
        - Gel purification tomorrow
    - PCR 58 (modification: changed reaction volume to 50uL)
        - 2.6uL FAS 4 (added after 10 cycles)
        - 2.6uL FAS 5C (added after 10 cycles)
        - 2uL Diluted Template (PCR37 & PCR38)
        - 25 Q5 Master Mix

        - 17.8µL sterile water
        - Overlap temperature: 58C (10 cycles)
        - Annealing temperature: 71C (26 cycles)
        - Gel purification tomorrow
    - Made 2.5mL overnight culture for previously failed Gib-22 for control for gfp promoter
        - 2.5mL LB
        - 2.5uL CAM
        - 17uL of Gib-22 A

8/21/19

- Nathan Sattah
    - Took OD600 of Gib-15 seed culture: 1.577
        - Kept remaining 2 mL culture in cold room
    - Took time to prepare for WB by writing out protocols and calculating dilutions
    - Made 100 mL 1M Tris-HCl (pH 6.5)
        - 12.1 g Trizma base
        - Up to 100 mL Milli-Q water
        - Added 6M HCl until pH of 6.5 was reached
    - Made 1 mL of Loading Dye for WB in microfuge tube (https://www.sigmaaldrich.com/technical-documents/protocols/biology/western-blotting/buffers-recipes/4x-sds-sample-loading-buffer.html)
        - 200 µL 1M Tris-HCl (pH 6.5)
        - 400 µL 1M DTT
        - 0.08 g SDS
        - 4 mg bromophenol blue
        - 320 µL glycerol
        - Adjust final volume to 1 mL with Milli-Q water
        - Stored in -20°C
    - Made 100 mL Cell Lysis Buffer
        - 6.5 mL 1M Tris-HCl (pH 6.5)
        - 2 g SDS
        - 10 mL glycerol
        - Up to 100 mL Milli-Q water
        - Stored in cold room
    - Transformation of Gib-12, Gib-22J, and Gib-22Q into s7002
        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated samples onto A++CAM plates
        - Sealed plates with tape and stored in photosynthetic incubator
    - Stored Primary Antibody and protein ladder in -20°C
    - Stored Secondary Antibody in cold room, wrapped in tinfoil

- Apple Lee
    - Ran PCR 36, 43, 60, and 58 on gel
        - Result: bands did not match simulation; gel discarded
    - Make 100mL overnight culture for gfp promoter
        - 100mL LB
        - 110uL CAM
        - 150mL seed culture (gfp promoter overnight)
    - Make 100mL overnight culture for gfp promoter
        - 100mL LB
        - 110uL CAM
        - 150mL seed culture (Gib-22 A overnight)
    - PCR 6B
        - 1.3uL Par-B A
        - 1.3uL VF2
        - 1uL Diluted Template (FAS1)
        - 12.5 Q5 Master Mix
        - 8.9µL dH20
        - Annealing temperature: 56C
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - PCR 18
        - 1.3uL Par-B R
        - 1.3uL Par-B F
        - 1.7uL Genomic DNA
        - 12.5 Q5 Master Mix
        - 8.2µL dH20
        - Annealing temperature: 67C
        - Gel result: band matched simulation; gel purified; sample stored in -20C
    - PCR 36
        - 1.3uL FAS 3 (added after 10 cycles)
        - 1.3uL VF2 (added after 10 cycles)
        - 1uL Diluted Template (FAS1 & FAS 2)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 72C (10 cycles)
        - Annealing temperature: 57C (30 cycles)
        - Gel purification tomorrow
    - PCR 43
        - 1.3uL FAS 8 (added after 10 cycles)
        - 1.3uL VR (added after 10 cycles)
        - 1uL Diluted Template (FAS6 & PCR38)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 61C (10 cycles)
        - Annealing temperature: 60C (30 cycles)

        - Gel purification tomorrow
    - PCR 58
        - 1.3uL FAS 4 (added after 10 cycles)
        - 1.3uL FAS 5C (added after 10 cycles)
        - 1uL Diluted Template (PCR37 & PCR38)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 58C (10 cycles)
        - Annealing temperature: 71C (30 cycles)
        - Gel purification tomorrow
    - PCR 60
        - 1.3uL FAS 9 (added after 10 cycles)
        - 1.3uL Cut 1 (added after 10 cycles)
        - 1uL Diluted Template (FAS6 & QC-29)
        - 12.5 Q5 Master Mix
        - 8.9µL sterile water
        - Overlap temperature: 59C (10 cycles)
        - Annealing temperature: 65C (30 cycles)
        - Gel purification tomorrow
    - Universal Promoter PCR
        - 12.5µL Q5 Master Mix
        - 1.3uL Fas-Uni
        - 1.3uL Fas 25
        - 1uL Diluted Template (Fas1)
        - 8.9µL sterile water
        - Annealing temperature: 57C
        - Gel purification tomorrow
    - PCR-45
        - 12.5 µL Q5 Master Mix
        - 1.3 µL Cut1
        - 1.3 µL Cut3
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
        - Gel purification tomorrow
    - PCR-46
        - 12.5 µL Q5 Master Mix
        - 1.3 µL Cut1
        - 1.3 µL Cut2
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
        - Gel purification tomorrow
    - PCR 62
        - 12.5µL Q5 Master Mix
        - 1.3uL VR

        - 1.3uL Alk 9
        - 1uL Diluted Template (NC64A-Fixed)
        - 8.9µL sterile water
        - Annealing temperature: 56C
        - Gel purification tomorrow
    - PCR 63
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-FLAG-1
        - 1.3uL VR
        - 1uL Diluted Template (NC64A-Fixed)
        - 8.9µL sterile water
        - Annealing temperature: 60C
        - Gel purification tomorrow
    - Gib-24 (FabF)
        - 0.75uL PCR46
        - 3uL PCR33
        - 1.25uL PCR48
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 20mins
    - Transformation of Gib-24
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-24 to JM109 tube, thawed on ice for 15 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Gib-25 (TesA)
        - 0.7uL PCR46
        - 2.5uL PCR33
        - 1.8uL PCR32
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 20mins
    - Transformation of Gib-25
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-25 to JM109 tube, thawed on ice for 15 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Gib-26 (FabD)

        - 1.75uL PCR46
        - 3uL PCR33
        - 1.25uL PCR49
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 20mins
    - Transformation of Gib-26
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-26 to JM109 tube, thawed on ice for 15 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)

8/22/19

- Nathan Sattah
    - Western Blot for Gib-15 (Attempt 1)
        - Sample Prep
            - Current OD600 of seed culture: 1.577
            - Target OD600 for sample prep: 0.5 (0.4 - 0.6)
            - Make a 15X dilution (down to OD of 0.1) using 28 mL LB + 28 µL CAM + 2 mL culture in an Erlenmeyer flask (cap with tinfoil)
            - Let grow in 37°C shaker for 30 minutes and take OD again
                - 1st OD: 0.230
                - 2nd OD: 0.375
                - 3rd OD: 0.508
            - Turn on 95°C heat block
            - Add 1 mL of culture to microfuge tube and centrifuge at 13,300 rpm for 3 minutes
            - Carefully discard supernatant
            - Repeat into same microfuge tube 4 more times (like Miniprep)
            - Resuspend in 50 µL cold Lysis Buffer
            - Boil the sample for 10 min at 95°C and freeze at -20°C for 10 min
            - Repeat 3 times
            - Stored in -20°C
        - Bradford Assay (BioRad protocol, 1 mL format)
            - Remove 1X Dye Reagent from 4°C storage and let it reach ambient temperature, invert tube before use
            - Prepare five 20 µL dilutions of BSA (Promega 10 mg/mL stock) in cuvettes
                - 0.1 mg/mL: 0.2 µL BSA + 19.8 µL water
                - 0.3 mg/mL: 0.6 µL BSA + 19.4 µL water
                - 0.5 mg/mL: 1 µL BSA + 19 µL water

        - 0.8 mg/mL: 1.6 µL BSA + 18.4 µL water
        - 1 mg/mL: 2 µL BSA + 18 µL water
        - Blank: 20 µL water
        - Sample: 20 µL sample
    - Add 1 mL of 1X Dye Reagent to each cuvette
    - Incubate at room temperature for 5 minutes (no longer than 1 hour)
    - Set spectrophotometer to 595 nm
    - Blank then measure absorbances
        - 0.1: 0.148
        - 0.3: 0.303
        - 0.5: 0.461
        - 0.8: 0.765
        - 1: 0.883
        - Sample: 1.065
    - Plot 595 nm values on y-axis with concentration in µg/mL on x-axis
    - Determine sample concentration using equation of standard curve
        - 63 µg/µL
- Gel Electrophoresis
    - Added 4X Loading Buffer to samples
        - 35 µL positive control (from Simone) + 11.6 µL Loading Buffer
        - 0.5 µL Gib-15 sample + 5 µL Loading Buffer + 14.5 µL 1X Running Buffer
    - Boil samples at 95°C for 7 minutes
    - Obtain bag containing gel from cold room, and open the bag
    - Carefully remove the green comb and tape
    - Clamp gel into place
    - If running one gel, clamp Buffer Dam into the opposite side
    - Fill apparatus with 1X Running Buffer to bottom fill line and fill the space in between the gel and the dam until wells are covered
    - Load 5 µL of protein ladder
    - Load 44.6 µL of control
    - Load 20 µL of sample
    - Close lid (ensure that electrodes match and that lid is fully closed)
    - Run at 150V for 50 minutes
        - Samples did not move through the gel
- RD-12
    - 5 µL CutSmart
    - 14.88 µL Template (BBa_J61100)
    - 0.5 µL SpeI-HF
    - 0.5 µL PstI-HF
    - 29.12 µL sterile water

        - Incubated at 37°C overnight
    - PCR-45
        - 12.5 µL Q5
        - 1.3 µL Cut1
        - 1.3 µL Cut3
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - PCR-46
        - 12.5 µL Q5
        - 1.3 µL Cut1
        - 1.3 µL Cut2
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - Gib-11
        - 1 µL PCR-46
        - 2 µL PCR-6B
        - 2 µL PCR-18
        - 5 µL 2x HiFi Assembly MasterMix
    - Gib-23
        - 1 µL PCR-60
        - 1 µL PCR-36
        - 3 µL PCR-58
        - 5 µL 2x HiFi Assembly MasterMix
    - Transformation of Gib-11 and Gib-23
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of Gib-15 to JM109 tube, waited for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Stored in 37°C overnight
- Apple Lee
    - Gfp promoter fluorescence testing with SImone
        - Prepared microsphere stock solution
            - Vortex silica beads for 30 secs
            - 100uL microspheres + 900uL ddH20
        - Performed serial dilution of microspheres and measured optical densities
            - 100uL total final volume in each well
        - Calibrated silicon beads (standard curve made)
        - Negative control and gfp promoter sample prepared through series of serial dilutions
            - 100uL seed solution + 900uL LB+CAM
            - 200uL total final volume in each well

        - 10-fold dilutions made for seed solution and negative control OD to reach 0.1
        - Calibrated fluorescein (standard curve made)
            - Vortex fluorescein for 30 secs
            - 100uL fluorescein + 900uL TBS
        - Performed serial dilution of microspheres and measured optical densities
            - 100uL total final volume in each well
    - Ran PCRs on gel
        - PCR 36: bands matched simulation; gel purified; sample stored in -20C
        - PCR 43: bands matched simulation; gel purified; sample stored in -20C
        - PCR 58: bands matched simulation; gel purified; sample stored in -20C
        - PCR 60: bands matched simulation; gel purified; sample stored in -20C
        - PCR 45: no bands present; gel discarded
        - PCR 46: no bands present; gel discarded
        - Universal Promoter PCR: no bands present; gel discarded
        - PCR 62: bands matched simulation; gel purified; sample stored in -20C
        - PCR 63: bands matched simulation; gel purified; sample stored in -20C
    - Colony PCR of Gibson products of FabF (20 colonies), FabD (20 colonies), TesA (10 colonies)
    - Ran colony PCRs on gel
        - FabF: Samples D and H matched simulation; overnights were made
        - FabD: Samples C, D, L, P, and T matched simulation; overnights were made
        - TesA: Samples D and H matched simulation; overnights were made

8/23/19

- Nathan Sattah
    - Made 500 mL liquid LB
    - Transformation of BBa_J61101
        - Pulled part from 2019 Kit Plate 3 well 16F using 10 µL water
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of BBa_J61101 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM plate
        - Stored in 37°C overnight
    - Ran RD-12, PCR-45, and PCR-46 on a gel with Apple's Universal Promoter PCR and gel purified
        - PCR-45: 45.9 ng/µL
        - PCR-46: 73.5 ng/µL
        - PCR-33: 66.6 ng/µL
        - RD-12: 27.7 ng/µL

- Redesigned parB-A and parB-R primers to adjust overhang temperatures since Gib-11 did not work (no colonies seen on the plate)
- Transformation of Gib-9 into s7002
  - Digested Gib-9B for 1 hour
    - 0.62 µL Gib-9B (1 µg)
    - 1 µL CutSmart
    - 0.2 µL EcoRI-HF
    - 8.18 µL sterile water
  - Added digested Gib-9B to 1 mL of 8/2/19 culture (OD730: 1.196)
- Made new s7002 liquid cultures of P-AlkL (from restreak plate 8/16/19), Failed Gib-9 (from restreak plate 8/16/19), and Gib-12
  - 5 mL A+ + 5 µL B12 + 1 µL CAM each
- Synechococcus Stock
  - Made a new 20 mL culture
    - Took 2 mL from 8/19/19 10 mL culture
    - Added 18 mL A+ media
    - Added 18 µL B12

- Apple Lee
  - Universal Promoter PCR
    - 12.5µL Q5 Master Mix
    - 1.3uL Fas-Uni
    - 1.3uL Fas 25
    - 1uL Diluted Template (Fas1)
    - 8.9µL sterile water
    - Annealing temperature: 57C
    - Gel result: band matched simulation; gel purified; sample stored in -20C
  - Gib-27 (FabZ)
    - 0.9uL PCR46
    - 2.8uL PCR33
    - 1.3uL PCR50
    - 5uL 2x HiFi Master Mix
    - 50C incubator for 20mins
  - Transformation of Gib-27
    - Took JM109 from -80°C and thawed for 20 minutes
    - Added 10µL of Gib-27 to JM109 tube, thawed on ice for 10 minutes
    - Heat shocked in 42°C bath for 45 seconds
    - Put on ice for 2 minutes
    - Added 300 µL of SOC media
    - Put into 37°C shaker for an hour
    - Plated 100 µL onto LB+CAM plate
    - Stored in 37°C overnight (18hrs)
  - Gib-28
    - 0.8uL PCR61
    - 1uL Alk-8

        - 3.2uL PCR62
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 20mins
    - Transformation of Gib-28
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-28 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Gib-29
        - 0.8uL PCR61
        - 1uL Alk-8
        - 3.2uL PCR63
        - 5uL 2x HiFi Master Mix
        - 50C incubator for 20mins
    - Transformation of Gib-29
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-29 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Miniprep overnight cultures for FabD, FabF, TesA colonies
        - Result: concentrations are high; graph looks good
    - Submit FabD (5), FabF (2), TesA (2) samples for sequencing
    - Colony PCR of Gib-23 (17 samples)
    - Ran colony PCR of Gib-23 on gel
        - Result: Colonies E and M matched simulation; overnights were made
    - Ligation-2
        - 1uL T4 ligase buffer
        - 1.6uL RD12 (Promoter BBa_J61100)
        - 3uL RD7 (gfp BBa_I746916)
        - 0.5uL T4 ligase
        - 3.9uL dH20
        - 1hr room temperature incubation
        - 10 mins 65C water bath incubation
    - Transformation of gfp promoter ligation
        - Took JM109 from -80°C and thawed for 20 minutes

        - Added 10µL of gfp promoter ligation to JM109 tube, thawed on ice for 15 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Plated 100uL of Gib-23 on LB + CAM
        - 37C incubator for 18 hours

8/24/19

- Cian Colgan
    - Took out all of the plates
        - TesA and Gib-23 plates regrew with overgrown colonies. Made liquid cultures

8/26/19

- Marti Gendel
    - PCR 64
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL Alk10
        - 1uL Diluted Template (NC64A-Fixed)
        - 8.9µL sterile water
        - Annealing temperature: 57C
    - Transformed Cians blue PCR tube labeled 12? Into JM109
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added the pcr tube product
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Made overnight culture of Alk-operon
        - 3mL of LB
        - 2.2uL CAM
        - Scraped top of NC64A Fixed tube stored in -80C
    - Made two overnight cultures from 1101 plate
        - 2mL LB and 2.2uL CAM + colony in each
- Nathan Sattah
    - Western Blot for Gib-15 (Attempt 2)
        - Sample Prep
            - Took OD600 of remaining culture: 0.501

        - Add 1 mL of culture to microfuge tube and centrifuge at 13,300 rpm for 3 minutes
        - Carefully discard supernatant
        - Repeat into same microfuge tube 4 more times (like Miniprep)
        - Resuspend in 50 µL cold Lysis Buffer
        - Boil the sample for 10 min at 95°C on heat block and freeze at -20°C for 10 min
        - Repeat 3 times
        - Store sample in -20°C
- Bradford Assay (BioRad protocol, 1 mL format)
    - Remove 1X Dye Reagent from 4°C storage and let it reach ambient temperature, invert tube before use
    - Prepare five 20 µL dilutions of BSA (Promega 10 mg/mL stock) in cuvettes
        - 0.1 mg/mL: 0.2 µL BSA + 19.8 µL water
        - 0.3 mg/mL: 0.6 µL BSA + 19.4 µL water
        - 0.5 mg/mL: 1 µL BSA + 19 µL water
        - 0.8 mg/mL: 1.6 µL BSA + 18.4 µL water
        - 1 mg/mL: 2 µL BSA + 18 µL water
        - Blank: 20 µL water
        - Sample: 20 µL sample
    - Add 1 mL of 1X Dye Reagent to each cuvette
    - Incubate at room temperature for 5 minutes (no longer than 1 hour)
    - Set spectrophotometer to 595 nm
    - Blank then measure absorbances
        - 0.1: 0.108
        - 0.3: 0.336
        - 0.5: 0.485
        - 0.8: 0.786
        - 1: 0.843
        - Sample: 1.063
    - Plot 595 nm values on y-axis with concentration in µg/mL on x-axis
    - Determine sample concentration using equation of standard curve
        - 62.67 µg/µL
- Gel Electrophoresis
    - Add 4X Loading Buffer to samples
        - 30 µL positive control (from Simone) + 10 µL Loading Buffer
        - 0.5 µL Gib-15 sample + 5 µL Loading Buffer + 14.5 µL 1X Running Buffer
    - Boil samples at 95°C for 7 minutes
    - Obtain bag containing gel from cold room, and open the bag

        - Carefully remove the green comb and tape
        - Clamp gel into place
        - If running one gel, clamp Buffer Dam into the opposite side
        - Fill apparatus with 1X Running Buffer to bottom fill line and fill the space in between the gel and the dam until wells are covered
        - Load 5 µL of protein ladder
        - Load 40 µL of control
        - Load 20 µL of sample
        - Loading order (left to right): ladder, control, sample
        - Close lid (ensure that electrodes match and that lid is fully closed)
        - Run at 150V for 40 minutes
- Transfer
    - Remove a drawer from transfer apparatus and open the lid
    - Open package containing transfer sandwich
    - Place bottom pad onto the bottom of the drawer
    - Use roller to smooth out air bubbles
    - Use green tool thing to open the gel casing
    - Use a razor blade to nick top right corner of the gel to keep orientation
    - Handle gel carefully so that the gel does not break
    - Place gel on top of bottom pad
    - Use roller to smooth out air bubbles
    - Place top pad over gel
    - Use roller to smooth out air bubbles
    - Select “Mini” running protocol
- Blocking
    - Make TBST + 5% milk solution
        - Take 20 mL of TBST and add to it 2.5 g of powdered milk
    - Use tweezers to remove membrane from sandwich and place in box lid
    - Use a razor blade to nick top right corner of the membrane to keep orientation
    - Fill box lid with 20 mL of TBST + 5% milk solution
    - Incubate on a shaker set to 3.5 and at room temperature for 1 hour
- Primary Antibody
    - Dilute Primary Antibody
        - 3 µL Primary Antibody + 3 mL TBST + 0.375 g powdered milk
        - Make in conical tube and store in cold room
    - Pour out Blocking Buffer from box lid
    - Transfer membrane carefully to vacuum sealable bag
    - Add 20 mL TBST into the bag and place back on shaker for about 5 minutes

                - Remove TBST from bag and repeat wash once more
                - Add all of the Primary Antibody solution to the bag
                - Place on shaker in cold room overnight
        - Transformation of pSB3C5
            - Pulled part from 2019 Kit Plate 6 well 4C using 10 µL water
            - Took JM109 from -80°C and thawed for 15 minutes
            - Added 1 µL of sample to JM109 tube, waited for 30 minutes
            - Heat shocked in 42°C bath for 45 seconds
            - Put on ice for 2 minutes
            - Added 300 µL of SOC media
            - Put into 37°C shaker for an hour
            - Plated 100 µL on an LB+CAM plate
            - Stored in 37°C overnight
        - Finished transformation of Gib-9 into s7002
            - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
            - Concentrated the culture to 100 µL by removing some supernatant
            - Resuspended by flicking the microfuge tube
            - Plated 100 µL of Gib-9 onto A++CAM plate
            - Sealed plates with tape and stored in photosynthetic incubator
        - Designed Cut primers for pSB1A2 and pSB3C5

8/27/19

- Marti Gendel
    - Miniprepped 2mL overnight culture of Alk-operon
        - Good nano-drop results: 256.9ng/uL
    - s7002 Transformations of Alk-Operon (NC64A from freezer stock)
        - Digested NC64A Alk-operon for 1 hour
            - 0.389 µL NC64A Alk-operon (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.41 µL sterile water
        - Boiled digested NC64A Alk-operon at 80C for 10 minutes
        - Took OD730 of 8/12/19 culture: 1.53
        - Added 1 mL of culture to a flask + 10 µL NC64A Alk-operon
        - Put in photosynthetic incubator for 24 hours (1:20pm)
    - Took out plate from transformed blue pcr tube → many colonies
        - Made overnights with 4 of the colonies using 2mL LB and 2.2uL CAM
    - PCR 67
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL PaII-1
        - 1uL Diluted Template (NC64A
        - 8.9µL sterile water

        - Annealing temperature: 57C
    - PCR 68
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL PalI-2
        - 1uL Diluted Template (NC64A
        - 8.9µL sterile water
        - Annealing temperature: 64C
    - PCR 69
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-Flag 2B
        - 1.3uL PalI-2
        - 9.9µL sterile water
        - Annealing temperature: 57C, extension time 30 seconds
- Nathan Sattah
    - Autoclaved water
    - Western Blot for Gib-15 (Attempt 2)
        - Secondary Antibody
            - Dilute Secondary Antibody
                - 3 µL Secondary Antibody + 3 mL TBST + 0.375 g powdered milk
                - Make in conical tube, wrap in tinfoil, and store in cold room
            - Open bag and pour out Primary Antibody solution into the original container and store it in -20°C
            - Add 20 mL TBST to the bag and place on room temperature shaker for about 5 minutes
            - Remove TBST and repeat 3 more times
            - Add all of the Secondary Antibody solution to the bag
            - Let shake at room temperature for 1 hour while covered in tinfoil
            - Open bag and pour out Secondary Antibody solution into the original container and store it in -20°C
            - Wash with TBST 3 times, cover with tinfoil
            - Wash one more time with TBS (different from TBST!!)
        - Imaging
            - Keep membrane submerged in TBS and bring it to the 9th floor Odyssey machine
        - Result: Unclear
        - Re-filled bag with Primary Antibody and put on shaker in the cold room
    - Made 1 L 1X TBST
        - 900 mL sterile water
        - 100 mL 10X TBS
        - 1 mL Tween-20
    - PCR-66
        - 12.5 µL Q5

        - 1.3 µL VR
        - 1.3 µL VF2
        - 1 µL Diluted Template (PCR-QC-29)
        - 8.9 µL sterile water
    - Gel purified PCR-66
        - Result: 83.6 ng/µL
    - Used 100 µL of same bacteria to replate pSB3C5 and BBa_J61101 on LB+CAM
    - Updated WB protocol with imaging instructions

8/28/19

- Marti Gendel
    - Miniprepped four overnights that came from colonies on the plate from the blue pcr tube → Nano drop results good
    - Finished transformation of Alk-Operon (NC64A from freezer stock) into s7002
        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated 100 µL of Alk-Operon (NC64A from freezer stock) onto A++CAM plate
        - Sealed plates with tape and stored in photosynthetic incubator
    - Ran PCR 67, 68,69 on gel → bad results except 67
    - Gel purified PCR-67 band→ nano drop results: 77ng/uL
    - Set up PCR 64 again
        - PCR 64
            - 12.5µL Q5 Master Mix
            - 1.3uL VF2
            - 1.3uL Alk10
            - 1uL Diluted Template (NC64A-Fixed)
            - 8.9µL sterile water
            - Annealing temperature: 57C
    - PCR 74
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL Alk-Flag2
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 57C
    - PCR-75
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water

        - Annealing temperature: 57C
    - Took 4 Miniprepped samples of blue-pcr and ran diagnostic digests
        - Single diagnostic digest
            - 1uL CutSmart
            - 1uL plasmid
            - 0.1uL EcoRI-HF
            - 7.9uL dH20
            - 1 hr digest at 37C incubator
            - Gel result:
        - Double diagnostic digest
            - 1uL CutSmart
            - 1uL plasmid
            - 0.1uL EcoRV-HF
            - 0.1uL KpnI-HF
            - 7.8uL dH20
            - 1 hr digest at 37C incubator
    - PCR 68
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL PaII-2
        - 1uL Diluted Template (NC64A
        - 8.9µL sterile water
        - Annealing temperature: 64C
    - s7002 Transformations of P-Alk-PS (28J)
        - Digested P-Alk-PS 1 hour
            - 0.54 µL P-Alk-PS (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.26 µL sterile water
        - Boiled digested P-Alk-PS (28J) at 80C for 10 minutes
        - Took OD730 of 8/16/19 culture: 0.87
        - Added 1 mL of culture to a flask + 10 µL P-Alk-PS (28J)
        - Put in photosynthetic incubator for 24 hours (4:05pm)
- Nathan Sattah
    - Made LB+CAM plates
    - PCR-65
        - 12.5 µL Q5
        - 1.3 µL Cut1B
        - 1.3 µL Cut3
        - 1 µL Diluted Template (pSB1A2)
        - 8.9 µL sterile water
    - Checked Apple’s sequencing results for FabD and FabF
        - Chose FabD-L and FabF-D to keep and relabelled them as P-FabD and P-FabF

- Troubleshooting Gib-23
  - Sequencing results show alignment with AlkL
  - Likely an issue with PCR-60
- PCR-70
  - 12.5 µL Q5
  - 1.3 µL FAS 12
  - 1.3 µL VF2
  - 1 µL Diluted Template (P-FabF)
  - 8.9 µL sterile water
- PCR-71
  - 12.5 µL Q5
  - 1.3 µL FAS 11
  - 1.3 µL VR
  - 1 µL Diluted Template (P-FabF)
  - 8.9 µL sterile water
- Gel purified PCR-65, PCR-70, PCR-71
  - PCR-65: 46.4 ng/µL, discarded
  - PCR-70: 114.3 ng/µL
  - PCR-71: 47.2 ng/µL, discarded
- PCR-60
  - 12.5 µL Q5
  - 1 µL each Diluted Template (FAS6 & QC-29)
  - 7.9 µL sterile water
  - Overlap temperature: 59°C (10 cycles)
  - 1.3 µL FAS 9 (added after 10 cycles)
  - 1.3 µL Cut1 (added after 10 cycles)
  - Annealing temperature: 65°C (30 cycles)
- Remade s7002 liquid cultures that spilled
  - Failed Gib-9, Gib-12, and Gib-9
  - 5 mL A+ + 5 µL B12 + 1 µL CAM
- Made an overnight culture from PCR-QC-29 plate to use as a negative control for Western Blot
  - 2 mL LB + 2.2 µL CAM
- Transformation of Failed Gib-9, NC64A Fixed, P-AlkL-PS, BBa_K2573000, pSB3C5, BBa_J61101, P-Alk-PS, P-FabD, P-FabF into JM109
  - Took JM109 from -80°C and thawed for 15 minutes
  - Added 1 µL of sample to JM109 tube, waited for 30 minutes
  - Heat shocked in 42°C bath for 45 seconds
  - Put on ice for 2 minutes
  - Added 300 µL of SOC media
  - Put into 37°C shaker for an hour
  - Plated 100 µL on an LB+CAM plate
  - Stored in 37°C overnight

8/29/19

- Marti Gendel
    - PCR 69
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-Flag 2B
        - 1.3uL PaII-2
        - 9.9µL sterile water
        - Annealing temperature: 64C, extension time 30 seconds
    - PCR 6C
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL ParB-A v2
        - 1.0uL Fas-1 template
        - 8.9µL sterile water
        - ™ 57
    - PCR18B
        - 12.5µL Q5 Master Mix
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R v2 primer
        - 1.62uL DNA template
        - 8.3uL water
        - ™ 63: Touchdown PCR
    - Ran gel with PCR64, PCR68, PCR69, PCR74, PCR75
        - Cut and gel purified PCR64, PCR68, PCR69
        - Gel purification good for PCR68 and PCR64
    - PCR 69
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-Flag 2B
        - 1.3uL PaII-2
        - 9.9µL sterile water
        - Annealing temperature: 64C, extension time 30 seconds, 40 cycles
        - Tube partially evaporated
    - Gib-33
        - 1.03uL PCR-46
        - 1.97 uL PCR-64
        - 1.98uL PCR-2
        - 5uL HiFI MasterMix
        - Put in 50C for 20 minutes
    - Transformation of Gib-33
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-33 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes

        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
        - Stored in 37°C overnight (18hrs)
    - Finished transformation of PAlk-PS (28J) into s7002
        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated 100 µL of PAlk-PS (28J) onto A++CAM plate
        - Sealed plates with tape and stored in photosynthetic incubator
    - Ran gel with PCR-6C, PCR-18B, PCR60
        - Gel results: Good band from PCR-6C, PCR60
        - Gel purified PCR-6C, PCR60
            - Nano-drop results: kept PCR60
    - PCR 74
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL Alk-Flag2
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 57C
    - PCR-75
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 57C
    - PCR-63
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1
        - 1uL 28J PN64A
        - 8.9µL sterile water
        - Annealing temperature: 60C
    - PCR-76
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 58C
    - PCR-69

        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-Flag 2B
        - 1.3uL PaII-2
        - 9.9µL sterile water
        - Annealing temperature: 64C, extension time 30 seconds, 40 cycles
- Nathan Sattah
    - Western Blot for Gib-15 (Attempt 3)
        - Sample Prep
            - Gib-15 culture is ready, need to prep negative control
            - Current OD600 of control culture: 1.038
            - Target OD600 for sample prep: 0.5 (0.4 - 0.6)
            - Turn on 95°C heat block for future use
            - Make a 5X dilution (down to OD of about 0.2) using 4 mL LB + 5.5 µL CAM + 1 mL culture in a Falcon tube
            - Let grow in 37°C shaker for 30 minutes
            - Add 1 mL of culture to microfuge tube and centrifuge at 13,300 rpm for 3 minutes (for both negative control and sample)
            - Carefully discard supernatant
            - Repeat into same microfuge tube 4 more times (like Miniprep)
            - Resuspend in 50 µL cold Lysis Buffer
            - Boil the sample for 10 min at 95°C on heat block and freeze at -20°C for 10 min
            - Repeat 3 times for both negative control and sample
            - Store sample in -20°C
        - Bradford Assay (BioRad protocol, 1 mL format)
            - Remove 1X Dye Reagent from 4°C storage and let it reach ambient temperature, invert tube before use
            - Prepare five 20 µL dilutions of BSA (Promega 10 mg/mL stock) in cuvettes
                - 0.1 mg/mL: 0.2 µL BSA + 19.8 µL water
                - 0.3 mg/mL: 0.6 µL BSA + 19.4 µL water
                - 0.5 mg/mL: 1 µL BSA + 19 µL water
                - 0.8 mg/mL: 1.6 µL BSA + 18.4 µL water
                - 1 mg/mL: 2 µL BSA + 18 µL water
                - Blank: 20 µL water
                - Sample: 20 µL sample
            - Add 1 mL of 1X Dye Reagent to each cuvette
            - Incubate at room temperature for 5 minutes (no longer than 1 hour)
            - Set spectrophotometer to 595 nm
            - Blank then measure absorbances
                - 0.1: 0.065
                - 0.3: 0.197
                - 0.5: 0.525

        - 0.8: 0.648
        - 1: 0.866
        - Sample: 1.043
    - Plot 595 nm values on y-axis with concentration in µg/mL on x-axis
    - Determine sample concentration using equation of standard curve
        - 58.78 µg/µL
- Gel Electrophoresis
    - Add 4X Loading Buffer to samples
        - 20 µL positive control + 7 µL Loading Buffer
        - 20 µL negative control +7 µL Loading Buffer
        - 20 µL sample + 7 µL Loading Buffer
        - 10 µL sample + 3.3 µL Loading Buffer
    - Boil samples at 95°C for 7 minutes
    - Obtain bag containing gel from cold room, and open the bag
    - Carefully remove the green comb and tape
    - Clamp gel into place
    - If running one gel, clamp Buffer Dam into the opposite side
    - Fill apparatus with 1X Running Buffer to bottom fill line and fill the space in between the gel and the dam until wells are covered
    - Load 5 µL of protein ladder
    - Load controls and samples
    - Loading order (left to right): ladder, positive control, negative control, sample
    - Close lid (ensure that electrodes match and that lid is fully closed)
    - Run at 150V for 40 minutes
- Transfer
    - Remove a drawer from transfer apparatus and open the lid
    - Open package containing transfer sandwich
    - Place bottom pad onto the bottom of the drawer
    - Use roller to smooth out air bubbles
    - Use green tool thing to open the gel casing
    - Use a razor blade to nick top right corner of the gel to keep orientation
    - Handle gel carefully so that the gel does not break
    - Place gel on top of bottom pad
    - Use roller to smooth out air bubbles
    - Place top pad over gel
    - Use roller to smooth out air bubbles
    - Select “Mini” running protocol
- Blocking
    - Make TBST + 5% milk solution
        - Take 20 mL of TBST and add to it 1 g of powdered milk

- Use tweezers to remove membrane from sandwich and place in box lid
- Use a razor blade to nick top right corner of the membrane to keep orientation
- Fill box lid with 20 mL of TBST + 5% milk solution
- Incubate on a shaker set to 3.5 and at room temperature for 1 hour

■ Primary Antibody

- Dilute Primary Antibody
  - 6 µL Primary Antibody + 3 mL TBST + 0.375 g powdered milk (tried 1:500 dilution of Primary Antibody)
  - Make in conical tube and store in cold room
- Pour out Blocking Buffer from box lid
- Transfer membrane carefully to vacuum sealable bag
- Add 20 mL TBST into the bag and place back on shaker for about 5 minutes
- Remove TBST from bag and repeat wash once more
- Add all of the Primary Antibody solution to the bag
- Place on shaker in cold room overnight

○ PCR-60

■ 12.5 µL Q5
■ 1 µL each Diluted Template (FAS6 & PCR-45)
■ 7.9 µL sterile water
■ Overlap temperature: 59°C (10 cycles)
■ 1.3 µL FAS 9 (added after 10 cycles)
■ 1.3 µL Cut1 (added after 10 cycles)
■ Annealing temperature: 65°C (30 cycles)

○ Made overnight cultures for P-FabF, P-FabD, P-Alk-PS, MetE, Gib-15 seed culture for WB, and PCR-QC-29 seed culture for WB

8/30/19

- Marti Gendel
  - Ran PCR63, PCR69, PCR74, PCR75, PCR76 in gel: results good for 69 and 74 cut those bands
  - Picked 10 colonies from Gib-33 Plate
    - Picked and dissolved each colony into 30 µL of water
    - Heated at 80°C for 10 minutes
    - To each tube
      - 3uL of dissolved colony
      - 15.4 µL water
      - 5 µL One Taq Buffer 5x
      - .5 µL dNTP’s
      - .5 µL VF2
      - .5 µL VR

                - 0.1 µL Taq Polymerase
            - Ran Colony PCR with 9:15 extension time
        - PCR 74
            - 12.5µL Q5 Master Mix
            - 1.3uL VF2
            - 1.3uL Alk-Flag2
            - 1uL 28J P-Alk-PS
            - 8.9µL sterile water
            - Annealing temperature: 57C
        - PCR-75
            - 12.5µL Q5 Master Mix
            - 1.3uL VR
            - 1.3uL Alk-Flag1
            - 1uL 28J P-Alk-PS
            - 8.9µL sterile water
            - Annealing temperature: 57C

- Nathan Sattah
    - Western Blot for Gib-15 (Attempt 3)
        - Secondary Antibody
            - Dilute Secondary Antibody
                - 3 µL Secondary Antibody + 3 mL TBST + 0.15 g powdered milk
                - Make in conical tube, wrap in tinfoil, and store in cold room
            - Open bag and pour out Primary Antibody solution into the original container and store it in -20°C
            - Add 20 mL TBST to the bag and place on room temperature shaker for about 5 minutes
            - Remove TBST and repeat 3 more times
            - Add all of the Secondary Antibody solution to the bag
            - Let shake at room temperature for 1 hour while covered in tinfoil
            - Open bag and pour out Secondary Antibody solution into the original container and store it in -20°C
            - Wash with TBST 3 times, cover with tinfoil
            - Wash one more time with TBS (different from TBST!!)
        - Imaging
            - Blot looked better, so progress… but image can be improved
    - Miniprepped overnight cultures for P-FabF, P-FabD, P-Alk-PS, and MetE
        - P-FabF: 201.8 ng/µL
        - P-FabD: 236.2 ng/µL
        - P-Alk-PS: 299.1 ng/µL
        - MetE: 230.6 ng/µL
    - Made glycerol stocks (750 µL) for P-FabF, P-FabD, and P-Alk-PS
    - Gib-23

        - 0.5 µL PCR-60
        - 1.25 µL PCR-36
        - 3.25 µL PCR-58
        - 5 µL 2x HiFi Assembly MasterMix
    - Transformation of Gib-23, BBa_J61101, and pSB3C5 into JM109
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 10 µL of Gib-23, 5 µL pSB3C5, and 1 µL BBa_J61101 to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL of Gib-23 and pSB3C5 on LB+CAM plates, and 100 µL BBa_J61101 onto LB+AMP plate
        - Stored in 37°C overnight
    - PCR-65
        - 12.5 µL Q5
        - 1.3 µL Cut1B
        - 1.3 µL Cut3
        - 1 µL Diluted Template (pSB1A2)
        - 8.9 µL sterile water
    - PCR-71
        - 12.5 µL Q5
        - 1.3 µL FAS 11
        - 1.3 µL VR
        - 1 µL Diluted Template (P-FabF)
        - 8.9 µL sterile water

8/31/19

- Nathan Sattah
    - Gib-23 plate had no colonies
    - Ran PCR-65 and PCR-71 on a gel
    - Gel purified PCR-65 and PCR-71
        - PCR-65: 65.3 ng/µL
        - PCR-71: 108.1 ng/µL
    - Ran Gib-33 Colony PCR on a 0.5% gel

9/3/19

- Marti Gendel
    - PCR 74
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL Alk-Flag2
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water

        - Annealing temperature: 58C
    - PCR-75
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 58C
    - PCR-76
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 58C
    - PCR-69
        - 12.5µL Q5 Master Mix
        - 2.6uL Alk-Flag 2B
        - 2.6uL PaII-2
        - 9.9µL sterile water
        - Annealing temperature: 64C, extension time 30 seconds, 40 cycles
    - PCR 6C
        - 12.5µL Q5 Master Mix
        - 1.3uL VF2
        - 1.3uL ParB-A v2
        - 1.0uL Fas-1 template
        - 8.9µL sterile water
        - ™ 57
- Jacob Wolf
    - Ran PCR-74 overnight
        - Recipe
            - 12.5µL Q5 Master Mix
            - 1.3uL VF2
            - 1.3uL Alk-Flag-2
            - 1uL 28J P-Alk-PS
            - 8.9µL sterile water
        - Annealing temperature: 57C
        - Ext Time 0:30
    - Prepared 6 ON cultures of Gib-33 resuspended colonies
        - Recipe
            - 2 mL LB
            - 2.2 uL CAM
            - 27 uL of resuspended colony
        - Placed in 37C shaker overnight

    - Gel purified PCR 69 and PCR 6C
        - Results:
            - PCR 69: 99.0 ng/uL
            - PCR 6C: 69.0 ng/uL
        - Capped and Stored on blue rack in -20C
    - Autoclaved Tips
- Nathan Sattah
    - Made 500 mL liquid LB
    - Went over to Simone's lab to pick Failed Gib-9 and P-AlkL-PS colonies for growth curve testing
    - Gib-30
        - 2 µL PCR-65
        - 3 µL PCR-66
        - 5 µL 2x HiFi Assembly MasterMix
    - Gib-31
        - 1 µL PCR-45
        - 2 µL PCR-70
        - 2 µL PCR-71
        - 5 µL 2x HiFi Assembly MasterMix
    - Transformation of Gib-30 and Gib-31 into JM109
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of sample to JM109 tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL on an LB+CAM (Gib-31) plate and on an LB+AMP (Gib-30) plate
        - Stored in 37°C overnight
    - Ran Marti's PCR-6C and PCR-69 on a gel
    - Made overnights for BBa_J61101 and pSB3C5
        - 2 mL LB + 2.2 µL CAM for pSB3C5
        - 2 mL LB+ 2.2 µL AMP for BBa_J61101
    - Picked 4 colonies from NC64A Fixed plate for overnight cultures for growth curve testing
        - 2 mL LB + 2.2 µL CAM
        - Stored in Mets Lab incubator to get blue light

9/4/19

- Marti Gendel
    - PCR-75
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1

        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 58C
    - PCR-76
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 58C
    - PCR18B
        - 12.5µL Q5 Master Mix
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R v2 primer
        - 1.62uL DNA template
        - 8.3uL water
        - ™ 62: Touchdown PCR
    - Gel purified and good nano drop results for PCR-74 only (33.1ng/uL)
    - s7002 Transformations of P-Alk-PS (28J)
        - Digested P-Alk-PS 1 hour
            - 0.54 µL P-Alk-PS (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.26 µL sterile water
        - Boiled digested P-Alk-PS (28J) at 80C for 10 minutes
        - Took OD730 of 8/16/19 culture: 1.1
        - Added 1 mL of culture to a flask + 10 µL P-Alk-PS (28J)
        - Put in photosynthetic incubator for 24 hours (3:30)
    - s7002 Transformations of Alk-Operon (NC64A from freezer stock)
        - Digested NC64A Alk-operon for 1 hour
            - 0.389 µL NC64A Alk-operon (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.41 µL sterile water
        - Boiled digested NC64A Alk-operon at 80C for 10 minutes
        - Took OD730 of 8/16/19 culture: 1.1
        - Added 1 mL of culture to a flask + 10 µL NC64A Alk-operon
        - Put in photosynthetic incubator for 24 hours (3:30)
    - Gib-34
        - 1uL PCR-45
        - 1.5 uL PCR-63
        - 2.5uL PCR-74
        - 5uL HiFI MasterMix
        - Put in 50C for 20 minutes

    - Transformation of Gib-34
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-34 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate
- Nathan Sattah
    - Growth Curve testing
        - At Simone's Lab using plate reader
        - Control (Failed Gib-9)
        - AlkL (P-AlkL-PS)
        - Alk (NC64A Fixed)
        - 4 replicates of each
        - Made 10X dilutions of the 4 NC64A Fixed overnight cultures
            - 900 µL LB + 100 µL culture + 1.1 µL CAM
        - Measured 150 µL of each sample in plate reader
        - Measured at 10:00 am, 12:00 pm, 2:00 pm, 4:00 pm, and 6:00 pm
    - Miniprepped pSB3C5 overnight culture
        - Result: 179.9 ng/µL
    - PCR-64
        - 12.5 µL Q5
        - 1.3 µL VF2
        - 1.3 µL Alk10
        - 1 µL Diluted Template (pNC64A)
        - 8.9 µL sterile water

9/5/19

- Marti Gendel
    - PCR-75
        - 12.5µL Q5 Master Mix
        - 1.3uL VR
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 57C
    - PCR-76
        - 12.5µL Q5 Master Mix
        - 1.3uL Alk-10
        - 1.3uL Alk-Flag1
        - 1uL 28J P-Alk-PS
        - 8.9µL sterile water
        - Annealing temperature: 63C

- Checked Gibson-34 plate: looks good with colonies, select 14 for colony pcr
- Picked 14 colonies from Gib-3 4Plate
  - Picked and dissolved each colony into 20 µL of water
  - Heated at 80°C for 10 minutes
  - To each tube
    - 3uL of dissolved colony
    - 15.4 µL water
    - 5 µL One Taq Buffer 5x
    - .5 µL dNTP's
    - .5 µL VF2
    - .5 µL VR
    - 0.1 µL Taq Polymerase
  - Colony PCR 2;30 extention time at 57 ™
- Ran PCR 64, 75,76 on gel → good band for 64
- Grab DNA protocol with Hanuel for 18B template
  - Transfer 1.5 ml of fresh, dense liquid culture of the cell into a microcentrifuge tube. Pellet cells by centrifugation at 20,000 × g for 5 minutes.
  - Add 200 µl of lysis buffer 0.2 M Lithium acetate
  - Freeze the cells either using liquid nitrogen or freezer. Boil at 95˚C for 2 min. Repeat; vortex 30 seconds.
  - Add 200 µl of 1:1 mixture of phenol and chloroform chloroform; vortex 2 minutes.
  - Centrifuge 5 minutes, room temperature, 20,000 × g.
  - Transfer the upper aqueous phase to a microcentrifuge tube containing 400 µl ice-cold 100% ethanol. Mix by inversion or gentle vortexing.
  - Incubate at room temperature, 5 minutes. Alternatively, precipitate at -20°C to increase yield.
  - Centrifuge 5 minutes, room temperature, 20,000 × g. Remove supernatant.
  - Wash the pellet with 0.5 ml 100% ethanol, spin down as described in step 8 above. Remove supernatant.
  - Air-dry the pellets at room temperature or for 5 minutes.
  - Resuspend in 25–50 µl water
- Set up gel to run colony PCR (.96 agarose)
- Finished transformation of PAlk-PS (28J) into s7002
  - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
  - Concentrated the culture to 100 µL by removing some supernatant
  - Resuspended by flicking the microfuge tube
  - Plated 100 µL of PAlk-PS (28J) onto A++CAM plate
  - Sealed plates with tape and stored in photosynthetic incubator
- Finished transformation of NC64A into s7002

        - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
        - Concentrated the culture to 100 µL by removing some supernatant
        - Resuspended by flicking the microfuge tube
        - Plated 100 µL of NC64A onto A++CAM plate
        - Sealed plates with tape and stored in photosynthetic incubator
    -
- Jacob Wolf
    - Gel Purified PCR-64 for MG
        - Faint Band was cut, yield of 16.1 ng/uL and poor absorbances
            - Sample was discarded.
- Nathan Sattah
    - Western Blot for Gib-15 (Attempt 4)
        - Sample Prep
            - Add 1 mL of culture to microfuge tube and centrifuge at 13,300 rpm for 3 minutes
            - Carefully discard supernatant
            - Repeat into same microfuge tube 4 more times (like Miniprep)
            - Resuspend in 50 µL cold Lysis Buffer
            - Boil the sample for 10 min at 95°C on heat block and freeze at -20°C for 10 min
            - Repeat 3 times
            - Store sample in -20°C
            - Also sample prepped negative control
        - Bradford Assay (BioRad protocol, 1 mL format)
            - Remove 1X Dye Reagent from 4°C storage and let it reach ambient temperature, invert tube before use
            - Prepare five 20 µL dilutions of BSA (Promega 10 mg/mL stock) in cuvettes
                - 0.1 mg/mL: 0.2 µL BSA + 19.8 µL water
                - 0.3 mg/mL: 0.6 µL BSA + 19.4 µL water
                - 0.5 mg/mL: 1 µL BSA + 19 µL water
                - 0.8 mg/mL: 1.6 µL BSA + 18.4 µL water
                - 1 mg/mL: 2 µL BSA + 18 µL water
                - Blank: 20 µL water
                - Sample: 20 µL sample
            - Add 1 mL of 1X Dye Reagent to each cuvette
            - Incubate at room temperature for 5 minutes (no longer than 1 hour)
            - Set spectrophotometer to 595 nm
            - Blank then measure absorbances
                - 0.1: 0.076
                - 0.3: 0.213
                - 0.5: 0.446

        - 0.8: 0.726
        - 1: 0.884
        - Sample: 0.977
        - Control: 1.9 (only had 500 µL of Bradford reagent remaining so this may not be accurate)
    - Plot 595 nm values on y-axis with concentration in µg/mL on x-axis
    - Determine sample concentration using equation of standard curve
        - This gives us µg, so divide by 20 µL to get µg/µL
        - Sample: 56.02 µg/µL
        - Control: 107.3 µg/µL
- Gel Electrophoresis
    - Add 4X Loading Buffer to samples
        - ~30 µg
            - Control: 0.3 µL control + 2 µL loading buffer + 5.7 µL water
            - Sample: 0.5 µL sample + 2 µL loading buffer + 5.5 µL water
        - ~45 µg
            - Control: 0.42 µL control + 2 µL loading buffer + 5.58 µL water
            - Sample: 0.8 µL sample + 2 µL loading buffer + 5.2 µL water
        - ~60 µg
            - Control: 0.55 µL control + 2 µL loading buffer + 5.45 µL water
            - Sample: 1 µL sample + 2 µL loading buffer + 5 µL water
    - Boil samples at 95°C for 7 minutes
    - Obtain bag containing gel from cold room, and open the bag
    - Carefully remove the green comb and tape
    - Clamp gel into place
    - If running one gel, clamp Buffer Dam into the opposite side
    - Fill apparatus with 1X Running Buffer to bottom fill line and fill the space in between the gel and the dam until wells are covered
    - Load 5 µL of protein ladder
    - Load controls and samples
    - Loading order (left to right): ladder, ~30 µg (control then sample), ladder, ~45 µg (control then sample), ladder, ~60 µg (control them sample, ladder
    - Close lid (ensure that electrodes match and that lid is fully closed)
    - Run at 150V for 50 minutes
- Transfer
    - Remove a drawer from transfer apparatus and open the lid

- Open package containing transfer sandwich
- Place bottom pad onto the bottom of the drawer
- Use roller to smooth out air bubbles
- Use green tool to open the gel casing
- Use a razor blade to nick top right corner of the gel to keep orientation
- Handle gel carefully so that the gel does not break
- Place gel on top of bottom pad
- Use roller to smooth out air bubbles
- Place top pad over gel
- Use roller to smooth out air bubbles
- Select “Mini” running protocol

■ Blocking
- Make TBST + 5% milk solution
  - Take 20 mL of TBST and add to it 1 g of powdered milk
- Use tweezers to remove membrane from sandwich and place in box
- Use a razor blade to nick top right corner of the membrane to keep orientation
- Fill box with 20 mL of TBST + 5% milk solution
- Incubate on a shaker set to 4 and at room temperature for 1 hour

■ Primary Antibody
- Dilute Primary Antibody
  - 6 µL Primary Antibody + 3 mL TBST + 0.15 g powdered milk
  - Make in conical tube and store in cold room
- Pour out Blocking Buffer from box
- Add 20 mL TBST into the box and place back on shaker for about 5 minutes
- Remove TBST from bag and repeat wash once more
- Add all of the Primary Antibody solution to the box
- Place on shaker in cold room overnight

9/6/19

- Jacob Wolf
  - PCR-2 (2 tubes)
    - Recipe
      - 17.8 uL dH2O
      - 25 uL Q5 2X MM
      - 2.6 uL Alk 3b
      - 2.6 uL Alk 4
      - 2.0 uL p-LUX @10 ng/uL
    - Anneal Temp 67.5C
    - EXT time 5:05

        - Gel: Both reactions yielded very bright bands near expected band size.
    - Gel purified PCR-64 for MG
        - Faint bands, smeared
        - Result: 25.5 ng/uL, A260: 0.5, 260/230: 0.3
        - Capped and stored in -20C.
    - Gel purified 2 lanes of PCR-2
        - Result:
            - Tube A: 91.6 ng/uL and good graph
            - Tube B:140.7 ng/uL and good graph
        - Capped and stored on blue rack in -20C

9/9/19

- Marti Gendel
    - PCR18B
        - 12.5µL Q5 Master Mix
        - 1.3uL of parB-F primer
        - 1.3uL of parB-R v2 primer
        - 1.08uL DNA template
        - 8.3uL water
        - ™ 62: Touchdown PCR
    - Redid PCC18B x4
        - 250ng
            - 12.5µL Q5 Master Mix
            - 1.3uL of parB-F primer
            - 1.3uL of parB-R v2 primer
            - 1.08uL DNA template
            - 8.3uL water
            - ™ 63
        - 100ng
            - 12.5µL Q5 Master Mix
            - 1.3uL of parB-F primer
            - 1.3uL of parB-R v2 primer
            - .44uL DNA template
            - 8.3uL water
            - ™ 63
        - 50ng
            - 12.5µL Q5 Master Mix
            - 1.3uL of parB-F primer
            - 1.3uL of parB-R v2 primer
            - .22uL DNA template
            - 8.3uL water
            - ™ 63
        - 10ng

                - 12.5µL Q5 Master Mix
                - 1.3uL of parB-F primer
                - 1.3uL of parB-R v2 primer
                - .04uL DNA template
                - 8.3uL water
                - ™ 63
        - Ligation-2
            - 1uL T4 ligase buffer
            - 1.8uL RD12 (Promoter BBa_J61100)
                - RD-12: 27.7 ng/µL
            - 2.55uL RD7 (gfp BBa_I746916)
            - 1 uL T4 ligase
            - 13.65uL dH20

9/10/19

- Marti Gendel
    - Transformation of Ligation-2
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of gfp promoter ligation to JM109 tube, thawed on ice for 15 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+AMP plate
        - Stored in 37°C overnight (18hrs)
    - Ran gel with varying concentrations of DNA template for PCR-18B (all results show primer dimers)
    - PCR-77
        - 12.5 µL Q5
        - 1.3 µL Cut1
        - 1.3 µL Cut3
        - 1 µL Diluted Template (pSB1A2)
        - 8.9 µL sterile water
        - Temp: 65 1:00 ext time
    - Ran PCR-77 in gel but results not great and realized the existence of PCR-65
    - Gib-30
        - 2 µL PCR-65
        - 3 µL PCR-66
        - 5 µL 2x HiFi Assembly MasterMix
    - Put Gib-30 for 20 minutes 50C
    - Transformation of Gib-30 into JM109
        - Took JM109 from -80°C and thawed for 20 minutes

        - Added 10µL of Gib-30 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+AMP plate

9/11/19

- Marti Gendel
    - Made overnights for Gib-30 and Ligation-2 (picked four colonies from each plate)
        - 3 mL LB + 2.2 µL Amp for Gib30
        - 3 mL LB+ 2.2 µL AMP for Ligation-2
    - Plated JM109 on LB+Amp plate to test effectiveness of antibiotic on plate
    - Set up Gib-35
        - Fasyn7 3.9uL
        - PCR-46 1.1uL
        - 5uL 5uL HiFI MasterMix
        - Put in 50C for 20 minutes
    - Transformation of Gib-35
        - Took JM109 from -80°C and thawed for 20 minutes
        - Added 10µL of Gib-35 to JM109 tube, thawed on ice for 10 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 2 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto LB+CAM plate

9/12/19

- Marti Gendel
    - Made new LB+AMP plates
    - Made Lysis buffer for E.coli in preparation for GCMS analysis
        - 602uL Tris HCl
        - .2922 grams NaCl
        - .015 grams EDTA
        - 2.5mL Triton x-100
        - 47.5mL dH_20
    - Made 6 overnights 2mL Lb + 2.2uL CAM for Gib-35
    - Replated 100uL Gib-30 and L2 on new LB+ Amp plate
    - Made Sample Overnight at stored in Mets incubator overnight to practice GCMS prep
    - Prepared PCR-78
        - 1.0uL NC64A template
        - 1.3uL VR
        - 1.3 uL VF2

        - 12.5uL Q5
        - 8.9uL dH2O

9/13/19

- Marti Gendel
    - Miniprepped Gib-34-4, Gib-30 A,B,C, Gib-35 A-F
    - Submitted for sequencing 3uL of sample 15uL primer
        - Gib-30 A-C
        - Gib-34-4
        - Gib-35 A,B,C, E,F
        - Gib-30 and 34 primers: VR, VF2, S-Alk-f10, S-alk-R11
        - Gib-35 primers: VR and VF2
    - Made Lysozyme solutions 10mg/mL in Tris HCl → 4.6mg in 460uL
    - Practiced GCMS prep
        - Centrifuge 1-2 ml samples of the overnight culture.
        - Resuspend the pellets in 350 µl of STET buffer (10 mM Tris-HCl, pH 8.0, with 0.1 M NaCl, 1 mM EDTA, and 5% [w/v] TRITON® X-100).
        - Add 25 µl of a freshly prepared lysozyme solution (500,000 units/ml in 10 mM Tris-HCl, pH 8.0).5. Mix by vortexing for 3 seconds.
        - Incubate the lysis mixture for 30 minutes at 37 ˚C
        - Centrifuge the lysis mixture at 14,000 x g.
        - Remove the pellet (cell debris) from the tube using a sterile toothpick
    - Run PCR78 in gel

9/15/19

- Made 4 overnights Gib-30
- Made 6 overnights L2
- Make 3 overnights P-Alk-PS (Mets incubator light overnight for 37C)
- Made 1 overnight Negative Control

9/15/19

- Took out overnights
- Made Lysozyme solutions 10mg/mL in Tris HCl
- GCMS prep on P-alk-Ps and negative control
    - Centrifuge 1-2 ml samples of the overnight culture.
    - Resuspend the pellets in 350 µl of STET buffer (10 mM Tris-HCl, pH 8.0, with 0.1 M NaCl, 1 mM EDTA, and 5% [w/v] TRITON® X-100).
    - Add 25 µl of a freshly prepared lysozyme solution (500,000 units/ml in 10 mM Tris-HCl, pH 8.0).5. Mix by vortexing for 3 seconds.
    - Incubate the lysis mixture for 30 minutes at 37 ˚C
    - Centrifuge the lysis mixture at 14,000 x g.
    - Remove the pellet (cell debris) from the tube using a sterile toothpick
    - Added 20uL of supernatant in 200uL of Hexane
- Performed GCMS analysis → results bad because P-alk-ps is wrong sample
- Mini prep 4 L2 samples

- Make Stock Solution from Ladder
    - Store 500uL in a vial
    - Place 500uL in stock solution container with 4.5mL of Hexane -
- Make overnight of NC64A-fixed 2mL LB+ 2.2uL Cam
- Make overnight of JM109 2mL LB+ 2.2uL Cam
- Place NC64A fixed and Negative control overnight in Mets incubator 37 with light overnight

9/17/19

- Marti Gendel
    - Took out overnights of negative control and NC64A- Fixed
    - Made Lysozyme solutions 10mg/mL in Tris HCl
    - GCMS prep on Nc64A Fixed and negative control
        - Centrifuge 1-2 ml samples of the overnight culture.
        - Resuspend the pellets in 350 µl of STET buffer (10 mM Tris-HCl, pH 8.0, with 0.1 M NaCl, 1 mM EDTA, and 5% [w/v] TRITON® X-100).
        - Add 25 µl of a freshly prepared lysozyme solution (500,000 units/ml in 10 mM Tris-HCl, pH 8.0).5. Mix by vortexing for 3 seconds.
        - Incubate the lysis mixture for 30 minutes at 37 ˚C
        - Centrifuge the lysis mixture at 14,000 x g.
        - Remove the pellet (cell debris) from the tube using a sterile toothpick
        - Added 20uL of supernatant in 200uL of Hexane
    - Took 4 Miniprepped samples of L2 and ran diagnostic digests
        - Single diagnostic digest
            - 1uL CutSmart
            - 1uL plasmid
            - 0.1uL EcoRI-HF
            - 7.9uL dH20
            - 1 hr digest at 37C incubator
            - Gel result:
        - Double diagnostic digest
            - 1uL CutSmart
            - 1uL plasmid
            - 0.1uL EcoRV-HF
            - 0.1uL KpnI-HF
            - 7.8uL dH20
            - 1 hr digest at 37C incubator
    - s7002 Transformations of P-Alk-PS (28J)
        - Digested P-Alk-PS 1 hour
            - 0.299 µL P-Alk-PS (0.1 µg)
            - 1 µL CutSmart
            - 0.2 µL EcoRI-HF
            - 8.26 µL sterile water
        - Boiled digested P-Alk-PS (28J) at 80C for 10 minutes

        - Took OD730 of 8/16/19 culture: 1.1
        - Added 1 mL of culture to a flask + 10 µL P-Alk-PS (28J)
        - Put in photosynthetic incubator for 24 hours (3:30)
    - Transferred synechococcus stocks to Mets backup incubator which was programmed to a 12 hours on/12 hours off light cycle at 34C
    - PCR-79
        - 1.0uL MetE template
        - 1.3uL MetE2
        - 1.3 uL VF2
        - 12.5uL Q5
        - 8.9uL dH2O
    - PCR-80
        - 1.0uL MetE template
        - 1.3uL MetE1
        - 1.3 uL VR
        - 12.5uL Q5
        - 8.9uL dH2O

9/18/19

- Marti Gendel
    - PCR-QC-81
        - Tube A (55°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL SigA1
            - 1uL P-AlkL
            - 10.75 µL Sterile Water
        - Tube B (57°C)
            - 12.5 µL Q5 MasterMix
            - 1.25 µL SigA2
            - 0.5 µL Gib-15
            - 10.75 µL Sterile Water
    - Mixed 2 tubes and added 1 µL DpnI
    - Ran pcr-79 and pcr-80 in a gel
        - Good nano-drop results from PCR-79
    - Transform PCR-QC-81 into JM109
        - Transformation of PCR-QC-81
            - Took JM109 from -80°C and thawed for 15 minutes
            - Added 10 µL of PCR-QC-29 to JM109 tube, waited for 30 minutes
            - Heat shocked in 42°C bath for 45 seconds
            - Put on ice for 2 minutes
            - Added 300 µL of SOC media
            - Put into 37°C shaker for an hour
            - Plated 100 µL onto LB+CAM plate
            - Stored in 37°C overnight

- Make new stock of Nathan's P-Alk-L liquid culture
  - Waffle Flask: 1mL of stock + 9mL of A+ media + 9uL B12
- Make DMSO stock of P-alk-L liquid culture
  - 1 tubes prepared at 1.8 mL
  - Recipe
    - 90 uL DMSO 100%
    - 1.7 mL Synechococcus NS P-AlkL culture
  - Flash-freezed in liquid nitrogen and stored in -80C
- Finished transformation of PAlk-PS (28J) into s7002
  - Transferred the 1 mL cultures into microfuge tubes and centrifuged at 5000 x g for 5 min
  - Concentrated the culture to 100 µL by removing some supernatant
  - Resuspended by flicking the microfuge tube
  - Plated 100 µL of PAlk-PS (28J) onto A++CAM plate
  - Sealed plates with tape and stored in photosynthetic incubator
- Made 5mL overnight of NC64A fixed and negative control
- Made 2mL overnight re-picked colony on Gib-34-4 plate and Gib-35

- Marti Gendel
  - Make DMSO stock of P-alk-L liquid culture
    - 2 tubes prepared at 1.8 mL
    - Recipe
      - 90 uL DMSO 100%
      - 1.7 mL Synechococcus NS P-AlkL culture
    - Flash-freezed in liquid nitrogen and stored in -80C
  - Took out overnights of negative control and NC64A- Fixed
  - Made Lysozyme solutions 10mg/mL in Tris HCl
  - GCMS prep on Nc64A Fixed and negative control
    - Centrifuge 1-2 ml samples of the overnight culture.
    - Resuspend the pellets in 350 µl of STET buffer (10 mM Tris-HCl, pH 8.0, with 0.1 M NaCl, 1 mM EDTA, and 5% [w/v] TRITON® X-100).
    - Add 25 µl of a freshly prepared lysozyme solution (500,000 units/ml in 10 mM Tris-HCl, pH 8.0).5. Mix by vortexing for 3 seconds.
    - Incubate the lysis mixture for 30 minutes at 37 ˚C
    - Centrifuge the lysis mixture at 14,000 x g.
    - Remove the pellet (cell debris) from the tube using a sterile toothpick
    - Added 40uL of supernatant in 400uL of Hexane
  - Analyzed standard (80uL in 400uL Hexane), negative control, and positive control on GCMS
  - Glycerol stock of Gib-34-4, Gib-30A, Gib-35A
  - Prepared two 20mL overnight cultures with JM109 as negative control and NC64A as sample
  - PCR-80
    - 1.0uL MetE template

        - 1.3uL MetE1
        - 1.3 uL VR
        - 12.5uL Q5
        - 8.9uL dH2O
    - Ran PCr-80 in gel

10/6/19

- Apple Lee
    - Made 500mL liquid LB
    - Made overnight cultures for L2 E and L2 F (gfp promoter seed cultures) and negative control (JM109)
        - 5mL LB
        - 5uL CAM

10/7/19

- Jacob Wolf
    - Made Stack of LB+CAM+AMP Agar plates
        - Followed Recipe for 500 mL LB+AGAR and added 500 uL AMP stock and 250 uL CAM stock
- Nathan Sattah
    - Double Transformation of Gib-30 and P-AlkL-PS into JM109
        - Took JM109 from -80°C and thawed for 15 minutes
        - Added 1 µL of Gib-30 and 1 µL of P-AlkL-PS to first JM109 tube, added 2 µL Gib-30 and 2 µL P-AlkL-PS to second tube, waited for 30 minutes
        - Heat shocked in 42°C bath for 45 seconds
        - Put on ice for 4 minutes
        - Added 300 µL of SOC media
        - Put into 37°C shaker for an hour
        - Plated 100 µL onto one LB+CAM+AMP plate, plated remaining liquid onto another plate
        - Stored in 37°C overnight
- Apple Lee
    - Gfp promoter fluorescence testing with SImone
        - Negative control (JM109) and gfp promoter sample prepared through series of serial dilutions
            - 100uL seed solution + 900uL LB+CAM
            - 200uL total final volume in each well
        - 2-fold dilutions made for seed solution and negative control OD to reach 0.1 initially
        - Optical densities of each well measured using plate reader
    - Plated 100uL of dilution 3, 4, and 5 for each sample (control, L2 E, and L2 F)
        - 37C incubator for 18 hours

10/7/19

- Nathan Sattah

- Made overnight cultures for Failed Gib-9, P-AlkL-PS, NC64A Fixed, and Gib-30 + P-AlkL-PS
    - 2 mL cultures with 2.2 µL CAM for Control, P-AlkL-PS, and NC64A Fixed
    - 2.2 µL AMP for Gib-30 + P-AlkL-PS
    - Made 4 replicates of each kind and stored in Mets lab incubator