Journal - Chlorophyll Extraction: Protein Production

Project: Journals

Authors: Nimaya De Silva

MONDAY, 5/20/2019

- Victoria Day (No iGEM)

TUESDAY, 5/21/2019

- Have decided to use *B. subtillis* as a chassis, the specific strain is WG800 (was also used by the 2016 Calgary iGEM Team) becease it is missing a number of proteases
- Promoter: is a Xylose inducible promoter because it is less expensive than IPTG, so better candidate for industrial purposes (this is in iGEM registry)
- Checked with the modelling group to maintain communication with them about what is happening with our subgroup vs. what the modelling group has accomplished for us
    - The modelling group will add 6HIS tags to the protein to the N-terminal and test in an emulsion scenario
- Still need to decide on a vector to use that is compatible in both *E. coli* and *B. subtilis*
- Still need to figure out ~3 different secretion tags that will be the most likley candidates for our use
- Decided against using the sfGFP since only one paper has characterized the protein for secretion
- Decided to use Golden Gate, but we might still need to make parts Golden Gate compatible

WEDNESDAY, 5/22/2019

    - Decided that we will be working in *E. coli* (DH5-alpha for cloning and BL21 for expression) for ease of use
- Looked at different golden gate collections that have been developed by previous teams, but they're not in the distribution kit and the registry isn't sending out parts until July (because they are moving offices) so we are likely going to have to create all of the parts of our genetic circuit from scratch
- Investigated different vectors that we could use for domestication and assembly
    - There are multiple ways to make pSB1C3 golden gate-compatible, which may be useful (we already have experience, primers, antibiotics, etc. that allow us to easily work with this vector)
- Did a practice transformation in the lab
- Attended the summer student welcome event hosted by the Charbonneau Institute where we networked with other researchers at our university

THURSDAY, 5/23/2019

- Attended a human practices/entrepreneurship meeting in the morning
- Began to work on some of the specific sequences in prepartion for ordering
    - Decided on the T7 promoter since we will be using BL21 - IPTG for induction is feasible within our lab, and we can consider alternatives for scaling up after proof of concept
    - Generated the cDNA sequence for our chlorophyll binding protein (PDB ID: 6GIX) that is codon optimized for expression in *E. coli* K12 (high) using EMBOSS backtranseq and added a double stop codon (UAAUAA)
    - Investigated adaptation of registry parts BBa_P10500 (universal acceptor plasmid) and BBa_K1467400 ('flipper' to make pSB1C3 destination plasmid)
    - Identified a few signal peptides to use - OmpA, MalE, PhoA, TorA, PelB, and YcbK signal peptide sequence from registry
    - Decided on the bidirectional terminator BBa_B0014

FRIDAY, 5/24/2019

- Created all of our constructs in Benchling and double checked them for compatibility
    - Used the fusion sites for each of our parts as described on the iGEM registry and in the MoClo method oulined by Weber et al. (2011)
    - Ensured that the 6XHis tag and our chlorophyll binding protein (PDB ID: 6GIX) would be read in-frame upon addition of each signal peptide (TorA, OmpA, MalE, PhoA, and YcbK signal peptide from the registry)
    - Added a thrombin proteolytic cleavage site after our 6XHis tag in case complications require it to be removed
- Used Pymol to add 6XHis tag and each of the signal peptides to our chlorophyll binding protein (PDB ID: 6GIX) so that we may model any potential interferences with the protein's structure
- Created a preliminary experimental timeline for our subgroup
- Got our TA to check over our parts before ordering them early next week

Week 3 (May 20th-24th) WEEKLY SUMMARY:

This week our subgroup discussed various chassis, plasmids, parts, and signal peptide sequences to ultimately come to a finalized decision on the technical aspects of our project and construct. At the start of the week, we decided to use *Bacillus subtillis* for our chassis, but then after researching the advantages and disadvantages of this chassis compared to *E. coli*, we ultimately decided to use *E. coli* strain BL21 (DE3) for our chassis. In regards to cloning, we will utilize the Golden Gate Assembly technique to put our construct together. In all, there will be four parts (promoter + RBS; signal peptide; 6XHIS + 6GIX; double terminator) that will be assembled for our construct. Additionally, we determined a part to improve for our gold medal requirements; the Golden Gate Flipper BBa_K1467400 by changing the construct to include a constitutive promoter, a stronger RBS site, and a bidirectional terminator. This part will be used to make pSB1C3 golden gate compatible. Finally, we worked with our modelling team to create PDB files of protein models which contain the various signal peptides with the 6XHIS tag and the 6GIX protein. The files will be used by our modelling team to figure out any problems that may arise during protein folding, and the protein interactions in the emulsion.

MONDAY, 5/27/2019

- The part BBa_K1467400 was ordered from the iGEM parts registry
- Made a timeline on paper for our summer months
- Prepped for making DH5 alpha competent cells for the next day and made overnight cultures from a DH5-alpha glycerol stock made from last year
- Team meeting

TUESDAY, 5/28/2019

- Made LB broth and transformed chemically competent DH5a with the "petrobrick" part to see if the part was still viable and for new members to practice transforming
- Made list of lab items to order
- Created parody of 'Old Time Road' - iGEM Road!
- Made chemically competent DH5a cells

WEDNESDAY, 5/29/2019

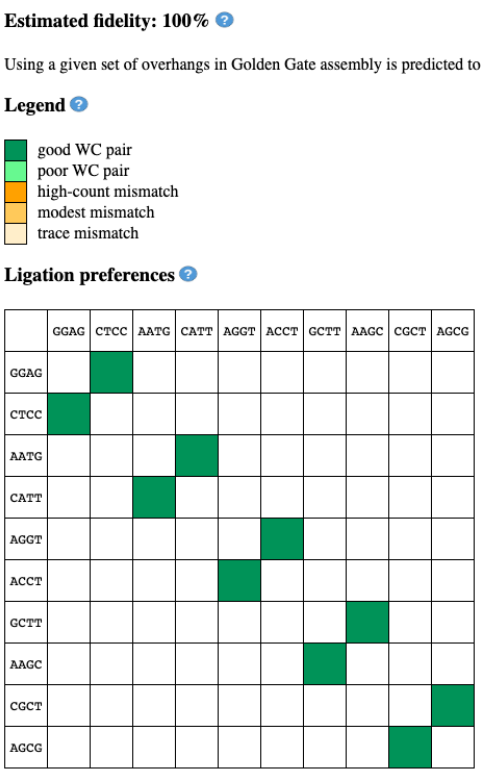
- Transformed RFP into the chemically competent DH5a cells to check for their viability
- Made a streak plate of the plate with the transformed DH5a with the petrobrick to get individual colonies
- A few of our plates in the fridge had strange growth, so we placed a few in the incubator to check if they were contaminated
- Worked on various human practices activities, including:
    - Development of our Synthetic Biology Training Package
    - Summary of the project for Innovate Calgary who is helping us investigate the IP landscape surrounding the project
    - Generation of a iGEM Calgary sponsorship package, specifically the levels of sponsorship

THURSDAY, 5/30/2019

- Ran a synthetic biology education activity at Webber Academy

- Checked on plates from the day before: red, black, and blue batches grew red colonies. Green plate had white colonies, not red, so it was put back in incubator for further growth.
- The Petrobrick streak plate looked good! We are awaiting confirmation from NDC-High River to pursue further verification
- Registered with IDT and finalized our parts for the first order!
- Used NEB's Ligase Fidelity Viewer tool to analyze the overhangs we chose to use for our parts. They showed 100% fidelity and high efficiency as demonstrated below!

Screen Shot 2019-05-30 at 11.23.31 AM.png



Estimated fidelity: 100%

Using a given set of overhangs in Golden Gate assembly is predicted to result in 100% of correctly ligated products.

Legend

good WC pair
poor WC pair
high-count mismatch
modest mismatch
trace mismatch

Ligation preferences

	GGAG	CTCC	AATG	CATT	AGGT	ACCT	GCTT	AAGC	CGCT	AGCG
GGAG		■								
CTCC	■									
AATG				■						
CATT			■							
AGGT						■				
ACCT					■					
GCTT								■		
AAGC							■			
CGCT										■
AGCG									■	

- The whole subgroup learned how to use Gravit for graphic design and we began to update some of the figures from our synthetic biology training package
- Work on the Mindfuel grant also began today

FRIDAY, 5/31/2019

- Ran a synthetic biology education activity at Webber Academy for another class
- Went through the timeline that we created and added questions that we still have
- We worked on updating some of the figures from our synthetic biology training package

Week 4 (May 27th-31st) WEEKLY SUMMARY:

This week, our subgroup finalized our parts design after receiving confirmation via protein modelling that our chosen tags shouldn't negatively impact protein structure stability. We registered with IDT and we will order our parts as gBlocks from them as soon as they contact us. In addition, we ordered the Golden Gate Flipper (BBa_K1467400) from the iGEM registry. In the lab, we prepared for cloning work by creating chemically competent *E. coli* DH5-alpha cells. Outside of the lab, members of our subgroup focused on many aspects of our human practices work including several visits to Webber Academy to give an educational talk about synthetic biology, work on our synbio education package, and entrepreneurship activities. We also pursued various funding opportunities and began to create a sponsorship package for the team.

MONDAY, 6/3/2019

- Began the long and painful process of lab cleaning, inventory, and stocking. This will continue tomorrow
- Continued updating figures in the synbio education package
- Created our subgroup summary for Innovate Calgary
- Team meeting

TUESDAY, 6/4/2019

- Found some BsaI in the enzyme box in the freezer, but we want to confirm that it works. We looked for distribution kit parts with BsaI sites, and decided to use BBa_K575008 from the 2018 kit. This part was transformed by Nimaya and Sravya
- Continued the lab cleaning, inventory, and stocking
- Sent a follow-up email to IDT to confirm our registration because we hadn't heard from them yet
- Some members of the subgroup attended a meeting with Genome Alberta

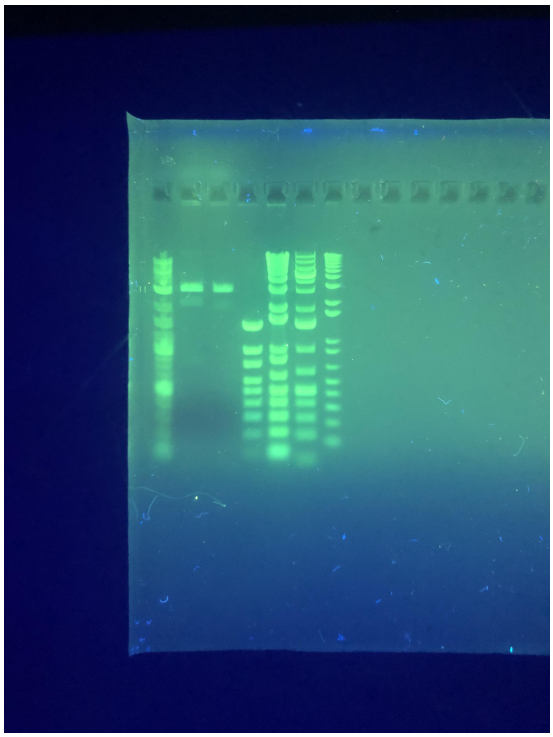
WEDNESDAY, 6/5/2019

- Day three of lab cleaning, inventory, and stocking. We can finally see the floor
- Ordered our parts from IDT!!!
- Continued updating figures in the synbio education package

THURSDAY, 6/6/2019

- Did restriction digest with BsaI/XbaI and PstI/XbaI
  - Success! BsaI works (as seen in gel lanes 2 and 3) - But we left the enzyme out overnight so RIP
  - We tested a bunch of other ladders that we found in the freezer and they work too!

IMG_1293.jpg



- Made more figures for education package

FRIDAY, 6/7/2019

- Met with farmer Craig via skype for HP
- Made more figures for education package

- Wet lab internal weekly meeting
- 7/9 of our parts arrived from IDT!

Week 5 (June 3rd-7th) WEEKLY SUMMARY:

We spent the first half of this week conducting a very thorough lab clean-up and taking inventory of our supplies. In the middle of the week, the wet lab ordered all of our parts from IDT. To our surprise, half of them arrived within the week! We will begin work to clone them into our donor plasmid (pSB1C3) next week. Outside of the lab, we continued various human practices activities, including meeting with canola farmers and working on our synthetic biology training package.

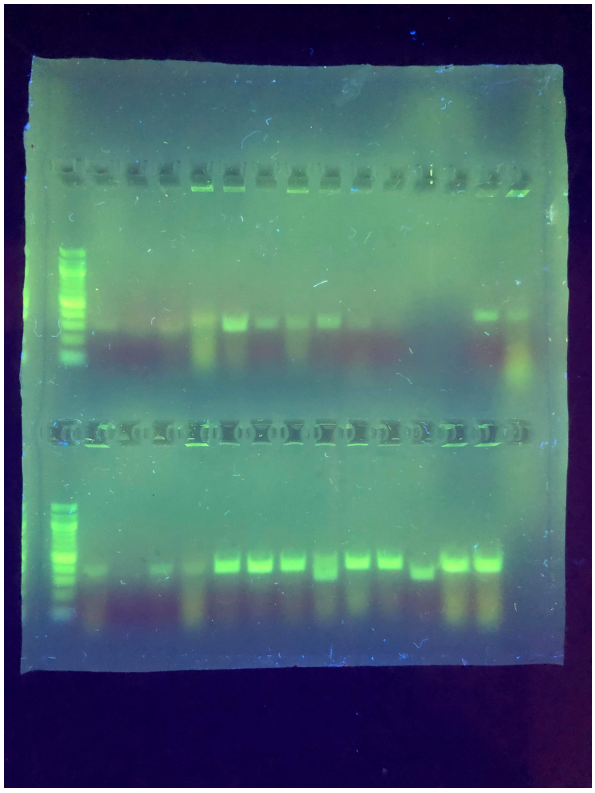
MONDAY, 6/10/2019

- Made linearized pSB1C3 mastermix and used it to digest the backbone
- Set up EcoRI/PstI digestions and subsequent ligations of the following IDT G-Blocks into pSB1C3:
    - T7-RBS
    - YcbK-SP
    - TorA-SP
    - MalE-SP
    - Terminator
    - OmpA-SP
    - PhoA-SP
- Transformed the ligations in *E. coli* DH5a
- THe 6xHis-6GIX part came in the mail in the afternoon, so we set up a digestion for that as well

TUESDAY, 6/11/2019

- Plates from yesterday's pSB1C3-PhoA and pSB1C3-T7-RBS transformations had colonies, so we did cPCR for 10 colonies of pSB1C3-T7-RBS and 15 colonies of pSB1C3-PhoA. After running on a 2% agarose gel, bands of the correct size were visible for some of the samples

June11cPCR.png



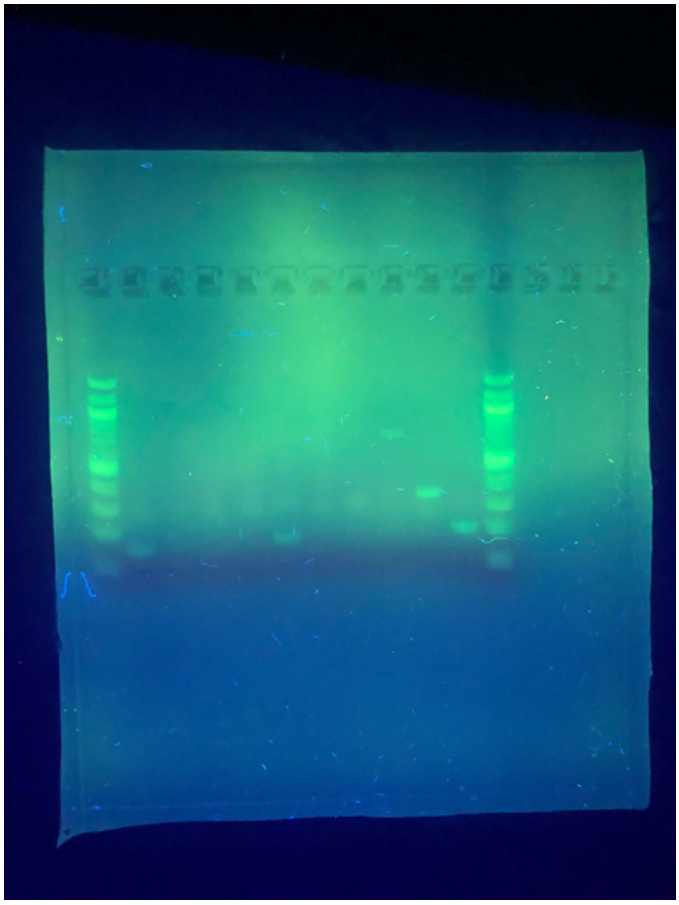
2% agarose gel, NEB 100bp ladder.
Top row lanes 2-11: pSB1C3-T7-RBS, lanes 12-13: empty, lanes 14-15: pSB1C3-PhoA. Bottom row lanes 2-14: pSB1C3-PhoA, lane 15: empty.

- Made overnights for pSB1C3-T7-RBS colonies #5-8 and pSB1C3-PhoA colonies #7, 8, 11, and 14
- Re-plated the transformations performed yesterday
- Made new linearized backbone master mix, then repeated the digestions, ligations and transformations of the following parts:
    - T7-RBS
    - YcbK-SP
    - TorA-SP
    - MalE-SP
    - Terminator
    - OmpA-SP
    - PhoA-SP
- Took team pictures
- Set up a PCR for the amplification of all 10 gblocks that have arrived thus far

WEDNESDAY, 6/12/2019

- Ran 10ul of each PCR product on a 2% gel. Bands of correct size are observed for:
    - T7-RBS (lane 2)
    - PhoA (lane 6)
    - 6GIX (lane 9)
    - End sequence (lane 10) (for the other subgroup)
    - Terminator (lane 11)

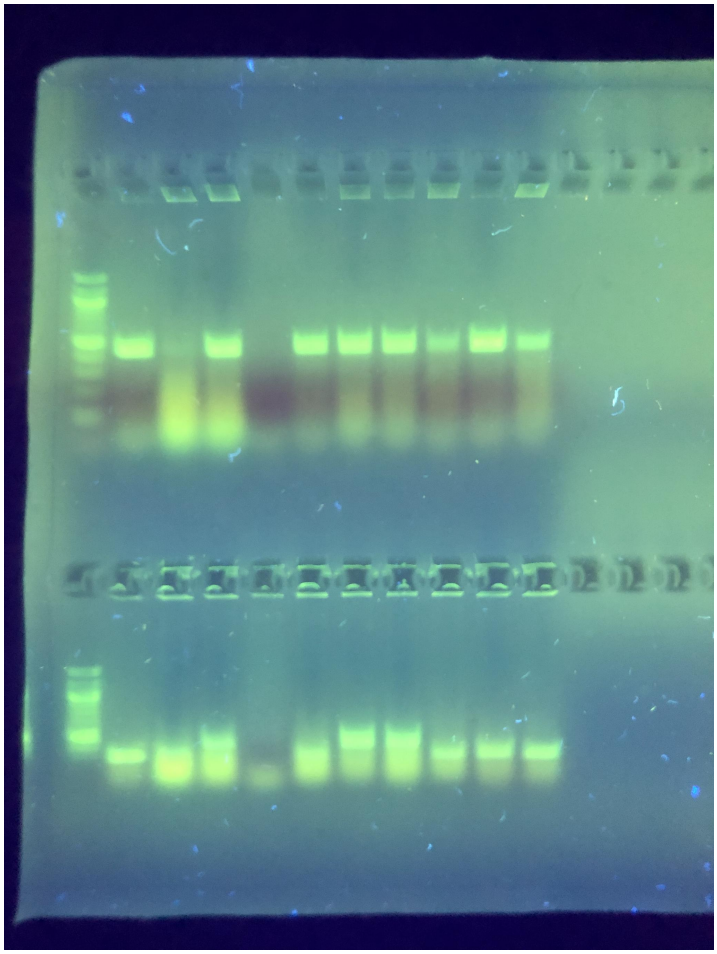
image.png



2% agarose gel, NEB 100bp ladder.

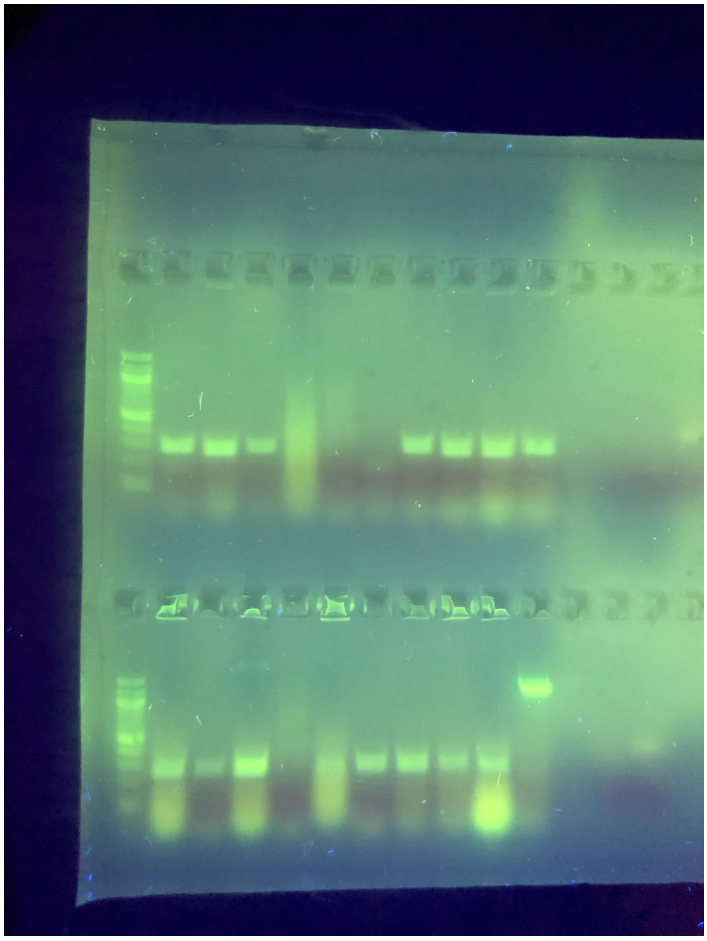
- Miniprepped the pSB1C3-T7-RBS and pSB1C3-PhoA overnights
- Some of the new ligation plates and replated transformations had growth, so we ran cPCR as follows:
    - 10 colonies of pSB1C3-6GIX ligation
    - 15 colonies of pSB1C3-OmpA
    - 16 colonies of pSB1C3-MalE
    - 10 colonies pSB1C3-YcbK
    - 10 colonies of pSB1C3-TorA
    - 10 colonies of pSB1C3-Terminator

IMG_1387.jpg



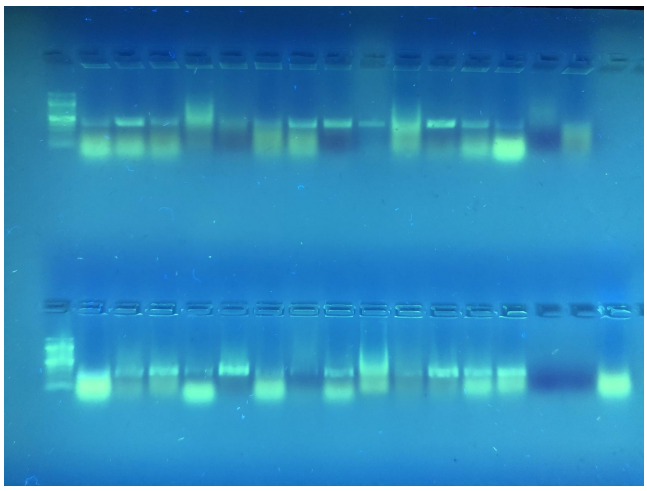
2% agarose gel, NEB 100bp ladder
Top row: pSB1C3-6xHis-6GIX colonies
Bottom row: pSB1C3-YcbK colonies

IMG_1388.jpg



2% agarose gel, NEB 100bp ladder
Top row: pSB1C3-TorA colonies
Bottom row: pSB1C3-Terminator colonies

IMG_1390.jpg



2% agarose gel, NEB 100bp ladder
Top row: pSB1C3-MalE colonies
Bottom row: pSB1C3-OmpA colonies

- The results from the cPCR gels show that a few of the colonies appear to contain the correctly ligated parts
- Our last part - the golden gate flipper - arrived today. We digested it and set up an overnight ligation into pSB1C3

THURSDAY, 6/13/2019

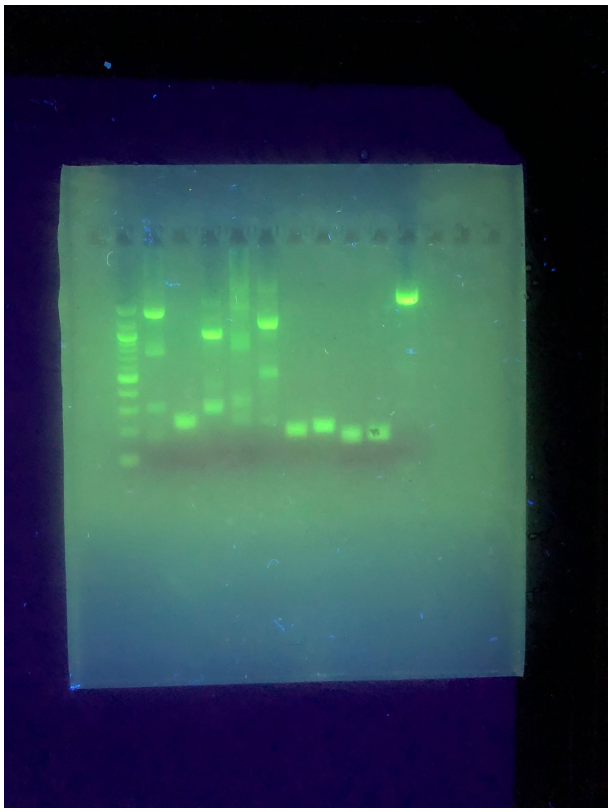
- There was some RFP contamination on the PhoA plate and YcbK masterplate (red colonies)
- Transformed pSB1C3-Flipper ligation into DH5a

- Made overnights for the colonies that looked promising from yesterday's cPCR
- After looking at our parts on Benchling, we realized that there are no internal restriction sites that we can use to digest confirm the ligations into pSB1C3. We therefore arbitrarily sent the pSB1C3-T7-RBS colony #7 and pSB1C3-PhoA colony #7 that were miniprepped yesterday for sequencing
- Worked on the faculty talk presentation
- Ran PCR for all the GBlocks using NEB taq and ran them on a 2% agarose gel as seen below:

FRIDAY, 6/14/2019

- Miniprepped pSB1C3-YcbK, pSB1C3-OmpA, and pSB1C3-MalE
    - Sent samples in for sequencing: MalE 4, YcbK 7, and OmpA 5
- Made new LB agar plates with chloramphenicol and then used nearly all of them
- Transformed new pSB1C3-TorA, pSB1C3-6GIX, pSB1C3-Flipper, and pSB1C3-Terminator ligation into DH5a. However, really old T4 DNA ligase (Invitrogen) for the ligation so we are not sure if this will work
- PCR'ed all of the GBlocks using Kapa Hi-Fi taq for 25 cycles and ran them on a 2% agarose gel as seen below:

image.png



2% agarose gel, NEB 100bp ladder

- Replated pSB1C3-6GIX and pSB1C3-RFP-flipper transformations from earlier in the week
- Worked on the faculty talk presentation
- Finished the Mindfuel Seed Fund application
- Recieved sequencing results for pSB1C3-T7-RBS colony #7 and pSB1C3-PhoA colony #7. pSB1C3-PhoA colony #7 was the correct part and the glycerol stock was retained, but pSB1C3-T7-RBS colony #7 was not correct

SATURDAY, 6/15/2019

- Our lord and saviour Saran came into the lab to take many plates out of the incubator today

SUNDAY, 6/16/2019

- Our lord and saviour Saran made 8x 2ml DH5a overnights 6x 2ml BL21 overnights, plus some other overnights for the other subgroup
- Took more plates out of the incubator

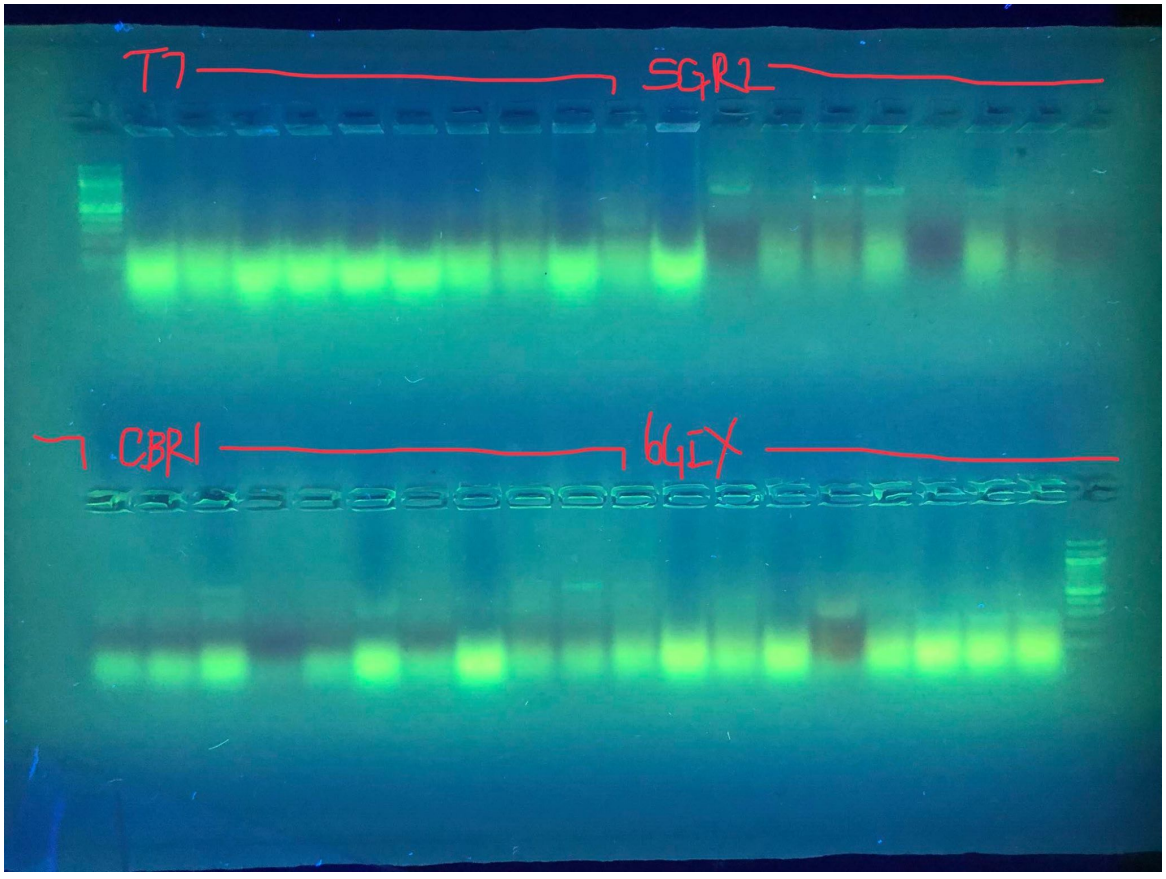
Week 6 (June 9th-14th) WEEKLY SUMMARY:

We received all of our parts from IDT throughout the course of this week, and began the process of cloning each part into our donor plasmid (pSB1C3) before use in Golden Gate reactions. Initial confirmation of cloning success was obtained via colony PCR reactions. By the end of the week, we were able to sequence confirm one of the signal peptides (PhoA signal peptide) in pSB1C3. We also PCR-amplified all of the IDT GBlocks in order to increase the amount of DNA that we have to work with. Cloning efforts will continue next week. Members of our subgroup also focused efforts on the Mindfuel Seed Fund application and preparation for our Faculty Talk presentation.

MONDAY, 6/17/2019

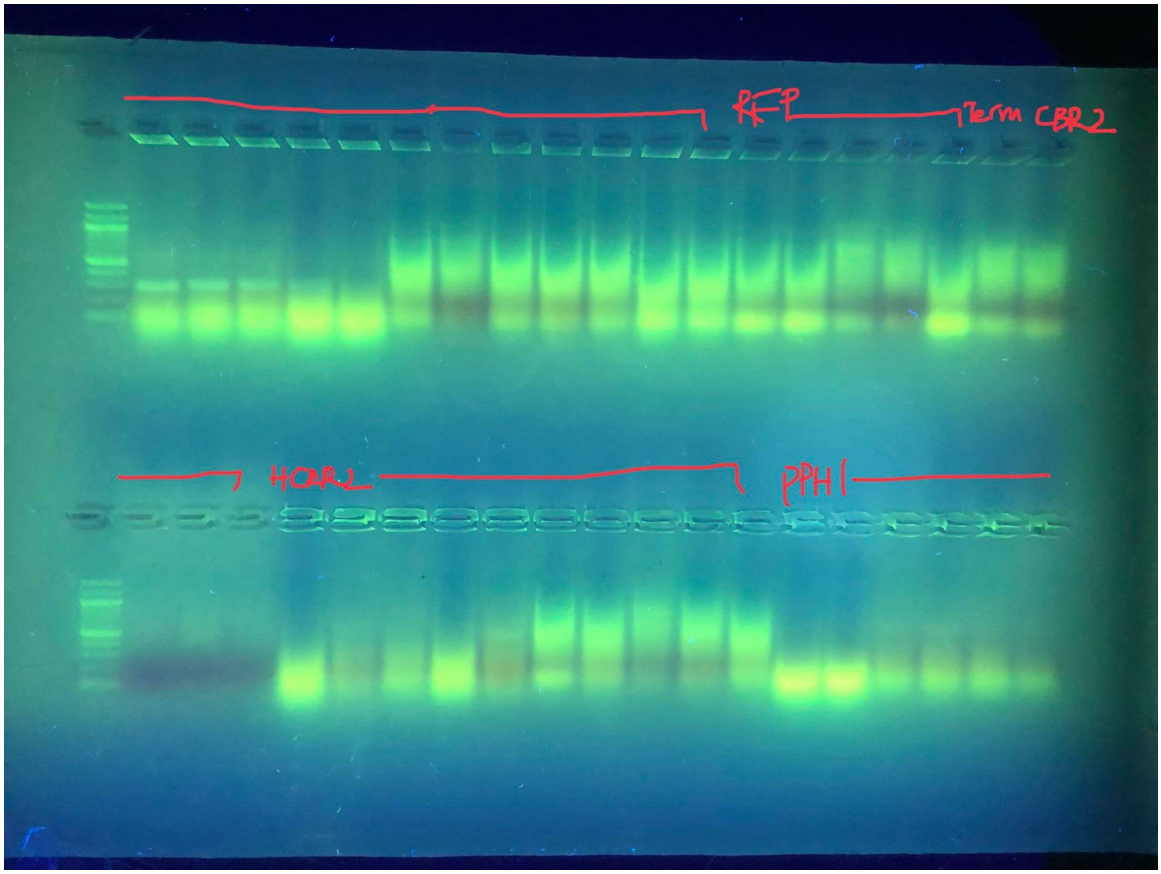
- Made tons of chemically competent DH5a and BL21 cells
- Ran cPCR for 106 colonies from plates that were incubated over the weekend. Unfortunately, too much colony was added to each well for the cPCR and when run on a 2% agarose gel, only smears of DNA were visible

image.png



2% agarose gel, NEB 100bp ladder

image.png



2% agarose gel, NEB 100bp ladder

- Sent pSB1C3-T7-RBS colony #8 for sequencing as #7 didn't work last week
- Recieved sequencing results for pSB1C3-YcbK colony #7, pSB1C3-OmpA colony #5, and pSB1C3-MalE colony #4
    - pSB1C3-MalE colony #4 was the correct part with a few reported SNPs that we believe are due to poor sequencing quality
    - pSB1C3-YcbK colony #7 and pSB1C3-OmpA colony #5 were not the correct parts
- Marija ordered our Golden Gate enzymes!
- Ran the PCR'ed GBlocks on a 2% agarose gel and excised them in preparation for use of the purification kit
- Made overnights of the sequence-confirmed pSB1C3-PhoA and pSB1C3-MalE so we can miniprep tomorrow and stock up on DNA
- Also made an overnight for pSB1C3-T7-RBS colony #10 from the new cPCR because it was the only one out of all our cPCRs that looked half resonable

TUESDAY, 6/18/2019

- Miniprepped the overnights and placed in the miniprep box for later use
- Decided that pSB1A3 should be our RFP-flipper destination vector for ease in colony selection following Golden Gate assembly
- Worked with Marija to optimize our dig-ligs and repeated them once more for the parts that have yet to be cloned in. Transformed and plated
    - pSB1A3-Flipper (1:1 ligation)
    - pSB1A3-Flipper (3:1 ligation)
    - pSB1C3-6xHis-6GIX (2:1 ligation)
    - pSB1C3-6xHis-6GIX (2:1 ligation)
    - pSB1C3-TorA (7:1 ligation)
    - pSB1C3-OmpA (7:1 ligation)

    - pSB1C3-Terminator (7:1 ligation)
    - pSB1C3-T7-RBS (7:1 ligation)
    - pSB1C3-YcbK (7:1 ligation)
- Used the Genscript gel purification kit on the gel excised PCR products from yesterday. After nanodropping, they appear to have usable concentrations

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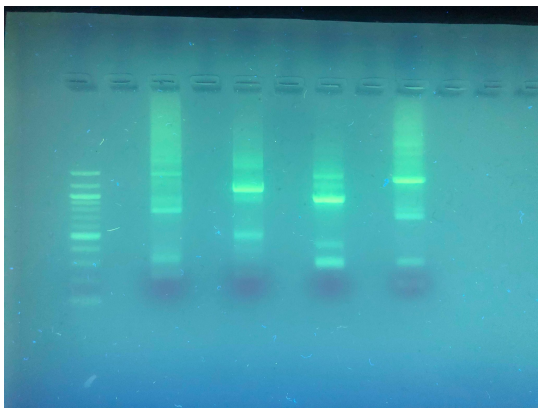
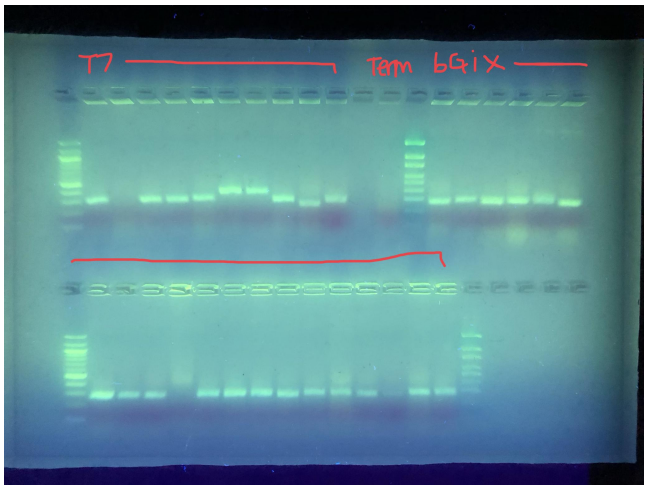


2% agarose gel, 100BP NEB Ladder

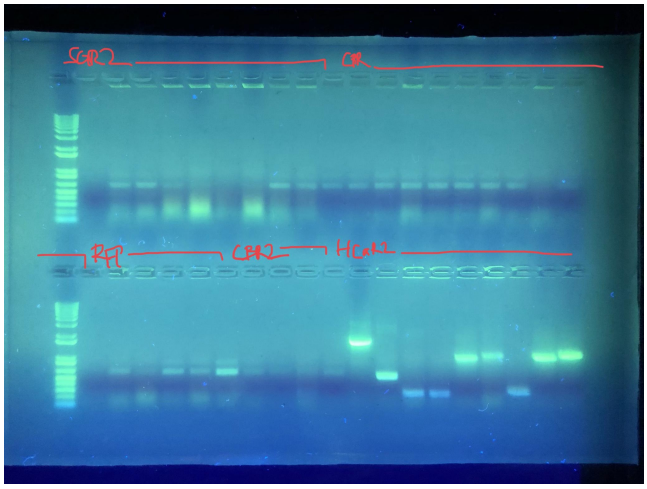
- Made ampicillin plates for using pSB1A3
- The masterplates from yesterday's cPCRs didn't grow well and a few were contaminated with some mystery GFP (yikes). Sara and Mike remade the masterplates and repeated the cPCR using a smaller amount of colony. This time we had actual bands!!! (See below)

IMG_1222.JPG



2% agarose gel, BioBasic 100bp-1500bp ladder
Top row, wells 2-11: T7-RBS
Top row, well 13: Terminator
Top row, wells 15-20: 6GIX
Bottom row, wells 2-15: 6GIX

IMG_1225.jpg



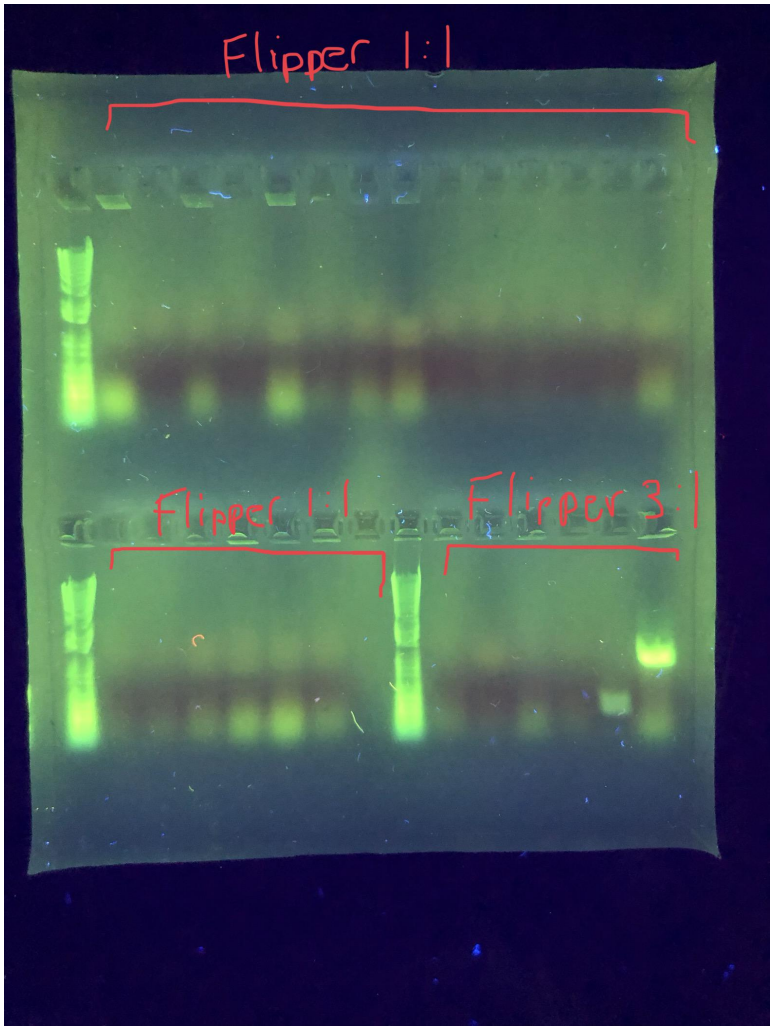
1% agarose gel, Invitrogen 1kb plus ladder
Top row: Other subgroup's parts
Bottom row, well 3-7: RFP-Flipper
Bottom row, wells 7-20: Other subgroup's parts

- Made overnights for pSB1C3-T7-RBS colonies 6 and 7 that looked okay on the cPCR gel above

WEDNESDAY, 6/19/2019

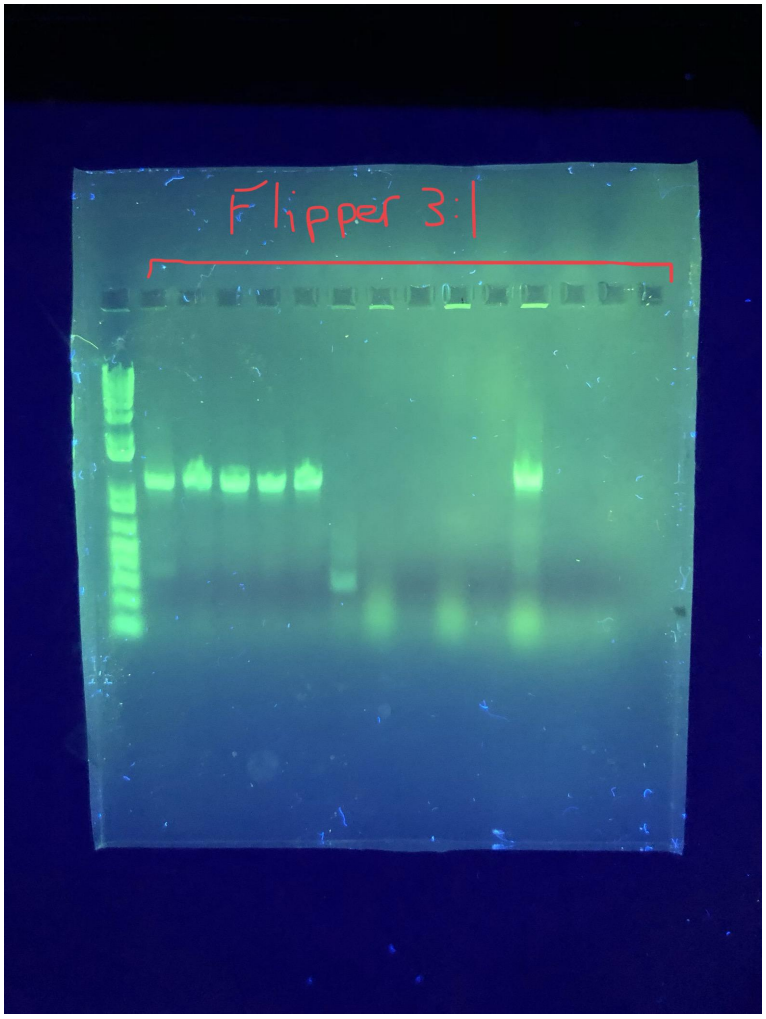
- As per Marija's instructions, we re-transformed yesterday's ligations after room temperature incubation overnight
- The plates from yesterday's dig-lig transformations all had growth. Ran 133 cPCRs total for the parts and ran on gels as follows:

IMG_1489.jpg



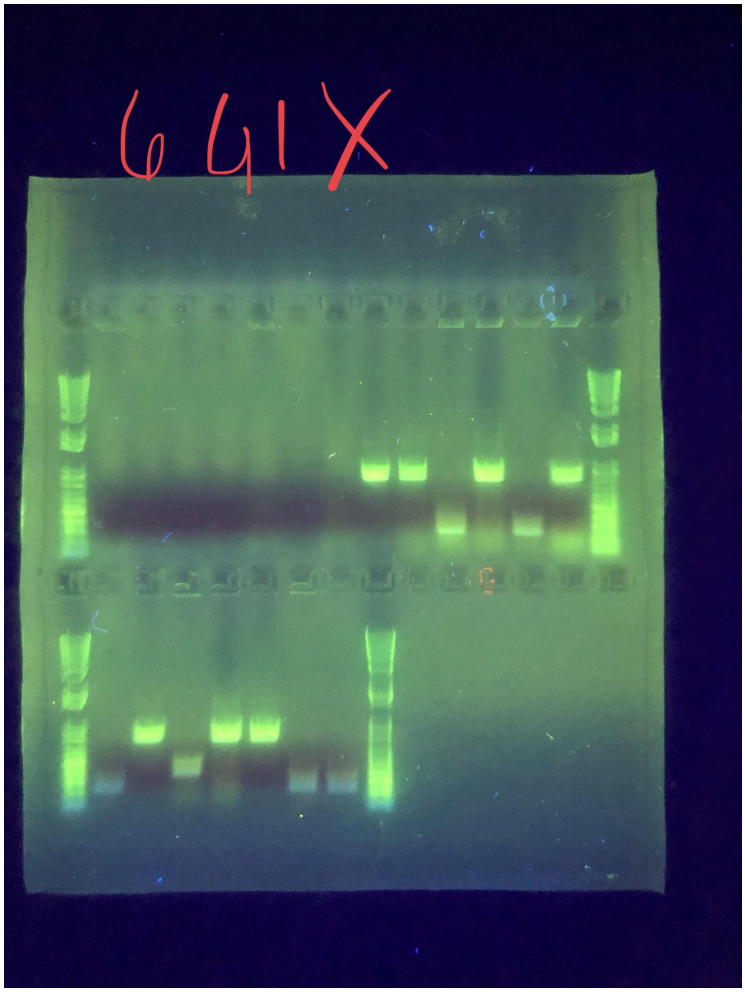
1% agarose gel, Invitrogen 1kb plus ladder

IMG_1491.jpg



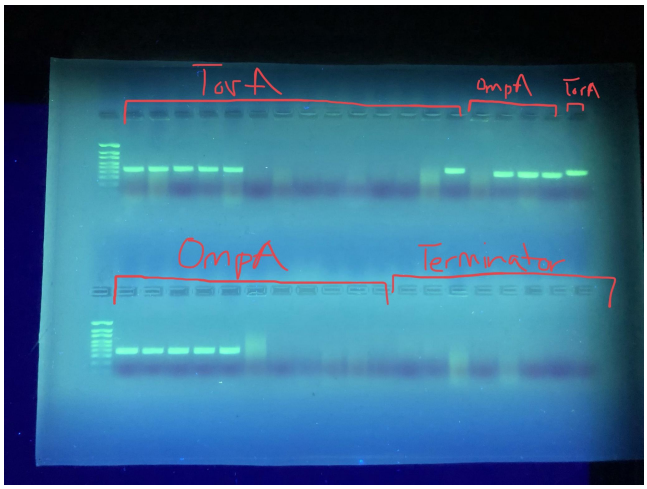
1% agarose gel, Invitrogen 1kb plus ladder

IMG_1501.jpg



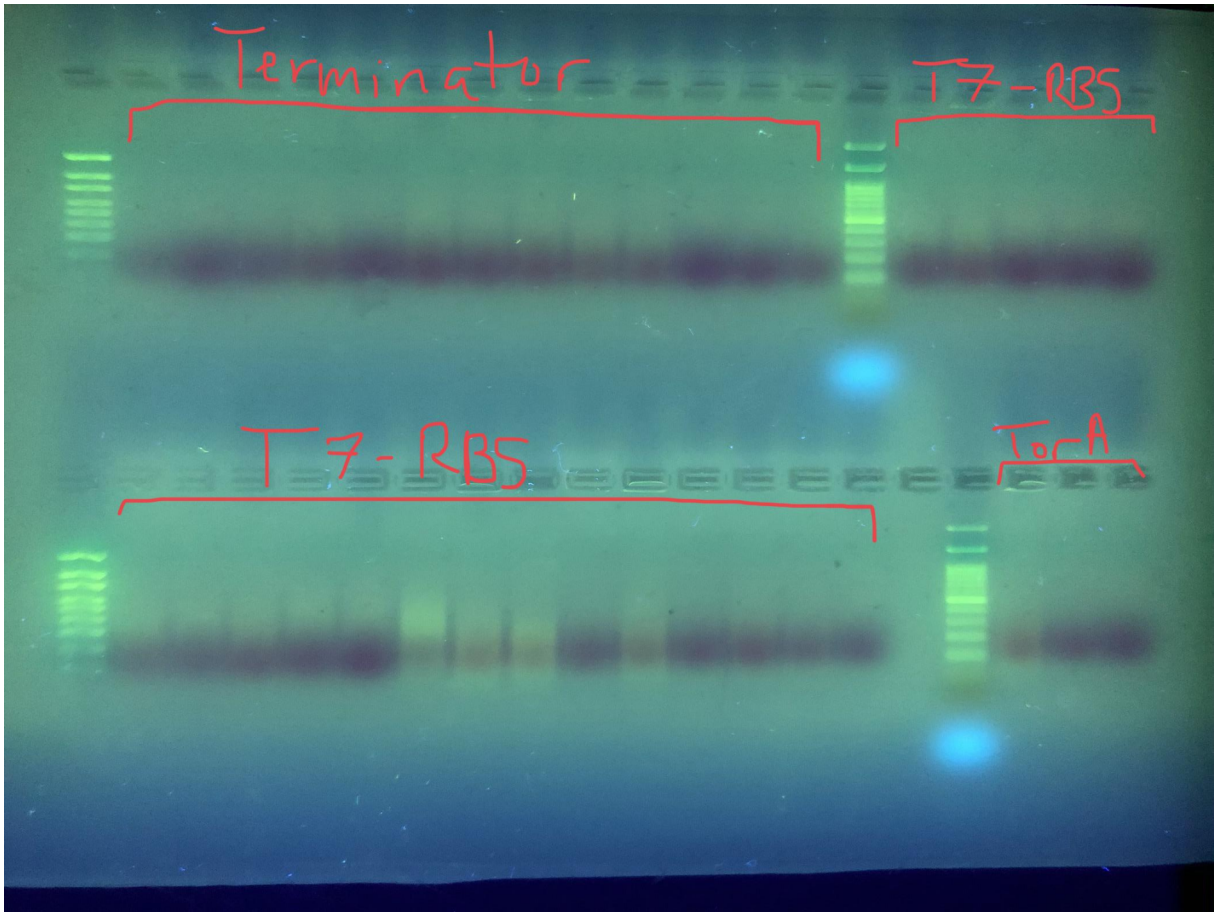
1% agarose gel, Invitrogen 1kb plus ladder

IMG_1493.jpg



2% agarose gel, BioBasic 100bp-1500bp ladder

IMG_1496.jpg



2% agarose gel, BioBasic 100bp-1500bp ladder
Top row lane 15 and bottom row lane 17 were used for testing GeneDireX 100bp DNA ladder H3 RTU that was found in the cabinet from many years ago.

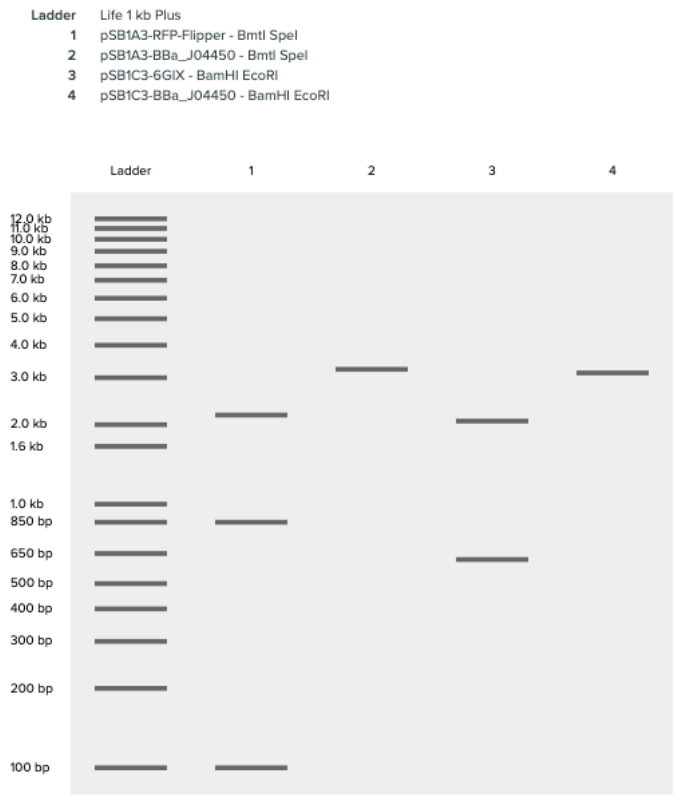
- Made 1x 4ml overnights for pSb1A3-Flipper (3:1) colonies 7, 8, 10, 11, and 17 in LB+Amp using the original colonies as the masterplate had been contaminated by condensation
- Made 1x 4ml overnights in LB+chlo for pSB1C3-6GIX colonies 9, 15, 11, and 18, pSB1C3-OmpA colonies 5-8, and pSB1C3-TorA colonies 3, 4, and 15
- Stocked up on cPCR mastermix
- Miniprepped yesterday's pSB1C3-T7-RBS colonies 6 and 7 overnights and sent #6 for sequencing
- Practiced for the faculty presentation
- Did preliminary chlorophyll extraction experiments using acetone and ethanol as solvents. 0.5g of tissue from spinach leaves was homogenized using a motar and pestle, and 10 ml of acetone or ethanol was added. The mixture was centrifuged for 15 mins at 14000 rpm, and the (very green) supernatant was transferred to a 2ml tube. This was used for multiple experiments:
    - Vacufuged samples of the chlorophyll-containing solvent and were left with chlorophyll at the bottom of the tube
    - Left the tubes open overnight to allow evaporation of the solvents, again were left with chlorophyll in the tube
    - Added to canola oil in 1:1 and 1:3 ratios. Observed the chlorophyll move into the oil phase after agitation. The solvent phase could be removed via vacufugation

THURSDAY, 6/20/2019

- The pSB1A3-flipper transformation and masterplates have red colonies, which is a really good sign!
- Made glycerol stocks and then miniprepped each of the overnights made yesterday.

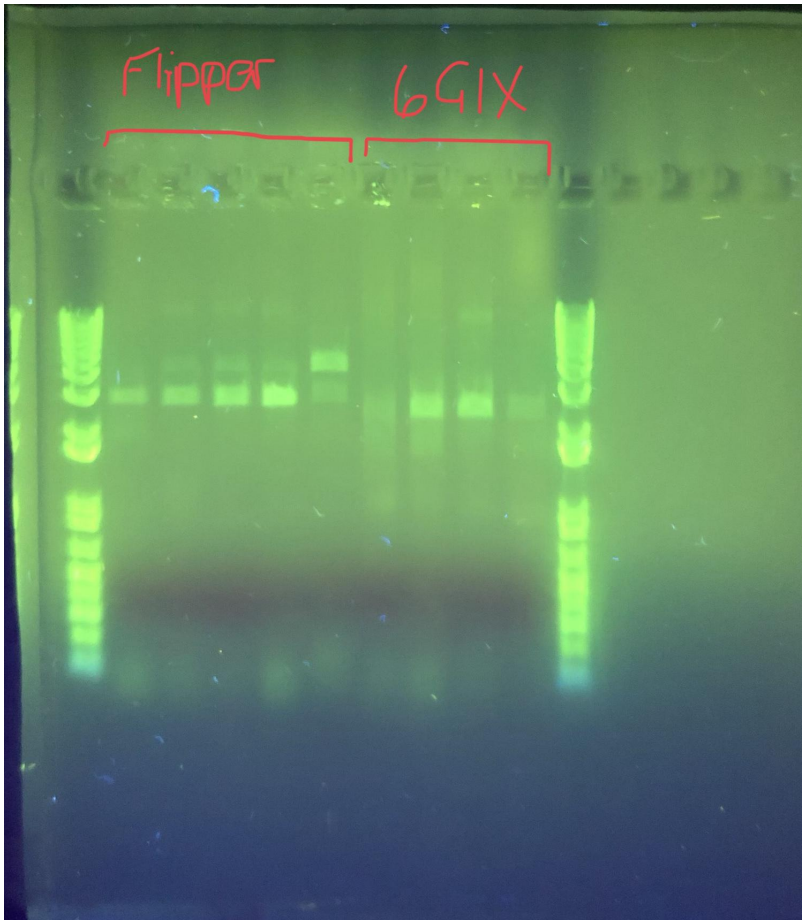
- Performed a digest confirmation of the pSB1A3-RFP-Flipper and pSB1C3-6GIX parts while we waited for sequencing results. Double digested pSB1A3-RFP-Flipper with BmtI-HF and SpeI-HF and pSB1C3-6GIX with BamHI and EcoRI-HF. Expected and actual results are shown below:

Screen Shot 2019-06-20 at 2.09.37 PM.png



In silico digestion of pSB1A3-RFP-Flipper with BmtI-HF/SpeI-HF & pSB1C3-6GIX with EcoRI-HF/BamHI pSB1A3-BBa_J04450 and pSB1C3-BBa_J04450 are included to demonstrate the potential "empty backbone" result (which may occur as the linearized backbone is derived from these parts)

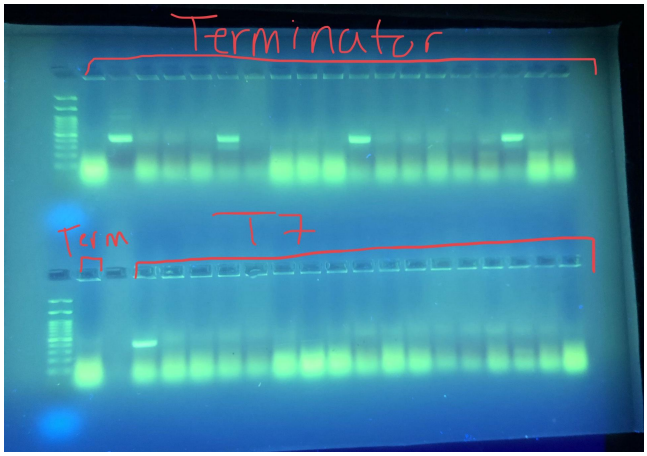
IMG_1517.jpg



1% agarose gel, Invitrogen 1kb plus ladder
Digestion of pSB1A3-RFP-Flipper colonies 7, 8, 10, 11, 17 with BmtI-HF/SpeI-HF and pSB1C3-6xHis-6GIX colonies 9, 11, 15, 18 with BamHI-HF/EcoRI-HF

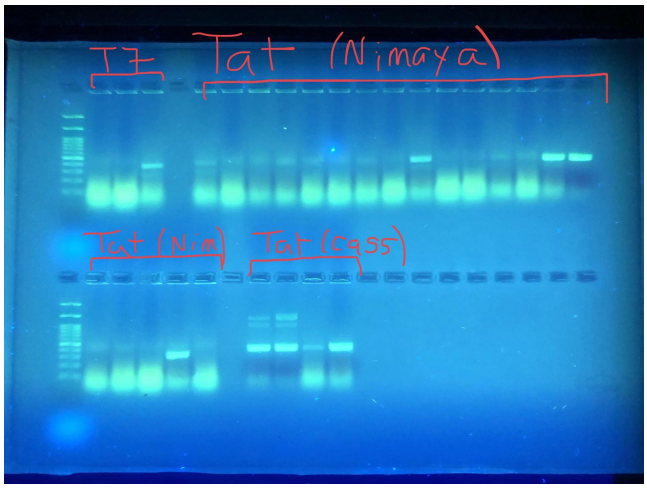
- Digestions do not appear as expected, but an error may have occurred with the enzymes or the incubation period may have been too short (only 1 hour). We will repeat the digest confirmation tomorrow.
- Cleaned excess plates out of the fridge
- Made more Chlo plates
- cPCR'd a bunch more colonies from previous transformed ligations. Ran on a 2% gel (results below)
    - 20 colonies from the pSB1C3-Terminator transformation performed by Nimaya yesterday
    - 20 colonies from the pSB1C3-T7-RBS transformation performed by Nimaya yesterday
    - 20 colonies from the pSB1C3-YcbK transformation performed by Nimaya yesterday
    - 4 colonies from the pSB1C3-YcbK transformation performed by Cassie on Tuesday

IMG_1512.jpg



2% agarose, GeneDireX 100bp DNA ladder H3 RTU

IMG_1510.jpg



2% agarose, GeneDireX 100bp DNA ladder H3 RTU

- Made O/NS for pSB1C3-Terminator colonies 2, 11, 17, pSB1C3-T7-RBS colony 1, pSB1C3-YcbK (06/19) colonies 14, 15, and pSB1C3-YcbK (06/18) colonies 5, 7
- Streaked pSB1C3-MalE #4 and pSB1C3-PhoA #7 as back-up long term storage. Also streaked a super pink contamination colony for fun
- Lots of practice for the faculty presentation!!!

FRIDAY, 6/21/2019

- pSB1C3-MalE #4 and pSB1C3-PhoA #7 streak plates grew well. They were parafilmed and placed in the fridge. The contamination plate didn't grow well, so it was left in the incubator
- Ordered our no-signal peptide control T7-RBS-6xHis-6GIX-Terminator complete construct from IDT so that we can start working on protein purification ASAP
- Even though the digest confirmation results from yesterday were questionable, sent the following for sequencing:
  - pSB1A3-Flipper #7
  - pSB1C3-6GIX #18
  - pSB1C3-OmpA #5
  - pSB1C3-TorA #15
- Repeated the digest confirmations from yesterday using slightly more DNA (and definitely the correct enzymes). Incubated for 4 hours, will run the gel Monday.

- Miniprepped yesterday's overnights
- Began gel purification of the rest of the GBlock PCR products that were made last week in preparation for the golden gate reactions we will conduct next week
- Presented our project to Dr. Church and recieved feedback from our RA and TA on how to improve the presentation

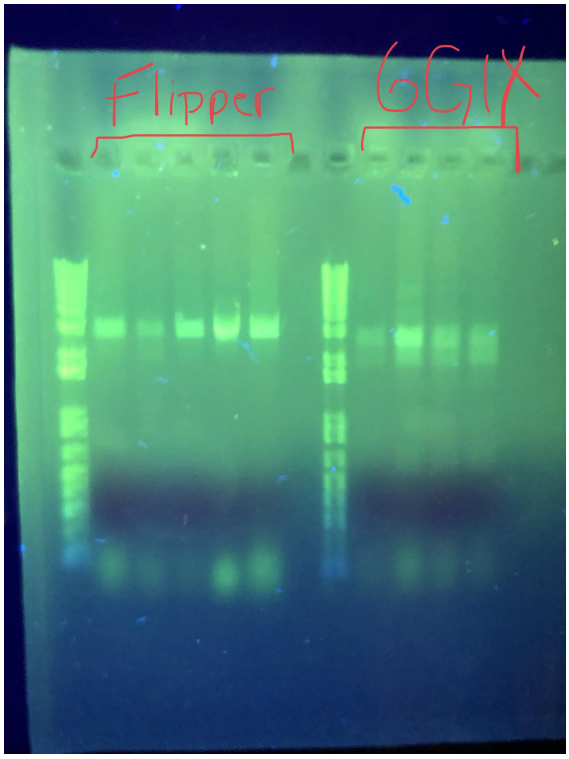
Week 7 (June 17th-21st) WEEKLY SUMMARY:

After optimization of our colony PCR protocol early in the week, we continued to clone all of our parts into the donor vector (pSB1C3) and our improved Golden Gate RFP Flipper into the destination vector (pSB1A3). We screened these ligations using colony PCR. Attempts at digest confirmations throughout the week were not successful, so we resorted to DNA sequencing. We were able to sequence confirm one more signal peptide in pSB1C3 (MalE), and we are waiting on the sequencing results for others. Aside from cloning, we gel-purified the IDT GBlocks that were PCR-amplified last week. We also began experiments to validate models that will be used to determine the chlorophyll concentration in canola oil. We also focused on preparations for our Faculty Presentation that will occur early next week.

MONDAY, 6/24/2019

- Ran the digest confirmation from friday on a gel:

IMG_1556.jpg



1% agarose gel, Invitrogen 1kb plus ladder
Digestion of pSB1A3-RFP-Flipper colonies 7, 8, 10, 11, 17 with BmtI-HF/SpeI-HF and pSB1C3-6xHis-6GIX colonies 9, 11, 15, 18 with BamHI-HF/EcoRI-HF

- The digest once again looks incorrect... But the colonies are pink, so we will have faith in the cPCR and proceed with Golden Gate while we wait for the sequencing results
- Gel purified all of the GBlock PCR products that were made last week in preparation for the golden gate reactions we will conduct this week
- Did an empty linearized backbone control ligation for pSB1C3 and pSB1SA3 and transformed them
- Recieved sequencing results for the pSB1A3-Flipper #7, pSB1C3-6GIX #18, pSB1C3-OmpA #5, and pSB1C3-TorA #15 samples sent in on Friday. All four parts were correct!!!
- Set up golden gate reactions for each of the signal peptides using a combination of miniprepped plasmid DNA and gel-purified PCR products. Ran the reactions in the thermocycler overnight as per Boston University iGEM 2018's protocol
- Had our faculty talk! Recieved lots of useful feedback about many aspects of our project

TUESDAY, 6/25/2019

- The ligation control plates had no colonies!
- Transformed samples from each of the golden gate reactions into chemically competent DH5-alpha
- Had some of the team members perform replicates of the ethanol chlorophyll extraction experiments
- Recieved our T7-RBS-6xHis-6GIX-Terminator no signal peptide control construct in the mail from IDT today

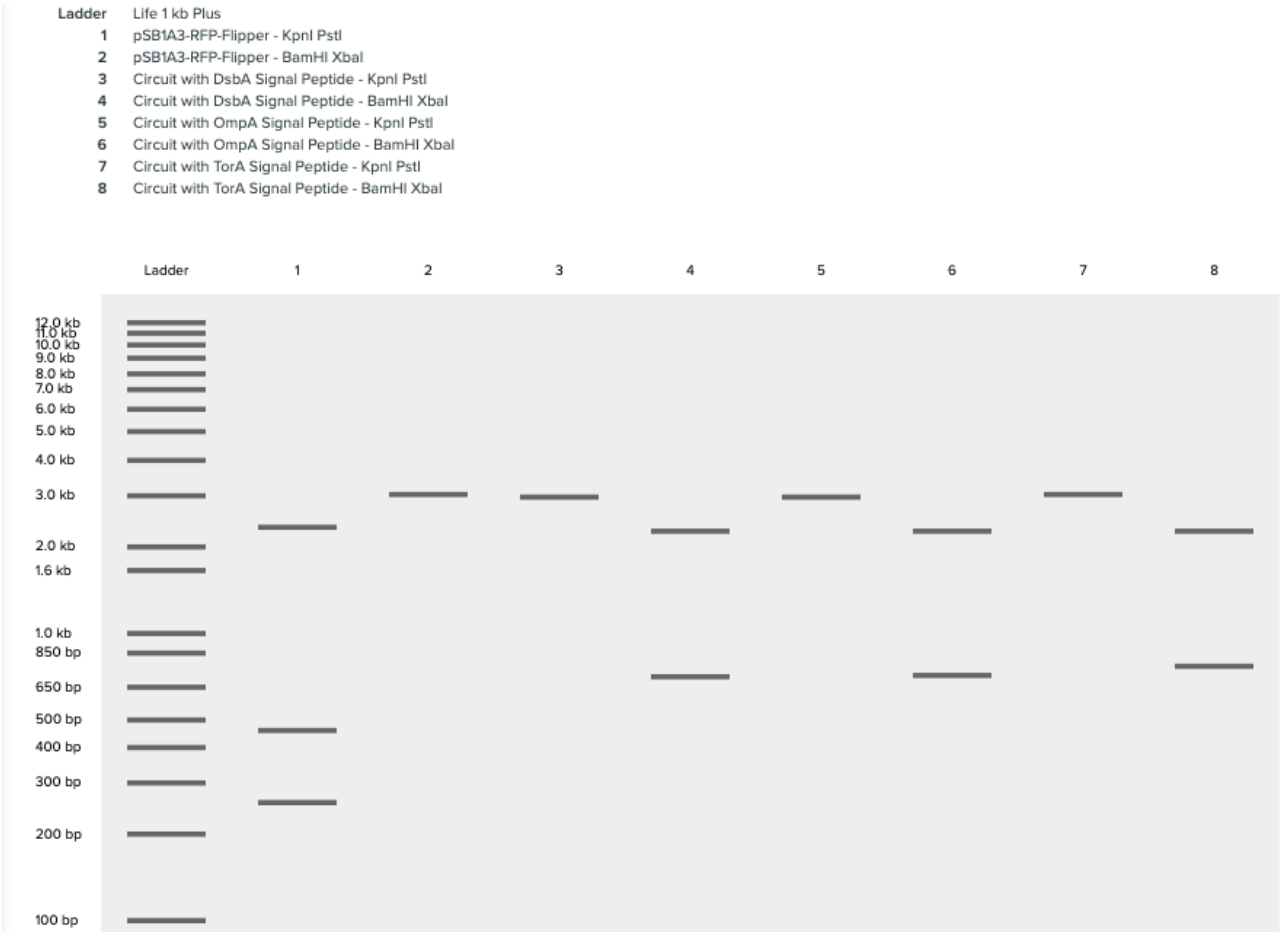
WEDNESDAY, 6/26/2019

- Travelled to Lacombe, AB to attend CanolaPALOOZA. Our team learned a ton about the local canola industry and where/how our project can be integrated
- All but one of the golden gate transformations had colonies grow. For three of the reactions, there were at least one white colony (which indicates the correct construct, as opposed to red which contain the RFP flipper part). Overnight cultures were made for these colonies.
    - Satellite colonies grew around all of the colonies on our ampicillin plates. Further analysis will need to be conducted to determine whether this is a function of leaving the plates in the incubator for too long or whether our plates do not contain enough/poor antibiotic

THURSDAY, 6/27/2019

- Prepared and digested our new T7-RBS-6xHis-6GIX-Terminator no signal peptide control construct DNA. Ligated it into pSB1A3 and transformed. Also transformed miniprepped pSB1A3-Flipper DNA as a positive control for the strange colony growth we observed on the ampicillin plates. If satellite colonies are present on that plate, it can be inferred that the satellite colony growth observed was not related to the Golden Gate reaction
- Made glycerol stocks and miniprepped the pSB1A3-OmpA GG construct, pSB1A3-DsbA GG construct, and pSB1A3-TorA GG construct
- Set up repeat Golden Gate reactions for all six signal peptides and ran them using a different thermocycler protocol found on the NEB website
- Miniprepped the overnights of the Golden Gate OmpA, TorA, and DsbA constructs. Ran digest confirmations using KpnI-HF/PstI and XbaI/BamHI. The digests will be run on a gel tomorrow. Expected results are below:

Screen Shot 2019-06-27 at 3.12.40 PM.png

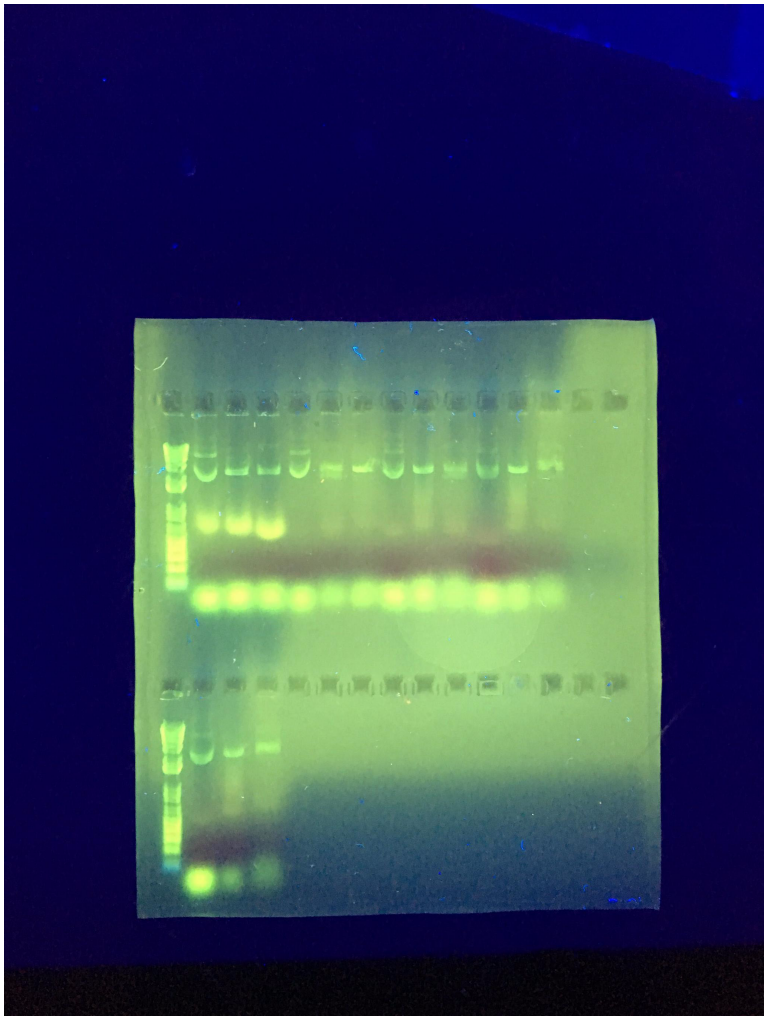


In silico digestion of pSB1A3-RFP-Flipper and Golden Gate DsbA, OmpA, and TorA constructs with KpnI/PstI and XbaI/BamHI

FRIDAY, 6/28/2019

- A digest confirmation was done with the golden gate constructs with OmpA, TorA, and DsbA. The digestion was done overnight, but the gel ran in the morning (1% gel with Invitrogen 1kb+ ladder) did not have the desired band sizes. The digest confirmation was repeated with more miniprepped DNA and a digestion of only 1 hour. XbaI, KpnI, PstI, and BamHI were used. The second gel was more clear and included control DNA that wasn't digested, but still didn't appear to have the right bands:

Image from iOS.jpg



Lane Order: *Invitrogen 1kb+ Ladder,* OmpA1-U, OmpA1-K/P, OmpA1-X/B, OmpA2-U, OmpA2-K/P, OmpA2-X/B, OmpA3-U, Omp3-K/P, OmpA3-X/B, TorA-U, TorA-K/P, TorA-X/B, *Invitrogen 1kb+ Ladder,* DsbA-U, DsbA-K/P, DsbA-X/B

U= Undigested Plasmid
K/P= KpnI + PstI
X/B= XbaI + BamHI

- cPCR was also performed for transformations using the 4:1 ligation of pSB1A3-T7-RBS-6GIX-Terminator (no SP) into pSB1C3, the 1:1 ligation of pSB1A3-T7-RBS-6GIX-Terminator (No SP), and golden gate using PhoA. The other golden gate contruct transformations didn't grow any colonies. However, after the PhoA colonies were cPCRed, some of the the colonies on the original plate started to appear red, indicating that the golden gate failed.
    - 4:1= 15 colonies
    - 1:1= 15 colonies
    - PhoA GG construct= 8 colonies

Image from iOS (1).jpg

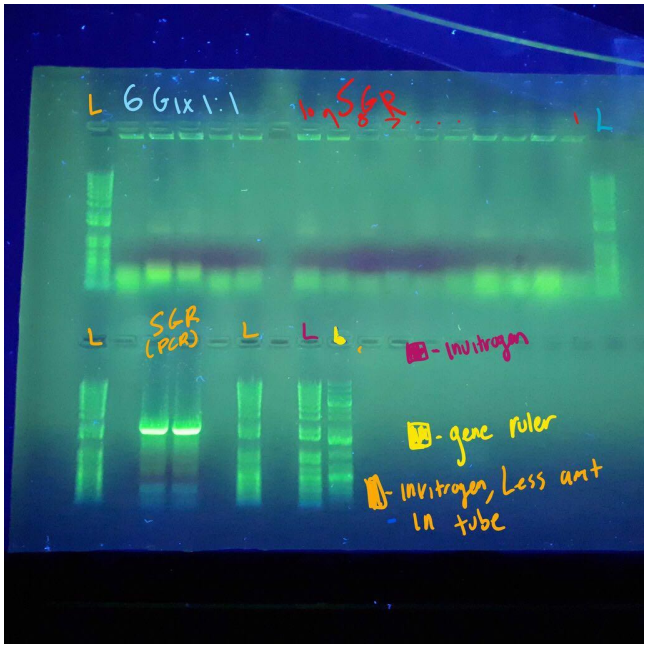
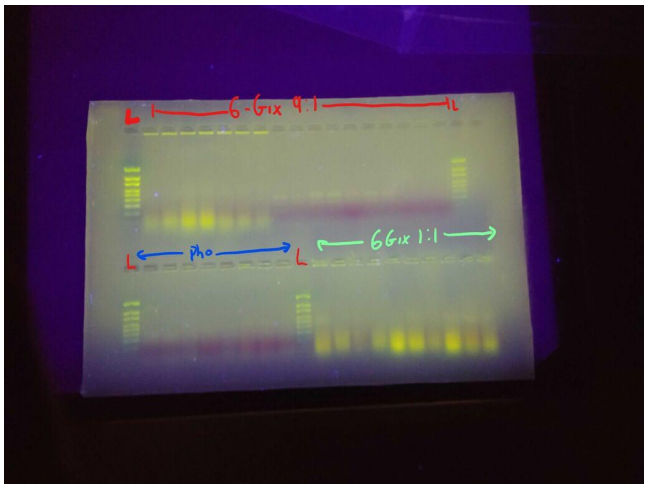


Image from iOS (2).jpg



- Transformations of the GG constructs were repeated because the ones done yesterday (inlcuding the ones that were used for cPCR) were not done completely correct
  - NoSP 6GIX 1:1 Ligation
  - No SP 6GIX 4:1 Ligation
  - MalE GG Contsruct
  - OmpA GG Construct
  - PhoA GG Construct
- Minipreps were aslo done of DNA confirmed parts

Week 8 (June 24th-28th) WEEKLY SUMMARY:

Early in the week, we received positive sequencing results for several signal peptides (OmpA, TorA, and DsbA) and the 6XHis-6GIX coding sequence in pSB1C3, as well as our improved Golden Gate RFP Flipper in pSB1A3 (our destination vector). Once we confirmed the destination vector, we were able to proceed with our first Golden Gate reactions. We used a combination of PCR products and parts in donor vectors for these reactions in order to create all six of our constructs (one for each different signal peptide). After transformation, we had some white colonies that putatively indicate successful Golden Gate reactions. Further analysis will be performed on these colonies. Efforts continued in cloning the other IDT parts into pSB1C3. We also received our 'no signal peptide' control construct from IDT and began cloning it into pSB1A3. We were observing some satellite colony growth on our LB agar plates containing ampicillin, so we spent some time on optimization to reduce the issue. We also performed a ligation control for the EcoRI/PstI digests, which showed no growth and therefore helped us determine that in the absence of a digested IDT GBlock, the digested linearized backbone will not religate closed. More replicates for the experiments validating the chlorophyll concentration in oil models were performed this week.

Our team gave a presentation to faculty members and other researchers at our university this week, and we received valuable feedback about our project and experimental plans. In addition, we attended CanolaPALOOZA in Lacombe, Alberta. This event brings together many members of the canola farming and canola oil industries, and therefore gave us a valuable opportunity to conduct some human practices work.

MONDAY, 7/1/2019

- Canada Day!

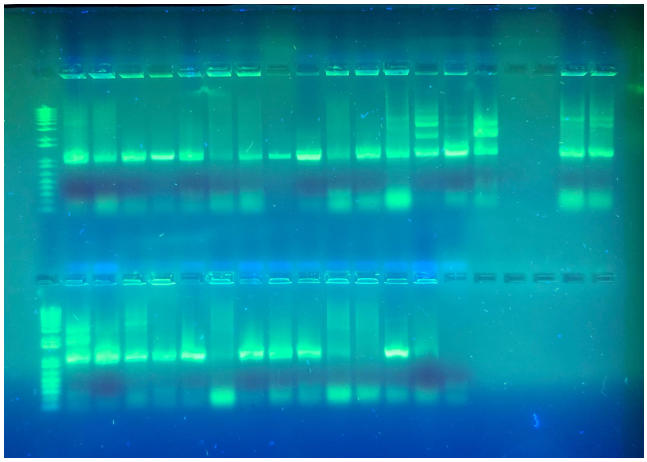
TUESDAY, 7/2/2019

- Made streak plates from all of the Golden Gate construct colonies that appeared white
- Made LB + Amp plates containing various concentrations of ampicillin in an attempt to minimize growth of satellite colonies. Transformed and plated miniprepped pSB1A3-Flipper
- Repeated cPCR for the pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) ligation colonies (from a masterplate) that was originally performed last Friday. Ran out of time to run on a gel, so placed in the fridge overnight
- Repeated Golden Gate reactions for the OmpA, MalE, PhoA constructs overngiht in the thermocycler
- Made overnights for each of the colonies that were white in appearance for previous Golden Gate reactions

WEDNESDAY, 7/3/2019

- Golden gate construct streak plates all had growth. Placed in the fridge
- Transformed the overnight Golden Gate reactions for the OmpA, MalE, PhoA constructs into ccDH5a
- The LB + Amp plates containing various concentrations of antibiotic with pSB1A3-Flipper transformation plated yesterday all had red lawns grow on them (the transformation was too efficient)
    - Transformed an old ligation (pSB1A3-Flipper) into ccDH5a and plated on more plates from this batch
- Ran pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide)cPCR samples from yesterday on a gel:

IMG_1648.jpeg



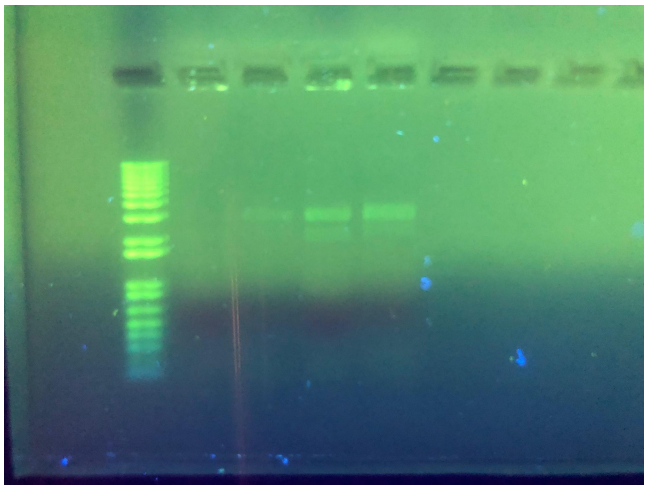
1% agarose, Invitrogen 1kb plus ladder
Top row, wells 2-16: pSB1A3-T7-RBS-Terminator (no signal peptide) 1:1 Top row, wells 19-20 and bottom row, wells 2-15: pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 3:1 ligation

- Made overnights for pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 colonies 4 and 8 as well as pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 3:1 colonies 6, 9, 10
- Made streak plates for each of the sequence-confirmed parts in pSB1C3 (or pSB1A3 in the case of the RFP Flipper) for long-term back-up storage
- Miniprepped all of yesterday's overnights of the colonies that were white in appearance for previous Golden Gate reactions, and set up overnight digest confirmations for each of them using KpnI/PstI and BamHI/XbaI
- Handed out flyers for our bottle drive!

THURSDAY, 7/4/2019

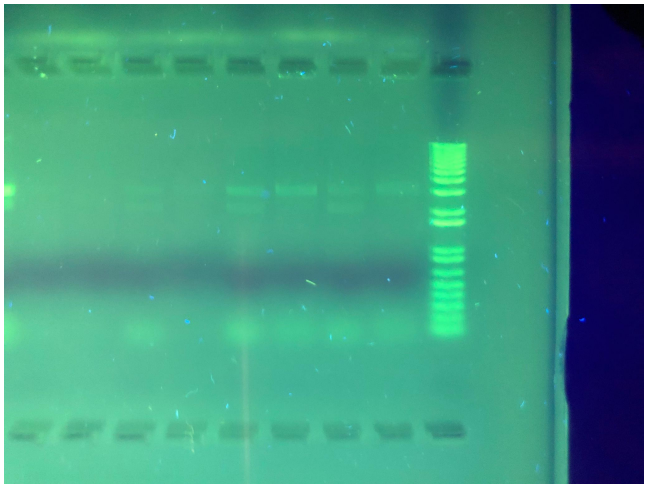
- Experiments using the pSB1A3-Flipper ligation product plated on agar with various concentrations of ampicillin once again showed growth of satellite colonies. We will need to make new ampicillin stocks to replace the ones in our freezer.
- Miniprepped the pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 colonies 4 and 8 as well as pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 3:1 colonies 6, 9, 10 O/Ns from yesterday. Sent pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 colony #4 and pSB1A3-MalE GG construct #3 for sequencing
- Repeated PCR amplification of our IDT GBlocks for use in Golden Gate reactions, but only the TorA-SP, PPH (other subgroup), and CBR (other subgroup) parts were visible on the gel. Extracted the bands and gel purified.
- Repeated digest confirmations of each of the Golden Gate constructs that appeared as white colonies. Results were once again not as expected:

IMG_1651.JPG



1% agarose, Invitrogen 1kb plus ladder
KpnI/PstI and BamHI/XbaI digestions of the Golden Gate complete constructs

IMG_1650.JPG



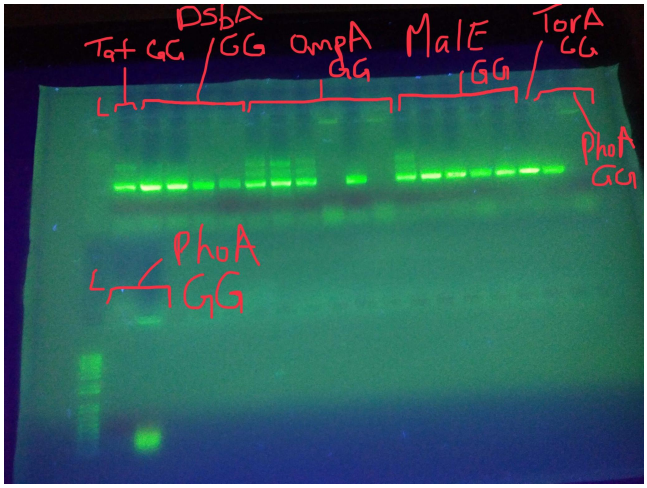
1% agarose, Invitrogen 1kb plus ladder
KpnI/PstI and BamHI/XbaI digestions of the Golden Gate complete constructs

- Repeated cPCR for all of the Golden Gate constructs that look promising. Placed in the fridge overnight to be run on a gel in the morning.
- Recorded tracks for our 'iGEM Road' Parody
- Handed out flyers for our bottle drive

FRIDAY, 7/5/2019

- Ran the cPCR samples from yesterday on a gel. The ladder did not appear well for the top row, but bands estimated to be ~1.2kb are present.

IMG_1783.jpg



1% agarose, Invitrogen 1kb plus ladder

- Gel-purified the PCR products for the TorA-SP, PPH (other subgroup), and CBR (other subgroup) parts. Nanodropped and placed in freezer.
- Continued with experiments aimed to test various concentrations of ampicillin in LB agar plates.
- Transformed chemically competent *E. coli* BL21 with miniprepped pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 colony #4 DNA
- Chlorophyll night (wooooooo!!)

SATURDAY, 7/6/2019

- Came into the lab to take plates out, but many of them had fungal contamination :(

- Team bottle drive for fundraising

Week 9 (July 2nd-5th) WEEKLY SUMMARY:

Throughout the week, we worked towards confirming all of our constructs via colony PCR. This analysis was followed by digest confirmations, but once again these did not work correctly. Colonies that appeared to contain the correct construct were sent for sequencing, and we are awaiting results. In the meantime, we began transforming the putative assembled constructs into BL21 (DE3), which is our expression chassis. We repeated the Golden Gate reactions for the constructs that have not yet been successfully assembled. We were still having difficulties with satellite colony growth on our LB agar with ampicillin plates, so we tested different antibiotic concentrations. We also tried to mitigate this issue by making streak plates of the promising colonies. Our team also ran a bottle drive this week to fundraise for our travel expenses.

MONDAY, 7/8/2019

- Since we have been having so many difficulties with satellite colony growth on our ampicillin plates, we attempted to ligate the RFP Golden Gate Flipper as well as the T7-RBS-6GIX-Terminator (no SP) parts into pSB1K3 because kanamycin is generally a better antibiotic. Performed a dig-lig using linearized pSB1K3 from the distribution kit and GBlocks from IDT. Transformed into ccDH5a
- The plate for pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 #4 in BL21 had fungal contamination over the weekend, so this transformation was repeated and plated.
- Recieved sequencing results for pSB1A3-T7-RBS-6GIX-Terminator (no signal peptide) 1:1 #4 that confirmed that this part was sucessfully cloned in. It will be referred to as pSB1A3-T7-RBS-6GIX-Terminator (no SP) from here on
- Also the sequencing results for pSB1A3-MalE GG construct #3 show that the sequencing reaction failed
- Repeated the PCR amplification for all of our GBlocks, which all worked this time. Excised and placed in the freezer for extraction tomorrow.

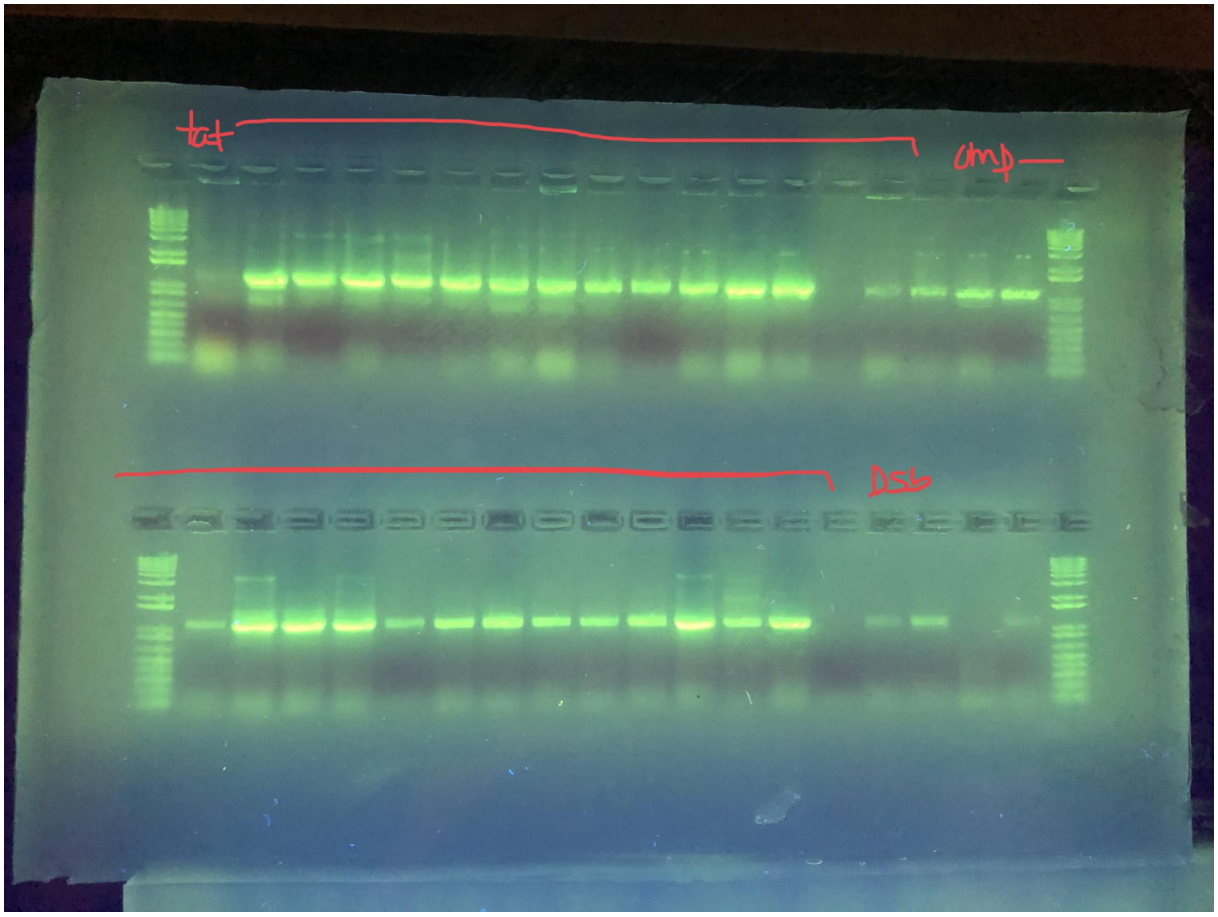
TUESDAY, 7/9/2019

- The new pSB1K3 ligation plates had no growth on them at all, so we re-transformed more ligation product into ccDH5a and plated, along with a positive pSB1K3 control from the registry
- The pSB1A3-T7-RBS-6GIX-Terminator (no SP) in BL21 plate had growth, so we streaked this and the same part in DH5a for long-term back up storage
- Made more chemically competent DH5a and tested using the chemically competent test kit from iGEM
- Continued gel purification of the PCR-amplified GBlocks
- Repeated Golden Gate reactions for TorA, YcbK, and DsbA constructs
- Ran overnight cPCR for all of the Golden Gate construct colonies that look promising (ie. are white)

WEDNESDAY, 7/10/2019

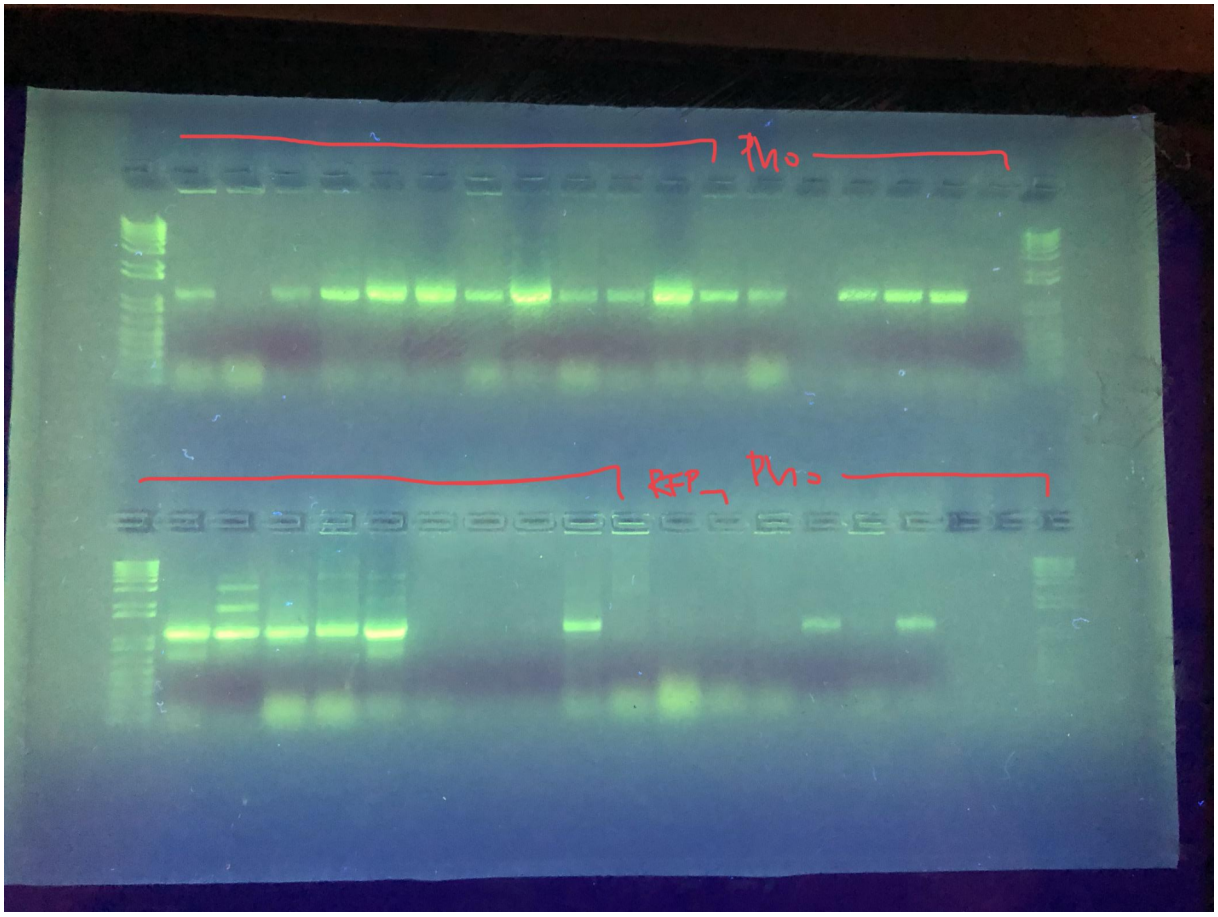
- pSB1K3 ligation plates did not grow again, but the positive control did. There was likely an issue with the ligation. However, we have had more success with the ampicillin plates and therefore feel confident in abandoning the effort to move into a new backbone. Work will continue in pSB1A3
- Made new ampicillin stock using amp powder from Marija's old lab and used it to make lots of new amp plates
- Cleaned all of our used plates out of the fridge (again)
- Made a giant batch of cPCR mastermix
- Ran the overnight cPCRs on 1% agarose:

IMG_1566.JPG



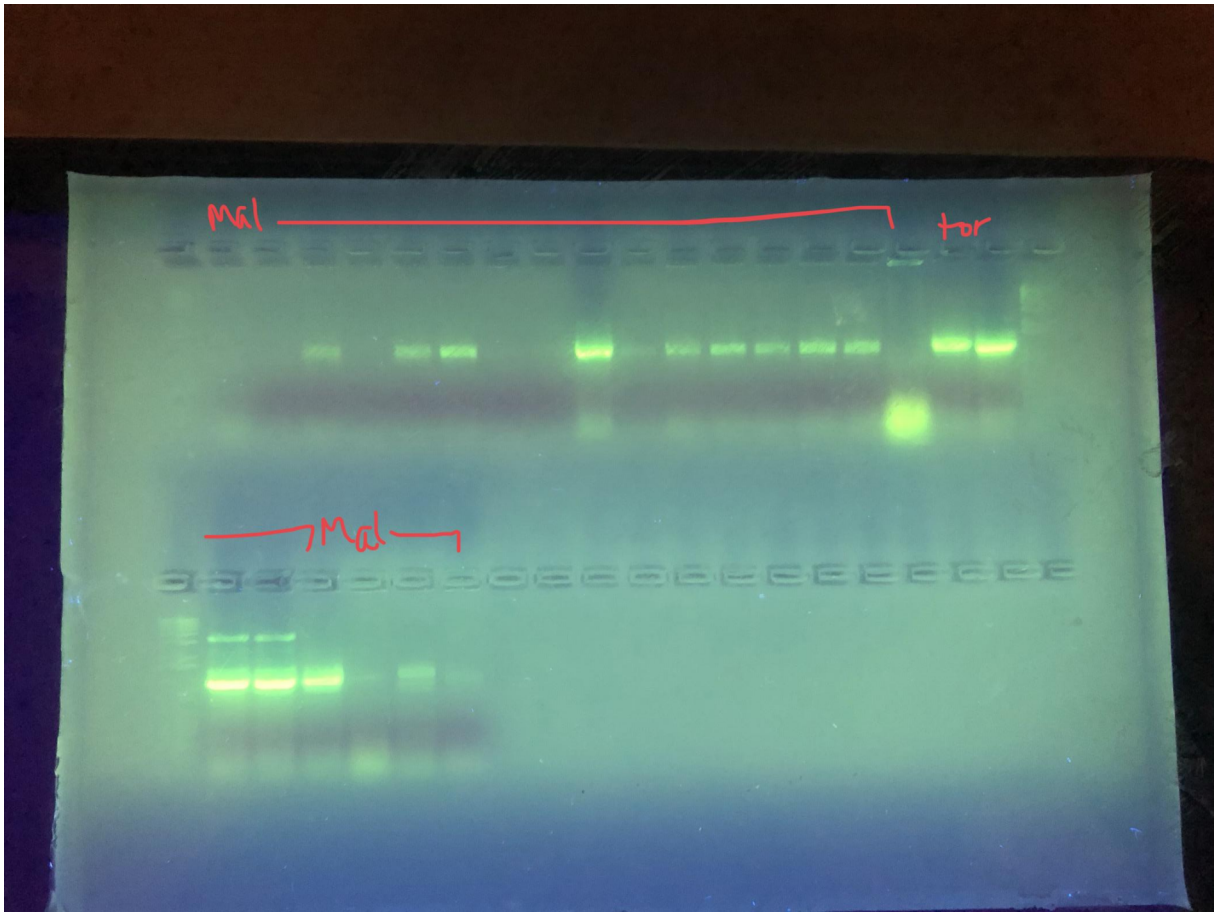
1% agarose, Invitrogen 1kb plus ladder

IMG_1567.JPG



1% agarose, Invitrogen 1kb plus ladder

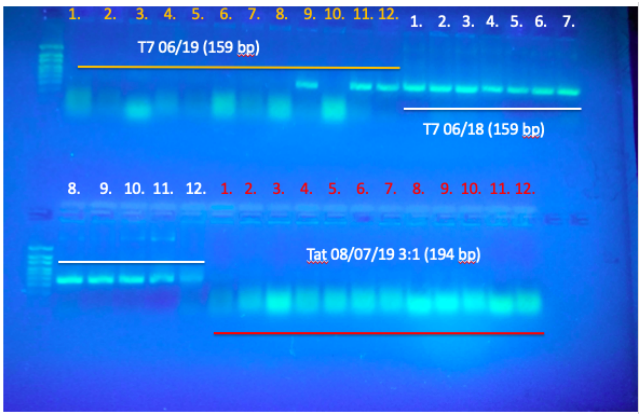
IMG_1568 2.JPG



1% agarose, Invitrogen 1kb plus ladder

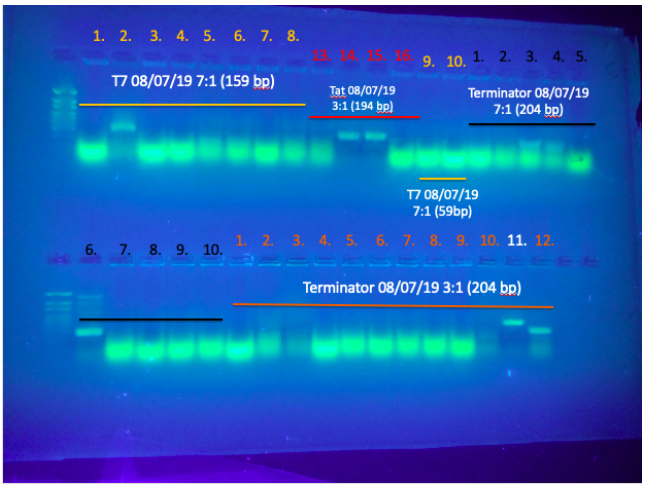
- Based on the results from the cPCR, sent the following (which were previously miniprepped) for sequencing:
  - pSB1A3-MalE GG construct #1
  - pSB1A3-PhoA GG construct #8
  - pSB1A3-TorA GG construct #1
  - pSB1A3-OmpA GG construct #3
  - pSB1A3-DsbA GG construct #8
- Transformed the most recent Golden Gate reactions into ccDH5a but didn't have time to plate. Placed in fridge and will plate tomorrow
- Finished the final round of gel purification of the IDT GBlocks. We now have stocks of all parts in the freezer
- In the interest of confirming that all of the IDT GBlock parts have been cloned into a donor plasmid for future Golden Gate, a cPCR was run on 100+ colonies of pSB1C3-T7-RBS, pSB1C3-YcbK-SP, and pSB1C3-Terminator using plates in the fridge from ligations done in June:

Screen Shot 2019-07-12 at 12.00.17 PM.png



1% agarose, Invitrogen 1kb plus ladder

Screen Shot 2019-07-12 at 12.00.27 PM.png



1% agarose, Invitrogen 1kb plus ladder

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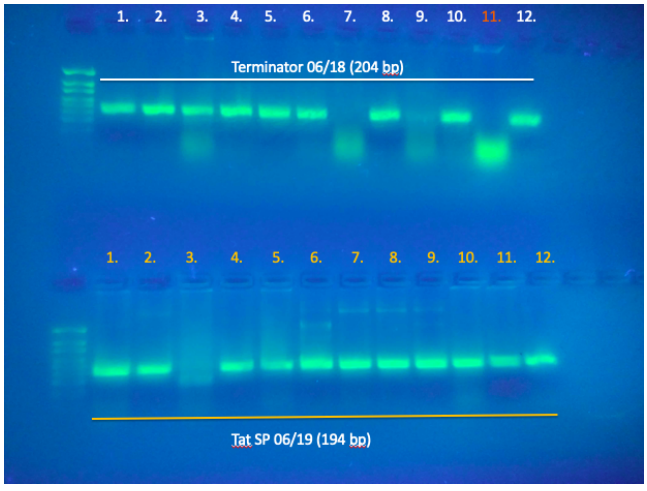


1% agarose, Invitrogen 1kb plus ladder

- Based on the results from the cPCR, made overnights from colonies that look promising

THURSDAY, 7/11/2019

- Plated yesterday's transformations
- Repeated all of the Golden Gate reactions once more using twice as much DNA in them this time. Placed in the freezer
- Miniprepped the pSB1C3-T7-RBS, pSB1C3-YcbK-SP, and pSB1C3-Terminator overnights
- Helped the other subgroup order their complete constructs from IDT
- Met with representatives from the Life Sciences Innovation Hub to plan the 'Bacteria Night' event that we will be hosting in September
- Refilled pipette boxes

FRIDAY, 7/12/2019

- Cleaned up the mess that was accumulating in the lab
- Transformed the Golden Gate reactions that were run yesterday into ccDH5a
- Recieved sequencing results for the golden gate constructs sent in on Wednesday. All five samples had the correct sequence! We are now only missing pSB1A3-YcbK GG construct before we can start protein experiments, but there were some candidates from the last cPCR that will continue to be analyzed
- Transformed previously miniprepped pSB1A3-MalE GG construct #1, pSB1A3-PhoA GG construct #8, pSB1A3-TorA GG construct #1, pSB1A3-OmpA GG construct #3, and pSB1A3-DsbA GG construct #8 (from now on, referred to without their number indentification) into chemically competent BL21 (DE3) and plated
- Made overnights for the following for glycerol stocks and/or miniprepping:
    - pSB1A3-MalE GG construct in DH5a
    - pSB1A3-PhoA GG construct in DH5a
    - pSB1A3-TorA GG construct in DH5a
    - pSB1A3-OmpA GG construct in DH5a
    - pSB1A3-DsbA GG construct in DH5a
    - pSB1A3-T7-RBS-6GIX-Term (no SP) construct in DH5a
    - pSB1A3-T7-RBS-6GIX-Term (no SP) construct in BL21 (DE3)
    - Putative pSB1A3-YcbK GG construct A in DH5a
    - Putative pSB1A3-YcbK GG construct A in DH5a
    - Untransformed BL21 (DE3)

SATURDAY, 7/13/2019

- Made glycerol stocks for all of the overnights made yesterday and miniprepped if in DH5a
- Only the pSB1A3-PhoA GG construct transformation into BL21 (DE3) from Friday had growth, so the MalE, OmpA, TorA, YcbK, and DsbA GG constructs were retransformed into BL21 (DE3)
- Yesterday's transformations were replated

SUNDAY, 7/14/2019

- The replated transformations didn't grow
- Made 2x 5ml O/Ns for pSB1A3-T7-RBS-6GIX-Term (no SP) in BL21 and pSB1A3-PhoA GG construct in BL21
- Transformed the MalE, OmpA, TorA, YcbK, and DsbA GG constructs once again, but accidentally transformed into DH5a instead of BL21 :(

Week 10 (July 8th-12th) WEEKLY SUMMARY:

Since we were having difficulties with ampicillin for use with pSB1A3, we started this week by attempting to switch our destination vector to pSB1K3. Unfortunately, none of the ligation transformations showed any growth, so we quickly abandoned this idea and stuck with streaking instead. We received sequencing results that showed that our 'no signal peptide' control construct had successfully been cloned into pSB1A3. Efforts to transform this part into *E. coli* BL21 (DE3) began this week. The sequencing reaction for our Golden Gate-assembled construct sent last week unfortunately failed. We PCR-amplified all of our GBlocks from IDT to create DNA stockpiles for performing Golden Gate, and then repeated all of the Golden Gate reactions again. In addition, we repeated our colony PCR screening for all of the colonies that are white in appearance, indicating that the assembled construct has replaced the RFP flipper in the destination vector. Constructs containing the signal peptides from DsbA, MalE, OmpA, PhoA, and TorA showed positive cPCR results and were sent for sequencing. The construct containing the 'YcbK' signal peptide was cPCR-confirmed later in the week and will be sent for sequencing next week. We also began the process of transforming all of the constructs containing signal peptides into *E. coli* BL21 (DE3). Members of our subgroup also took part in education and outreach work this week by beginning to plan an informational 'Bacteria Night' event.

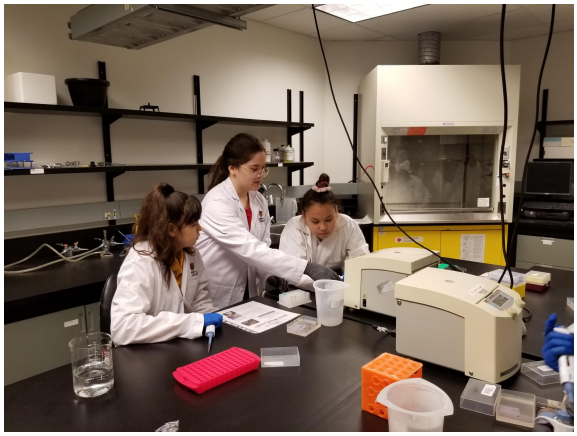
MONDAY, 7/15/2019

- Subcultured the pSB1A3-T7-RBS-6GIX-Term (no SP) in BL21 O/N 1:100, then induced with 100mM IPTG. Left overnight at 30 degrees celcius, shaking. We also subcultured an untransformed BL21 chemically competent aliquot for use as a negative control, and kept an uninduced 1:100 subculture of pSB1A3-T7-RBS-6GIX-Term (no SP) in BL21
- Sent samples of our IDT GBlocks in pSB1C3 that were cPCR'd last week for sequencing
    - pSB1C3-T7-RBS #12 from 06/19/19
    - pSB1C3-Terminator #10 from 06/18/19
    - pSB1C3-YcbK-SP #3 from 06/19/19
- Retransformed all GG constructs into BL21 (DE3) (OmpA, TorA, PhoA, DsbA, MalE) as well as replated previous transformations

TUESDAY, 7/16/2019

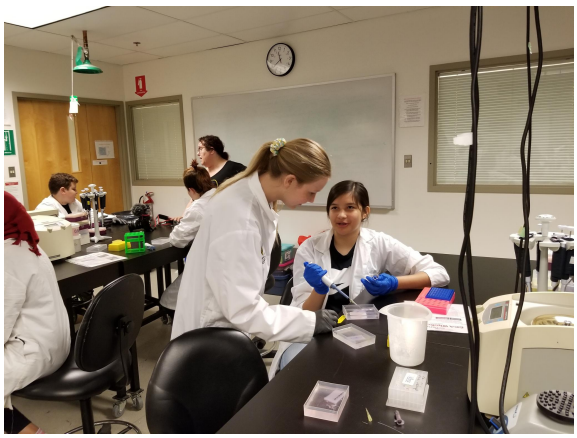
- Made more LB with amp
- New BL21 pSB1A3-OmpA GG construct and pSB1A3-MalE GG construct transformation plates did not have growth, so we replated. The other plates had growth (PhoA, Dsba, TorA GG constructs)
    - Made overnights for the constructs that grew so we can glycerol stock tomorrow
- Spun down the induced cultures and proceeded with three freeze-thaw cycles, followed by resuspension in 5ml of 1X PBS (pH 7.2). The resuspended cells were then treated with lysozyme (500µl of 10mg/ml lysozyme with 1% tween80) for 1 hour at 37°C
- Took 500µl aliquots from each resuspension and spun down, then seperated supernatant and pellet. Resuspended pellet in 1X SDS-loading buffer (made by the 2017 Calgary iGEM team). After doing these steps, we realized that the buffer used was very old and shouldn't be trusted. We discarded these samples and will repeat tomorrow
- Made the Tris buffers, 10% SDS, and 10% APS solutions that we will need to run SDS-PAGE tomorrow
- Recieved sequencing results for pSB1C3-T7-RBS #12, pSB1C3-Terminator #10, and pSB1C3-YcbK-SP #3.
    - OnlypSB1C3-Terminator #10 was the correct part. Made an O/N and will glycerol stock tomorrow
- Sent pSB1A3-YcbK GG construct A for sequencing
- Birth of Anthony the Snowman
- Helped with Dr. Hutchinson's Power to Choose IndigeSTEAM Camp

IMG_2085.JPG



IMG_2086.JPG

IMG_2087.JPG



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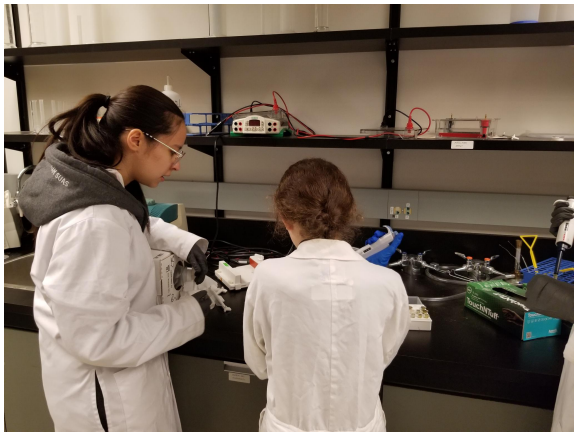

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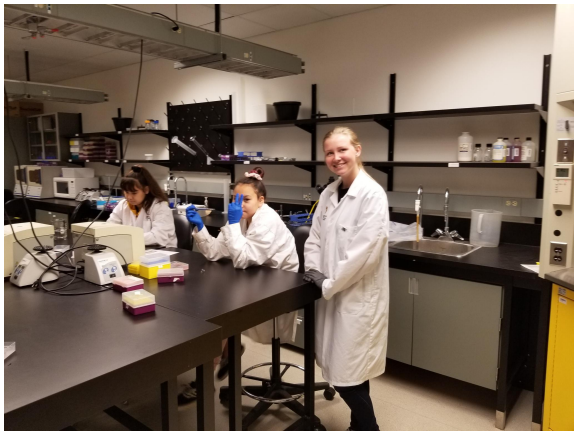


IMG_2090.JPG

WEDNESDAY, 7/17/2019

- Made glycerol stocks of the pSB1A3-PhoA, Dsba, TorA GG constructs in BL21 (DE3)
- Recieved sequencing results for pSB1A3-YcbK GG construct A, which show that this part is correct too! All of our constructs have now been made and successfully inserted into pSB1A3. Glycerol stocks of each of these parts in DH5a also exist.

pSB1A3-T7-RBS-6GIX-Terminator (Control)

pSB1A3-T7-RBS-DsbA-6GIX-Terminator

pSB1A3-T7-RBS-MalE-6GIX-Terminator

pSB1A3-T7-RBS-OmpA-6GIX-Terminator

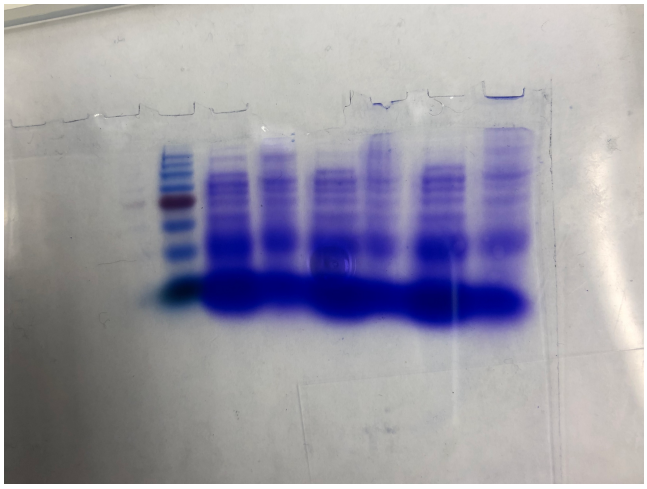
pSB1A3-T7-RBS-PhoA-6GIX-Terminator

pSB1A3-T7-RBS-Tat-6GIX-Terminator

pSB1A3-T7-RBS-TorA-6GIX-Terminator

- Retransformed the GG constructs into BL21 (DE3) (OmpA, TorA, Dsba, MalE, YcbK)
- Made new 4x SDS-loading buffer to replace Lalit's
- Made more P1 and P3 buffer for miniprepping
- Made SDS-PAGE gel using a protocol from Dr. Samuel's lab. Took 500µl aliquots from each resuspension (from yesterday) and spun down, then seperated supernatant and pellet. Resuspended pellet in 1X SDS-loading buffer and added 4x SDS-loading buffer to the supernatant. Loaded the SDS-PAGE gel and ran, but something went wrong because we had to run for >3 hours. We believe that this occured due to the glycerol in the gel recipe from Dr. Samuel's lab.
    - 6GIX is ~20 kDa, which means that it would be in that giant blob at the bottom. Clearly we were not able to visualize this on the gel, so we will need to try something different tomorrow

Untitled (1).png



10% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): Protein standard, no transformation control soluble fraction, no transformation control insoluble fraction, induced culture soluble fraction, induced culture insoluble fraction, uninduced culture soluble fraction, uninduced culture insoluble fraction

- Made overnights for sequence-confirmed pSB1C3-Terminator in DH5a and pSB1C3-YcbK GG construct in DH5a for glycerol stocking tomorrow

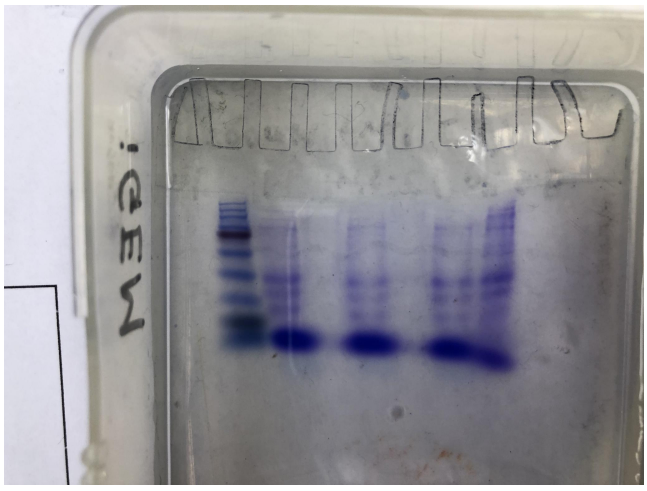
THURSDAY, 7/18/2019

- Sent another miniprepped pSB1C3-T7-RBS and pSB1C3-YcbK sample for sequencing
- BL21 (DE3) transformations of the YcbK, TorA, MalE, OmpA GG constructs on plates had colony growth, so made overnights of those
- Made new 10% SDS-PAGE gels as per a protocol from CMMB 451 and a protocol from Dr. Samuel's lab. Ran all of the protein samples on both gels again as previously described overnight (again, running time was extremely long)
- Made glycerol stocks of pSB1A3-YcbK GG construct (DH5a), pSB1C3-Terminator in DH5a

FRIDAY, 7/19/2019

- Stained one of the SDS-PAGE gels from yesterday as per protocol. Once again, our results were unclear as the gel didn't resolve very well. We also were not able to visualize protein for the pellets, which were 'goopy' upon loading. We will sonicate the samples before centrifugation next time in order to remove the DNA and hopefully improve the quality of our results. We also learned from Marija that we were probably using the gel apparatus incorrectly

IMG_1897.jpg



10% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): Protein standard, no transformation control soluble fraction, no transformation control insoluble fraction, induced culture soluble fraction, induced culture insoluble fraction, uninduced culture soluble fraction, uninduced culture insoluble fraction

- Discussed doing purification or a western blot as an alternative to the current protocol. If we continue to have poor results, we may have to move on to one of these alternatives before the intial confirmation of 6GIX production
- Upon Marija's reccomendation, we remade all of the buffers used for SDS-PAGE using deionized water
- Made glycerol stocks for our final pSB1A3-GG constructs in BL21. At this point, we have glycerol stocks of all of the IDT GBlocks ligated into pSB1C3 in DH5a (except for T7-RBS and YcbK-SP), as well as all of the golden gate constructs in both BL21 and DH5a
- Began work on wiki content, specifically the attributions for professors that we have worked with

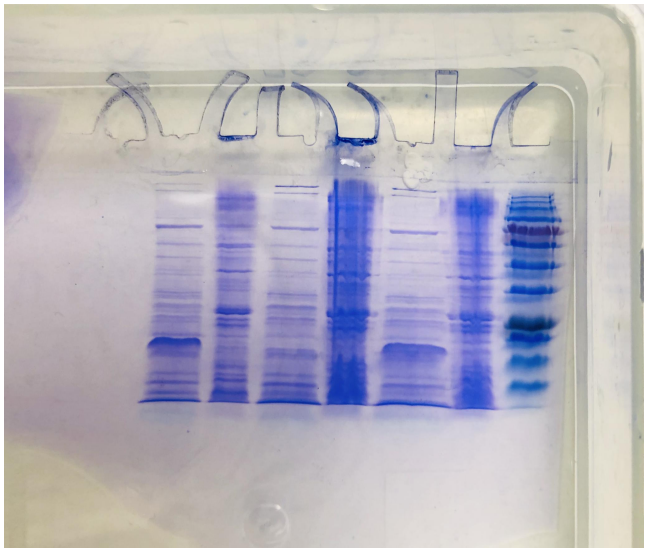
Week 11 (July 15th-19th) WEEKLY SUMMARY:

By the end of this week, we were able to assemble all of our constructs using Golden Gate Assembly. And we were able to sequence confirm all of our constructs and transform them into *E. coli* BL21 (DE3). We began preparing the necessary reagents for SDS-PAGE and became aquainted with many new protocols. We cultured and induced *E. coli* BL21 (DE3) that were untransformed or transformed with our 'no signal peptide' construct, then processed these samples and ran whole cell lysate fractions on SDS-PAGE gels. Unfortunately, we were unable to produce an SDS-PAGE gel that was able to be effectively run our samples. We will continue to experiment with different SDS-PAGE gel protocols in the weeks to come.

MONDAY, 7/22/2019

- Made a 12% SDS-PAGE gel as per protocol provided by Dr. Mayi
- Sonicated 1ml aliquots of the cell lysates collected last week, then spun them down and prepared them for running. Loaded 20ul samples onto the gel and ran it for ~40 mins. During the run, we realized that we forgot to add B-mercaptoethanol, which will be done for the next gel. Stained for 45 min and destained for 1.5 hours. Even though we forgot the B-mercaptoethanol, a band around 20 kDa is visible for both the induced and uninduced soluble fractions, but not the no-transformation control! The 6GIX produced in the uninduced culture is likely due to leaky expression

IMG_1909 2.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): Uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction, induced culture soluble fraction, induced culture insoluble fraction, protein standard

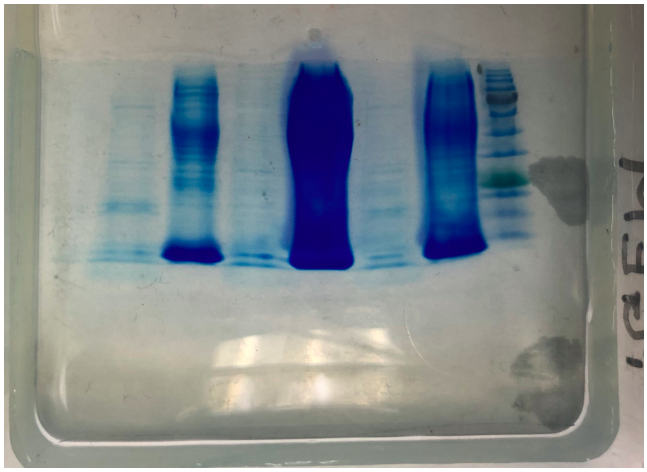
- Made O/Ns of the BL21 pSB1A3 contructs for T7-RBS-6GIX-Term (no SP), OmpA construct, MalE construct, YcbK construct, DsbA construct, PhoA construct, TorA construct, and untransformed BL21
- Team meeting

TUESDAY, 7/23/2019

- Wrote Telus spark Chlorophyll Cocktail funding proposal
- worked on pitch presentation for JulyGEM
- Made more LB media with Amp, Coomassie blue stain, and destaining buffer

- Ran another SDS-PAGE gel with the same samples from yesterday but remembered to include 5% B-mercaptoethanol this time. We used the new staining and destaining buffers, and the staining did not go as well. There are still visible bands near 20 kDa for the induced and uninduced soluble fractions.

gel.png



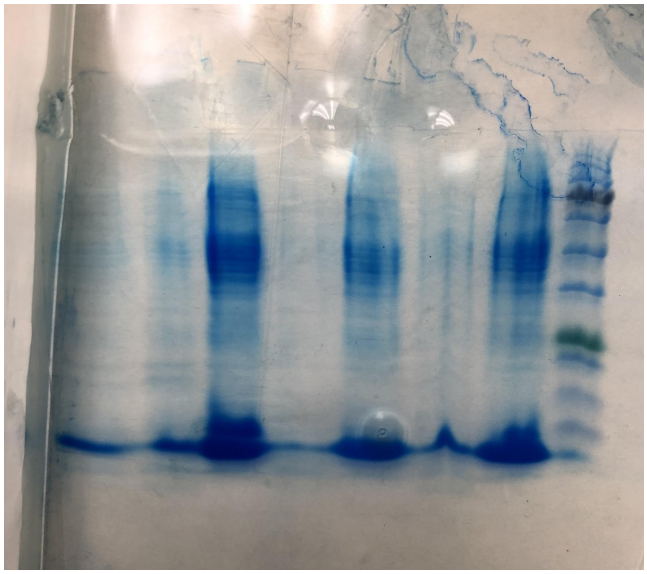
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): Uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction, induced culture soluble fraction, induced culture insoluble fraction, protein standard

- Subcultured the BL21 pSB1A3 contructs for T7-RBS-6GIX-Term (no SP), OmpA construct, MalE construct, YcbK construct, DsbA construct, PhoA construct, TorA construct, and untransformed BL21, but they all grew at variable rates. We will have to account for this and repeat before induction tomorrow

WEDNESDAY, 7/24/2019

- Worked on pitch presentation for JulyGEM
- Made new LB + Amp and plain LB media
- Took small samples of the cultures of the BL21 pSB1A3 contructs for T7-RBS-6GIX-Term (no SP), OmpA construct, MalE construct, YcbK construct, DsbA construct, PhoA construct, TorA construct, and untransformed BL21 from yesterday that had been left out on the counter overnight. Normalized the OD_{600} values of these samples via dilution to 0.3-0.4. Subcultured 1:100 into 50ml of LB and added Amp (80 ug/ml) to the appropriate cultures. Grew until OD_{600} reached about 0.6-0.7, which again took a variable amount of time depending on the construct. Once OD_{600} = 0.6-0.7, cultures were placed in the fridge. At the very end of the day, they were induced with 100mM IPTG and grown overnight at 30 degrees Celcius for periplasmic isolation tomorrow
- Ran another SDS-PAGE using the same samples as yesterday to test whether our new Coomassie stain is working. Once again, had poor results (smudges, low band sensitivity)

IMG_2095.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): Uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction, induced culture soluble fraction, induced culture insoluble fraction, protein standard

- Made solutions required for the periplasm preparation protocol that we will perform tomorrow

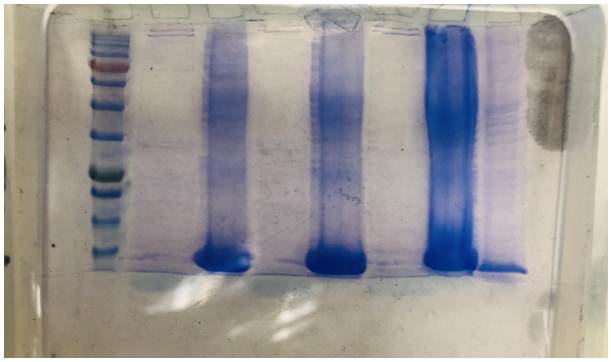
THURSDAY, 7/25/2019

- Measured OD_{600} of the overnight cultures, and found them all to be around 1.6
- Performed periplasmic isolation for all of the overnight cultures. Retained the periplasm samples in the fridge to run on a gel later
- Resuspended the remaining pellet and isolated the cytoplasmic fraction using freeze-thaw cycles and lysozyme treatment. Retained the cytoplasm samples in the fridge to run on a gel later
- Also prepared 'whole cell lysate' samples for each culture by performing the freeze-thaw cycles and lysozyme treatment only. Retained the whole cell lysate samples in the fridge to run on a gel later
- Set up a digestion and ligation of empty pSB1A3 for use as an empty vector control. Left ligation overnight at room temperature.
- Practiced SDS-PAGE gel making techniques and created a stockpile of pre-made gels
- Cleaned out fridge
- Attended the Life Sciences Innovation Hub Community Barbeque where we were able to network with many members of the life sciences community
- Finally had an education subgroup meeting (yay!)

FRIDAY, 7/26/2019

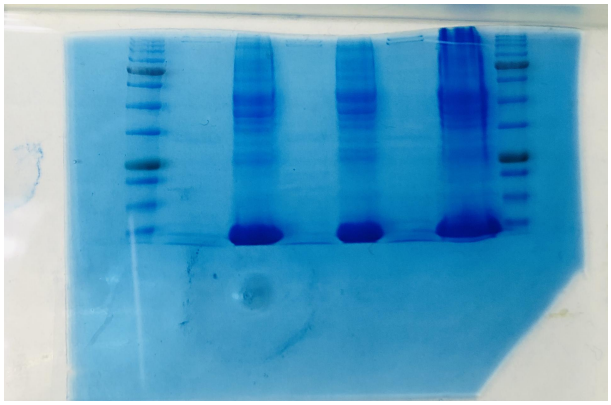
- Sonicated samples of the induced and uninduced No SP 6GIX construct cultures as well as the BL21 no transformation control. Ran on SDS-PAGE gels with B-mercaptoethanol. Each gel was stained with a different coomassie staining solution to determine which is the most effective. During this process, we learned that Coomassie G-250 and R-250 vary in their qualities and destaining time. In the future, we will use Coomassie R-250 to make our stain.

IMG_2107.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction, spillover
Stained with Coomassie R-250 solution

IMG_2109.jpg



% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction, protein standard
Stained with Coomassie G-250 solution

- Transformed yesterday's empty pSB1A3 empty vector control ligation into chemically competent DH5a and BL21

SATURDAY, 7/27/2019

- Hosted the JulyGEM meetup
- There was growth for the pSB1A3 empty vector control transformed into DH5a, but not in BL21
- Made overnights for the pSB1A3 empty vector control in DH5a
- Replated Friday's transformation of the pSB1A3 empty vector controlinto BL21

SUNDAY, 7/28/2019

- Miniprepped pSB1A3 empty vector control, then transformed into BL21
- Made overnights for repeating the SDS-PAGE gel for the induced and uninduced No SP soluble vs. insoluble fraction analysis, as well as DH5a for making chemically competent cells and pSB1A3 empty vector control in BL21

Week 12 (July 22nd-28th) WEEKLY SUMMARY:

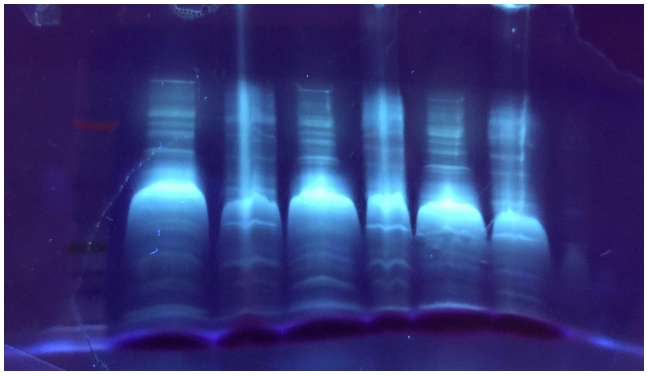
At the beginning of the week, we got evidence that our 6GIX protein was successfully produced both in induced and uninduced cultures. However, when we attempted to replicate our results we ran into troubles regarding the staining and destaining of the gels. After multiple trials of the SDS-PAGE gel we ultimately discovered that the coomassie gel staining solution using Coomassie R-250 is the best at staining the gel, and will be using this stain for future gels.

MONDAY, 7/29/2019

- Repeated the miniprep for the pSB1A3 empty vector control, then transformed into BL21

- Subcultured the DH5a overnight culture 1:50 for making chemically competent cells, but these subcultures didn't grow throughout the day. We'll have to try again tomorrow
- Ran 12% SDS-PAGE gels for all of the signal peptide samples collected last week. Stained using used Coomassie that we got from the Lab Tech Deirdre, but this stain didn't work. Placed in dH_2O overnight to re-stain tomorrow
- Ran soluble and insoluble fractions of induced and uninduced 'no signal peptide' constructs as well as BL21 on a 12% SDS-PAGE gel containing trichloroethanol. Dr. Turner works with this compound as it is useful for visualizing proteins that contain tryptophan residues after UV-exposure. Our protein is not particularly tryptophan-rich, but we thought we would give it a try. The results are not extremely clear, but it is possible that there is a faint band around 20 kDa for the soluble fractions of the induced and uninduced 'no signal peptide' constructs

IMG_2119.jpg



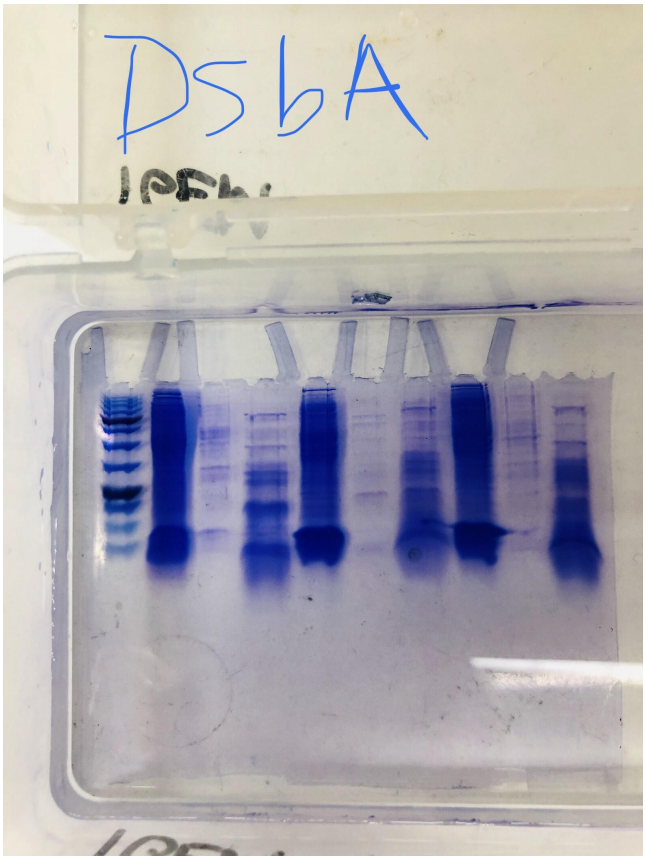
% SDS-PAGE, NEB Colour Protein Standard, under UV light
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction

- Subcultured the 'no signal peptide' and BL21 overnight cultures 1:100 and grew to OD_{600} = 0.6. Kept in the fridge until the end of the day, then induced one of the 'no signal peptide' cultures using 100mM IPTG. Incubated overnight, shaking.

TUESDAY, 7/30/2019

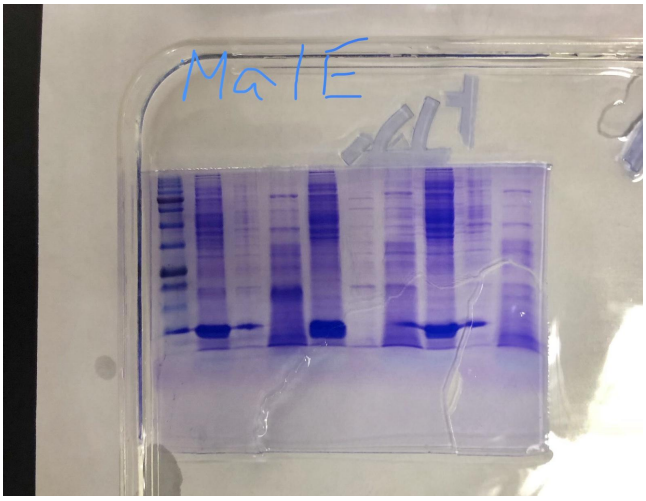
- Re-stained yesterday's signal peptide analysis gels using newly-made Coomassie R-250 stain. Unfortunately, there is no visible difference between the experimental and control samples for any of the gels. In addition, the periplasm fractions do have very high concentration of proteins for visualization. It is possible that there was cross-contamination in the subculturing step or that our induction didn't work properly. We will need to repeat.

IMG_2130.jpg



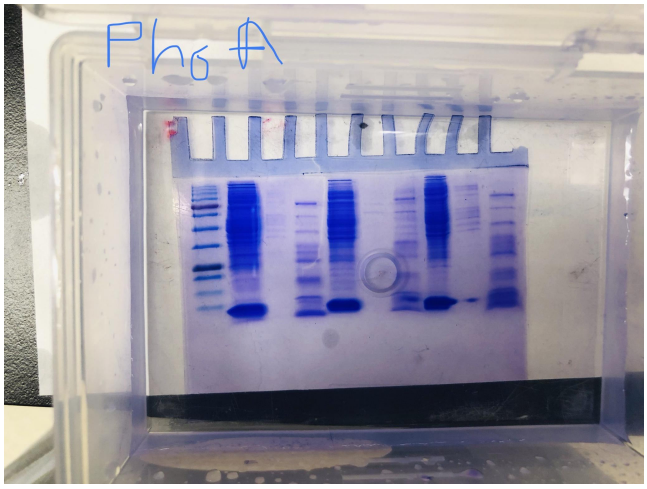
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, DsbA construct whole cell lysate, DsbA construct periplasmic fraction, DsbA construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

IMG_2137.jpg



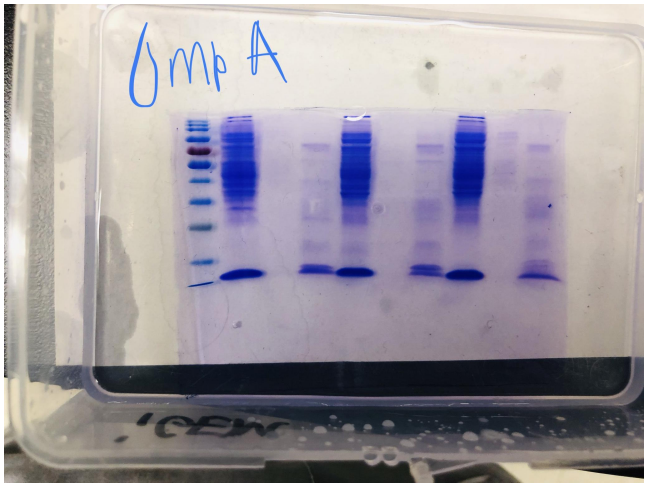
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, MalE construct whole cell lysate, MalE construct periplasmic fraction, MalE construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

IMG_2138.jpg



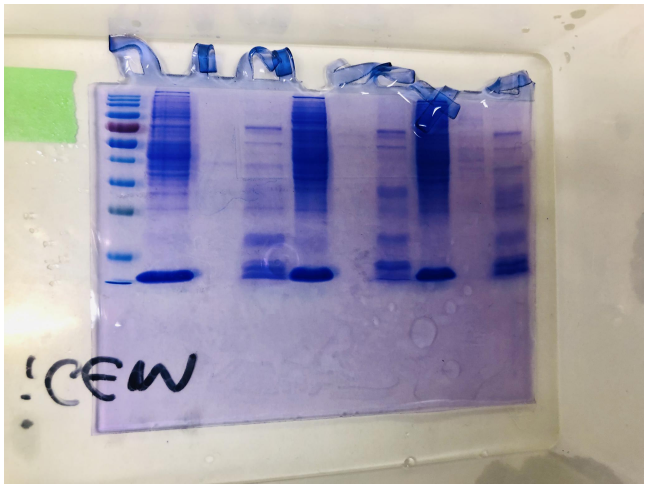
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, PhoA construct whole cell lysate, PhoA construct periplasmic fraction, PhoA construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

IMG_2142.jpg



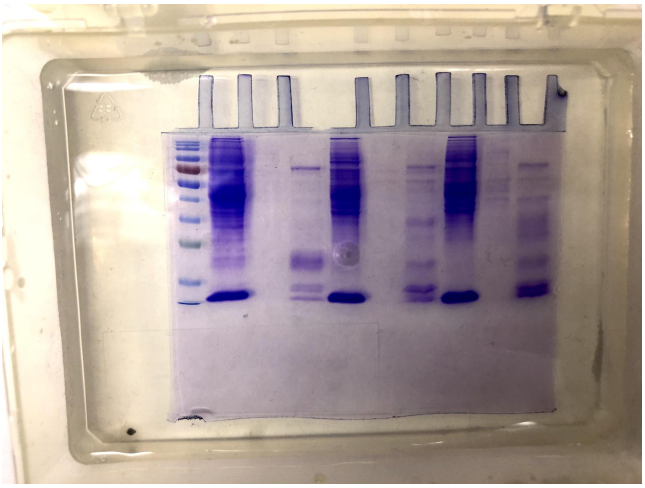
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, OmpA construct whole cell lysate, OmpA construct periplasmic fraction, OmpA construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

IMG_2145.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, YcbK construct whole cell lysate, YcbK construct periplasmic fraction, YcbK construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

IMG_2148.jpg



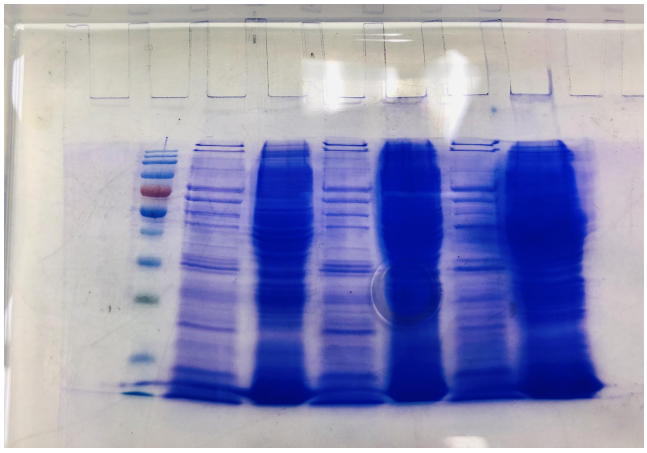
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, TorA construct whole cell lysate, TorA construct periplasmic fraction, TorA construct cytoplasmic fraction, no SP control construct whole cell lysate, no SP control construct periplasmic fraction, no SP control construct cytoplasmic fraction, no transformation control whole cell lysate, no transformation control periplasmic fraction, no transformation control cytoplasmic fraction

- The subcultures that were induced overnight were grown at 37 degrees Celcius rather than 30 degrees Celcius as they were supposed to. We proceeded with processing of these cultures, though it is likely that significant cell death or formation of inclusion bodies occurred.
- Processed overnight induced cultures by performing centrifugation, freeze-thaw cycles, and lysozyme treatment. Sonicated 800ul of each of the cultures, and seperated the soluble and insoluble fractions. Ran samples with B-mercaptoethanol on a 12% SDS-PAGE gel for ~40 mins. Stained using the new Coomassie R-250 stain for ~45 mins, then placed in dH_2O overnight for destaining tomorrow.
- There was growth for yesterday's BL21 transformation of pSB1A3 empty vector control
- Made overnights for the pSB1A3 empty vector control in BL21, 'no signal peptide' construct, and BL21
- Subcultured the DH5a overnight culture 1:50 for making chemically competent cells, but these subcultures didn't grow throughout the day once again. We have concuded that there may be something wrong with our glycerol stock, so we made streak plates with this.
- Helped the other subgroup with some of their experiments
- Had a meeting for our 'iGEM Road' parody

WEDNESDAY, 7/31/2019

- Took team pictures in a lovely wild canola field
- De-stained yesterday's SDS-PAGE gel. Insoluble fractions are too concentrated to properly analyze. Supernatant fractions show no differences between the different samples except for a band present at ~32 kDa for the induced and uninduced 'no signal peptide' soluble fractions that is absent for the BL21 control. Upon literature review, we were able to determine that this is the ampicillin resistance protein. This is good confirmation that there was no cross-contamination between experimental and control cultures this time.

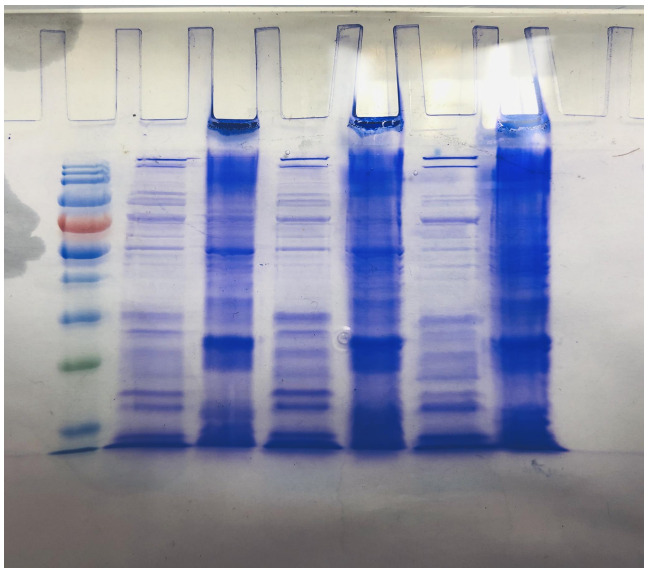
IMG_2313.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction

- Ran another set of these samples on a 12% SDS-PAGE gel without B-mercaptoethanol for ~40 mins. Stained using the new Coomassie R-250 stain for ~45 mins, and destained. Similar results were observed for this gel.

IMG_2316.jpg



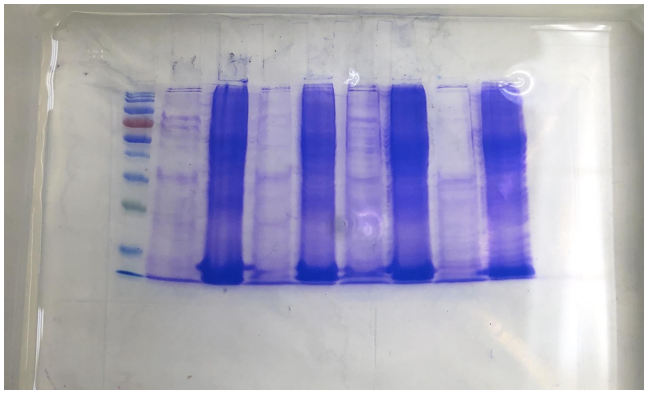
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction

- Since we didn't see a significant amount of our protein, we likely have an issue with our induction protocol. The IPTG that was used is very old and has been freeze-thawed many times. Due to this, we made new IPTG stocks and aliquoted for future use.
- Made a glycerol stock from the overnight of the pSB1A3 empty vector control in BL21
- Met with Magda and Callista from Mindfuel to talk about the geekStarter program. We discussed what resources would be helpful for us, as well as what we can contribute to their program.
- Using the overnights made yesterday, subcultured 1:100 into 50ml of media. Grew at 37 degrees Celcius until OD_{600} = 0.5 and induced one of the 'no signal peptide' cultures and the pSB1A3 empty vector control culture with new 100mM IPTG
- Using the overnight made yesterday, subcultured the 'no signal peptide' construct 1:100 into 250ml of media. Grew at 37 degrees Celcius until OD_{600} = 0.5 and induced with new 100mM IPTG. Following processing tomorrow this, cell lysate from this sample will be used in the preliminary emulsion experiments.
- Made overnights from the DH5a streak plate
- Sara joined the blue man group

THURSDAY, 8/1/2019

- Processed samples from the cultures that were induced overnight and ran on a 12% SDS-PAGE gel as per protocols. The gel didn't run very well, but results were once again not as expected.

IMG_2374.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, induced soluble fraction, induced unsoluble fraction, uninduced soluble fraction, uninduced insoluble fraction, empty pSB1A3 control soluble fraction, empty pSB1A3 control insoluble fraction, no transformation control soluble fraction, no transformation control insoluble fraction

- Made more chemically competent DH5a
- Prepared a 250mL culture of the no-SP 6GIX construct and induced overnight, then ran a freeze-thaw cycle and resuspended in 50mM phosphate buffer pH 7.8
- attempted to follow the emulsion protocol in [[link of paper]] using the green oil provided by Milligan Biofuels
    - a few changes were made to the procedure: instead of mineral oil, the green oil provided by Milligan Biofuels was used
    - we didn't have a tissue homogenizer, so we vortexed the mixture at max speed for 2 minutes instead to create the emulsion
- emulsion created a foam that could not be separated out completely by centrifugation
    - as per Sebastian's recommendation, we heated the samples overnight at 37 degrees to better separate the mixture

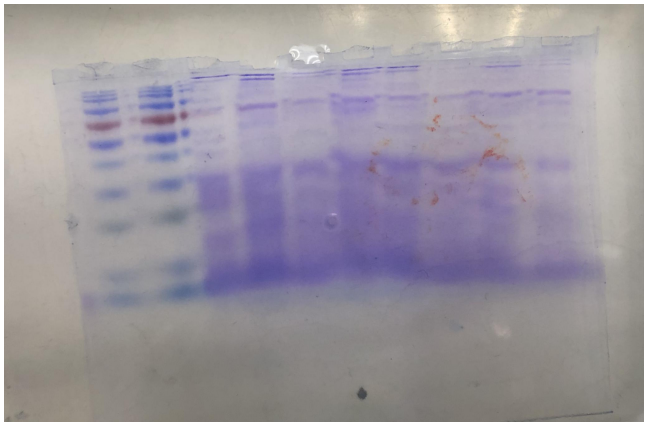
FRIDAY, 8/2/2019

- The emulsion system did not separate in the 37 degree incubator overnight
    - Did not separate after a 10 minute spin in the centrifuge either
- subculturing and induction
- Ran gel with same samples from yesterday
- "HP" meeting at mooses...

SATURDAY, 8/3/2019

- Stained and destained gel from yesterday. The gel didn't run properly, which can likely be attributed to using the SDS-running buffer too many times. We will try again on Tuesday.

IMG_2375.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, protein standard, BL21 control, empty pSB1A3 control, uninduced no SP construct, induced no SP construct, uninduced no SP construct, BL21 control, empty pSB1A3 control, induced no SP construct

Week 13 (July 29nd- August 3rd) WEEKLY SUMMARY:

This week we continued trying to replicate our gel results from last week which had showed successful expression of the 6GIX protein. However, even after using the correct staining compound, the gels did not convey a significant amount of protein was being produced. We also ran samples from the periplasmic extractions from the signal peptide constructs, but again did not see any significant results. To attempt to figure out the source of our troubles regarding protein expression, we obtained and used new tubes of IPTG when inducing, and discarded the old stocks of IPTG. Additionally, we also started trying to create an emulsion system, but did not obtain any promising results. Next week, we will continue troubleshooting our protein expression and emulsion protocols.

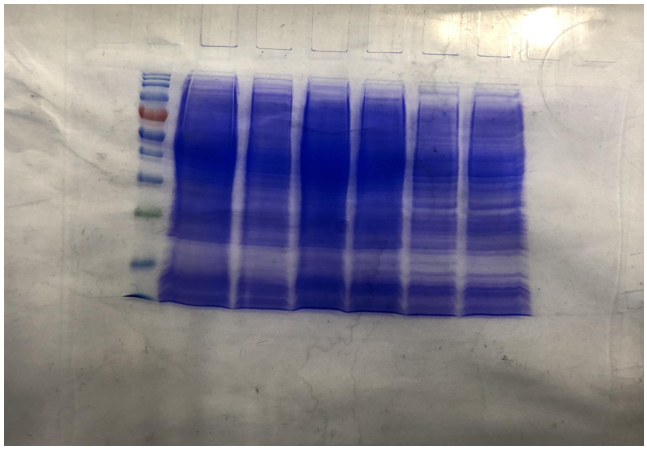
MONDAY, 8/5/2019

- Heritage Day (No iGEM)

TUESDAY, 8/6/2019

- Followed up with iGEM HQ regarding our requested parts
- Tried the emulsion experiment using the cell lysate prepared last thursday
    - This time, did not use the surfactants and used green oil prepared by mixing spinach-ethanol with oil
    - This emulsion mixture separated out well by centrifuge, but did not show any difference in OD of the aqueous layer between the cell lysate and buffer only control
        - This result is to be expected, as the gel run on thursday shows that our protein was not sufficiently expressed in this culture
- Resuspended the pellets from Friday's subculture in 500ul of 1X SDS loading dye, and then sonicated the samples. Spun down and ran on a 12% SDS-PAGE gel. Once again, there was no observable difference between experimental and control lanes:

IMG_2369.jpg



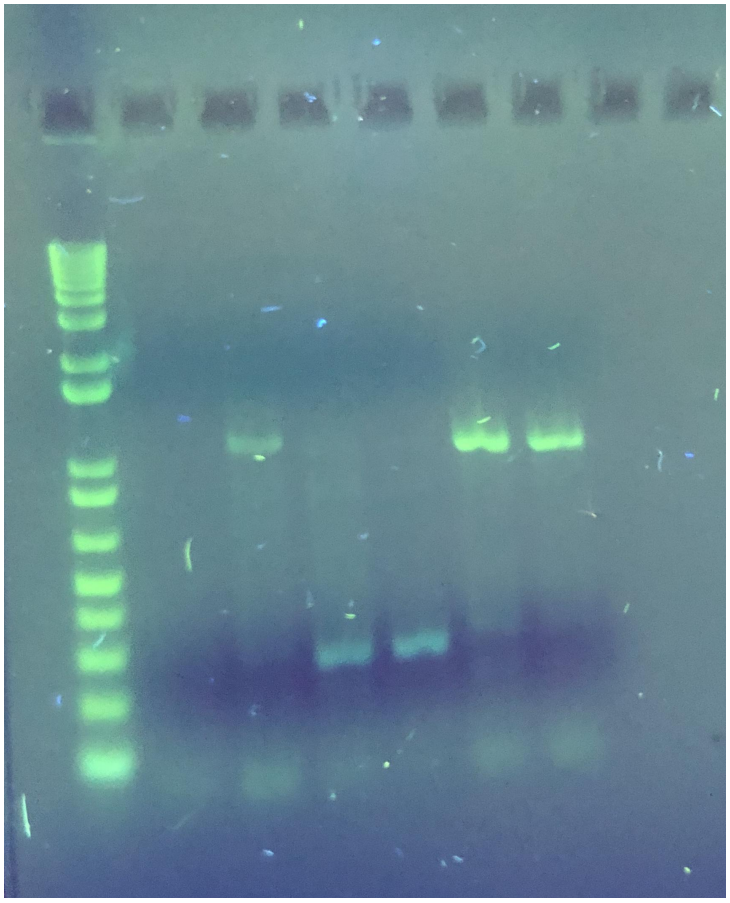
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, uninduced BL21 control, induced BL21 control, uninduced empty-pSB1A3 control, induced empty-pSB1A3 control, uninduced No-SP construct, induced No-SP construct

- We have to troubleshoot the reason for the lack of 6GIX expression. Tomorrow we will test our induction protocol using a postive control from Marija's old lab and different IPTG samples. In order to do so, overnights were made today.
- We are also concerned about contamination in our glycerol stocks, so we set up a cPCR that will run overnight to check
- Made 10X and 1X stocks of SDS-PAGE running buffer
- Team meeting

WEDNESDAY, 8/7/2019

- Ordered the modified 6GIX sequence (called 'modGIX' construct from here on) that Andrew generated via modelling from Twist Bioscience
- Ran the cPCR samples on a 1% gel. It appears that our BL21 glycerol stock is likely contaminated

IMG_2377.jpg



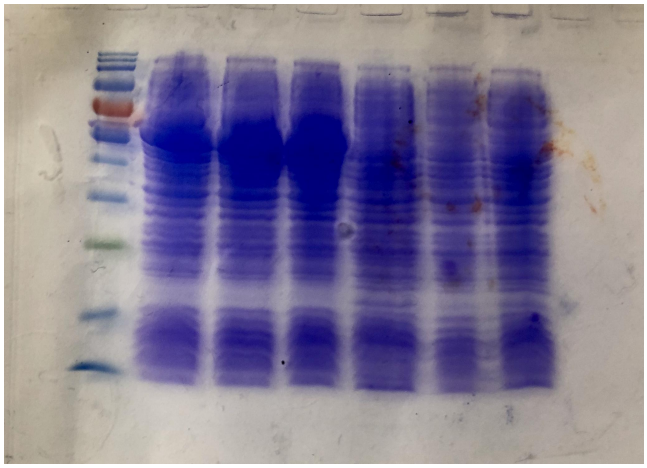
1% agarose gel, Invitrogen 1kb+ ladder
Lane order: Ladder, Bl21 glycerol stock, Bl21 glycerol stock, empty pSB1A3 glycerol stock, pSB1A3 glycerol stock, no SP construct glycerol stock, no SP construct glycerol stock

- Subcultured no signal peptide, BL21, postive control, and pSB1A3 empty vector control cultures, and induced for three hours with different samples of IPTG in order to test if the induction is the problem. Spun down and left pellets at -80 degrees celcius overnight
    - These will be used for SDS-PAGE analysis as well as protein purification
- Had a meeting to plan wiki content and plan the remainder of our workflow this summer and fall

THURSDAY, 8/8/2019

- Created all of the necessary buffers for protein purification
- Began preparing samples from yesterday for purification
- Processed the induced pellets from yesterday and ran a 12% SDS-PAGE gel in order to test if IPTG was the issue for protein expression. Results from this gel suggest that the IPTG was not the problem, since the postive control (a construct from Marija's old lab) show lots of protein production. We are also able to see a very faint band for the experimental sample (and the BL21 control due to the contamination we detected earlier) that corresponds to No SP 6GIX. Marija suggested that we run smaller amounts of these samples again, since this gel is difficult to interpret. This will be done tomorrow.

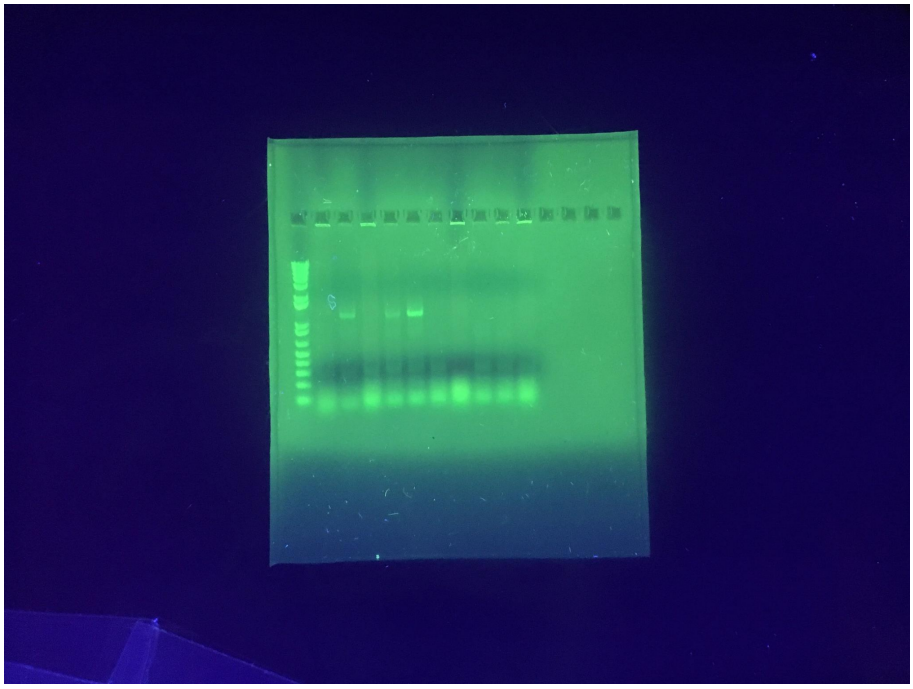
IMG_2421.jpg



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, postive control with IPTG sample #1 (from Marija), positive control with IPTG sample #2 (ours), No SP 6GIX with IPTG sample #1 (from Marija - likely not the correct sample or overspill), No SP 6GIX with IPTG sample #2 (ours), empty pSB1A3 control, BL21 control (later discovered to be contaminated)

- Sara did goil stuff
- Ran cPCR for colonies from BL21 streak plates and made a masterplate. A few of the colonies showed a band at 1.2kb, confirming contamination. Glycerol stocks will be made from the masterplate for colonies that did not produce any bands

Image from iOS (20).jpg



1% agarose gel, Invitrogen 1kb+ ladder
Lane order: Ladder, colonies 1-5 from original BL21 streak plate, colonies 1-5 from re-streaked BL21

FRIDAY, 8/9/2019

- Re-did SDS-PAGE gel from yesterday, but ran 5 microlitre samples instead of 10 microlitres. This gel showed the same things as yesterday's
- Performed a purification from cultures with empty pSB1SA3 (control) and No SP 6GIX. Ran fractions on a 12% SDS-PAGE gel, which will be stained tomorrow
- Cassie MCATed

SATURDAY, 8/10/2019

- Sara came in to stain yesterday's gel. She may have also left an entire glycerol stocks box out on the counter

Week 14 (August 5th-10th) WEEKLY SUMMARY:

This week we began our first attempt at protein purification using a Ni-NTA column. We also modified different components of our current protein expression method to figure out where we have been going wrong with protein expression. We tested two different IPTG samples (from another lab and our stocks) and also ran a positive control (a construct from our RA, Marija's old lab) to test our protein expression protocol's validity. We also detected contamination in our BL21 glycerol stocks, so made a new glycerol stock after confirming with cPCR.

MONDAY, 8/12/2019

- Restained the gels from the purification samples that Sara stained on Saturday. Samples appear to be too dilute to visualize the protein. We will run another gel with twice as much of each sample loaded tomorrow
- Reran the gel from Friday and it displayed the same results (Marija's positive control and our inductions). A faint band was present for the experimental samples and absent from the control. The positive control shows that both the IPTG aliquots are functional.
- Made new glycerol stocks for BL21 from overnights that Chris made us from the cPCR masterplate
- Subcultured empty pSB1A3 and No SP 6GIX as per protocol (4 cultures total). Induced with either 1mM IPTG or 2mM IPTG and incubated for 18 hours at 25 degrees celcius, as the T7 promoter characterization on the iGEM registry has data that suggests that this is most efficient
- Team meeting

TUESDAY, 8/13/2019

- Began processing of samples that were induced day before, including 3x freeze-thaw, resuspension buffer, lysozyme treatment and 4 rounds of sonication
- Received the 'registru flipper' (BBa_K1467400) that we improved from the iGEM registry. It was transformed into chemically competent DH5a and plated on an LB + Chlo plate
- Re-ran the purification samples from the weekend on a 12% SDS-PAGE gel with more sample loaded. Stained and destained as per protocol, but the concentrations were still too low to visualize

WEDNESDAY, 8/14/2019

- Sonicated samples for 2 more rounds in the morning, and did protein purification protocol all day
- Made O/Ns of pSB1C3-BBa_K1467400 ('registry flipper') for miniprepping tomorrow

THURSDAY, 8/15/2019

- Made SDS-PAGE gels to run purified samples of te No SP 6GIX and pSB1A3 empty vector control (1mM and 2mM IPTG)
- Ran gel, and stained, and destained. Once again, all of the samples and controls appeared identical
- Miniprepped two different colonies of pSB1C3-BBa_K1467400 ('registry flipper') and nanodropped.
- Set up a digestion of pSB1C3-BBa_K1467400 ('registry flipper') and linearized pSB1A3. Ran on a 1% LMP agarose gel, but an error was made and these samples were lost
- Made more Coomassie Blue stain

FRIDAY, 8/16/2019

- After helping us to analyze our gel, our wonderful RA Marija made some recommendations for troubleshooting that will be conducted next week
- Repeated the digestion and ligation of pSB1C3-BBa_K1467400 ('registry flipper') and linearized pSB1A3 without running on a gel. Left to incubate at room temperature over the weekend
- Helped the other group with some cPCR
- Worked through our pipeline to figure out where the problems in protein expression are arising. Made a plan to test each step that will be implemented next week

- Made more SDS page gels and stored in fridge

SATURDAY, 8/17/2019

- Took out Bl21 streak plate

SUNDAY, 8/18/2019

Week 15 (August 12- August 18th) WEEKLY SUMMARY:

This week we focused on protein purification of the no signal peptide construct and pSB1A3 empty vector control as well as preparing the 'registry flipper' BBa_K1467400 for comparison with the flipper we created. Initially, we had thought the problem for our protein purification had been our IPTG sample, but after comparing samples with our IPTG and Marija's IPTG,we realized they were both functional. From this, we decided to experiment on the amount of IPTG added to induce the samples and compared samples with 1mM and 2mM IPTG. However, we still did not see a band of the correct size for our protein. Thus, next week we will be sending the No-SP BL21 glycerol stock for sequencing to check for any mutations. In regards to the 'registry flipper', the week was spent following the procedures to get the flipper into pSB1A3 in order to compare it with the flipper we have been using.

MONDAY, 8/19/2019

- Sara was unable to make overnights on Sunday :(
- Made overnights of pSB1A3 empty vector control and 'No SP' constructs
- Transformed pSB1A3-BBa_K1467400 ('registry flipper') into DH5a, and previously sequence-confirmed 'No SP' construct from the miniprepped stock into BL21

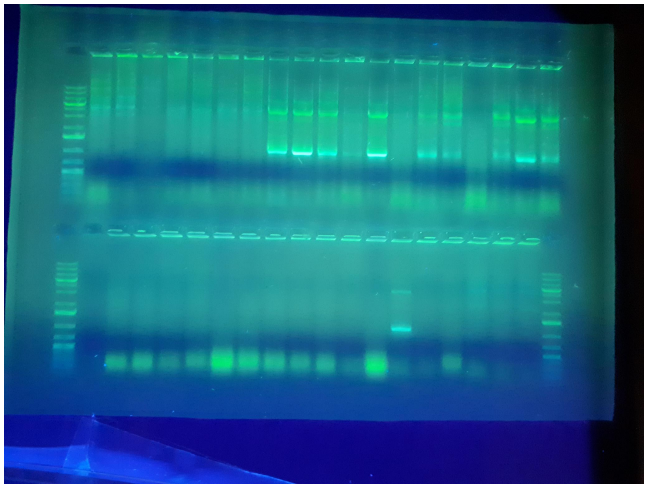
TUESDAY, 8/20/2019

- Miniprepped 'No SP' constructs made from glycerol stock streak plate and sent to sequencing
- cPCR of pSB1A3-BBa_K1467400 ('registry flipper'), and No SP BL21 transformation
    - Both were unsuccessful :(
- Had no growth in one of the 'No SP' BL21 overnights - unfortunately, we had to use the one with growth to miniprep and sequence, so we could not subculture today
- Remade overnights

WEDNESDAY, 8/21/2019

- Repeated cPCR from yesterday
    - Gel shows no bands for pSB1A3-BBa_K1467400 ('registry flipper') so we will perform a new digestion and ligation into pSB1A3 the next day
    - Gel shows correct band size for 'No SP' construct at ~853 bp, but there is also a larger band present at ~5kb which is suspicious (might be unspecific binding)

cPCR gel.jpg



1% agarose gel with cPCR of No SP BL21 transformation (wells 2-20), pSB1A3-BBa_K1467400 ('registry flipper') (last 3 wells before ladder)

- Subcultured 'No SP' construct from old streak plate, new streak plate, and pSB1A3 empty vector control
    - Tried three different induction times: 8 hours, 17 hours, and 24 hours

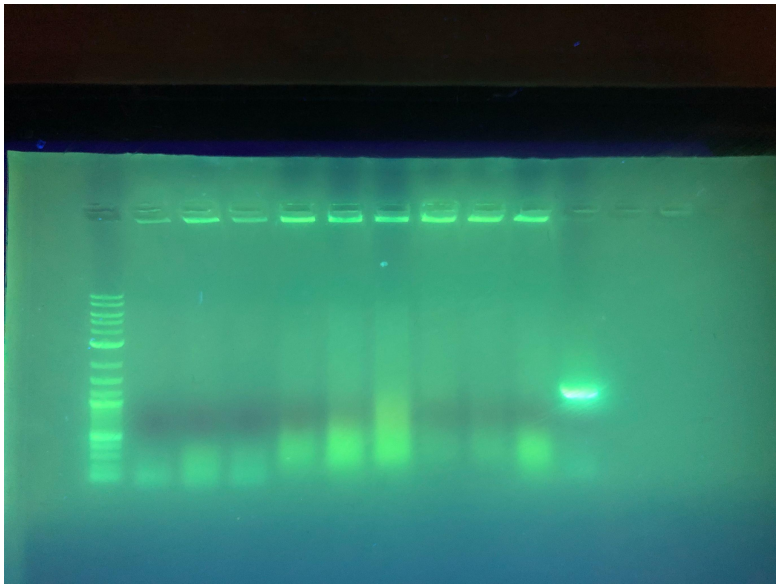
THURSDAY, 8/22/2019

- Got sequencing results for 'No SP' construct new streak plate which indicated mutations and a frameshift that led to a premature start and stop codon
    - New plan: made O/Ns for 'No SP' construct DH5a glycerol stock, 'No SP' construct new BL21 transformation (colonies 1, 8, 12), and another streak plate of 'No SP' construct DH5a to miniprep next day and send for sequencing
- Aso made O/N for 'No SP' construct new transformation BL21, and pSB1A3 empty vector control for subculturing tomorrow
- Performed a new digestion and ligation of BBa_K1467400 ('registry flipper') into pSB1A3

FRIDAY, 8/23/2019

- Miniprep of 'No SP' construct DH5a glycerol stock, 'No SP' construct new BL21 transformation (colonies 1, 8, 12) and sent colony 12 for sequencing
- Also sent 'No SP' construct original miniprep for sequencing
- Did another ligation of the 'No SP' construct into pSB1A3
- Subcultured 'No SP' construct and pSB1A3 empty vector control (8hrs and 16 hrs), will freeze pellet tomorrow after induction times. We did 2:50 mL subculture, put in shaker at 37 Celcius, induced at ~0.4 OD, once induced placed in shaker at 25 degrees Celcius
- Did cPCR of 'No SP' construct DH5a streak plate. The correct band at ~ 850 bp was only found in the one lane. We will make O/N for that on Sunday, and miniprep and send for sequencing Monday.

Image from iOS (1).jpg



1% agarose gel with cPCR of colonies on streak plate of DH5a

SUNDAY, 8/25/2019

- Made overnights of new transformation of 'No SP' construct and pSB1A3 empty vector control
- Transformed ligations from Friday

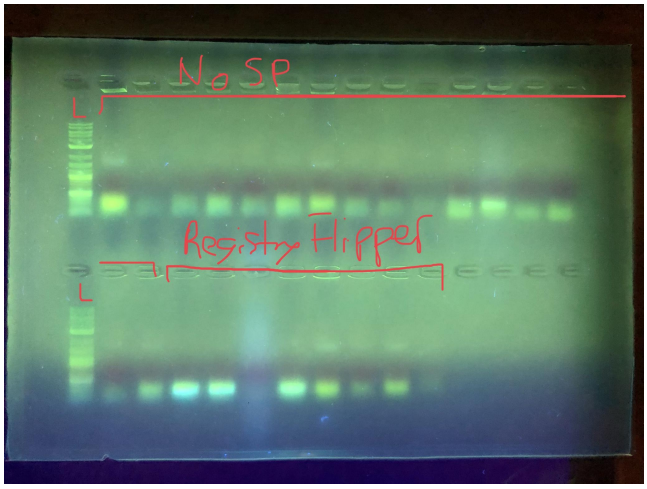
Week 16 (August 19th - 24th) WEEKLY SUMMARY:

This week we continued to troubleshoot our protein purification protocol, and sent a sample of No signal peptide construct from a new BL21 streak plate to sequencing. In regards to protein purification, we subcultured and induced 1mM IPTG for No SP old plate, No SP new streak plate, and pSB1A3 empty vector control. However, we tested the OD and processed samples at different induction times including after 8 hours, 16 hours, and 24 hours to see if there was an optimum induction period for protein expression. Our efforts in protein expression halted on Thursday, when our sequencing results returned showing mutations which caused a frameshift interfering with protein production. We finally discovered the source of all our issues with protein production and purification! Now the plan is to send the original miniprepped sample of No SP construct, No SP in DH5a, and No SP in new BL21 cells to sequencing to see if the mutations are present at all stages of our experimental process and go from there.

MONDAY, 8/26/2019

- Processed and purified the 'No SP' construct and pSB1A3 empty vector control pellets from last Friday as per protocol
- Ran cPCR for 'No SP' construct new ligation and pSB1A3-BBa_K1467400 ('registry flipper'). None of the lanes showed bands of the correct size

IMG_2647.jpg



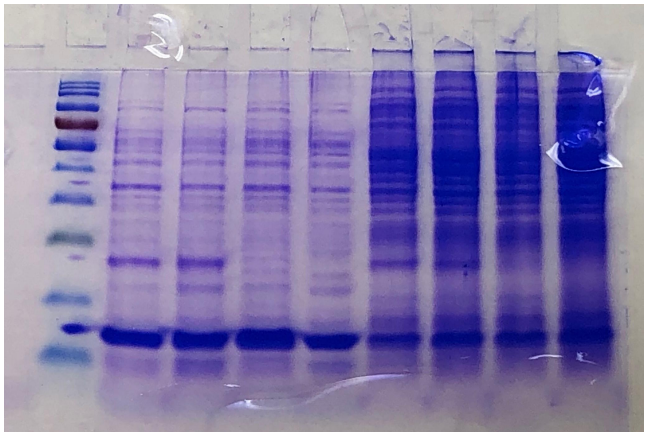
1% agarose gel with cPCR of colonies of 'No SP' construct and pSB1A3-BBa_K1467400 ('registry flipper')

- Subcultured the 'No SP' construct and pSB1A3 empty vector control overnights 2:50 as per protocol and induced for 8 hours when OD_{600} = 0.4 as per protocol. Spun down and placed pellet into the freezer overnight
- Re-transformed as well as re-plated previous transformations

TUESDAY, 8/27/2019

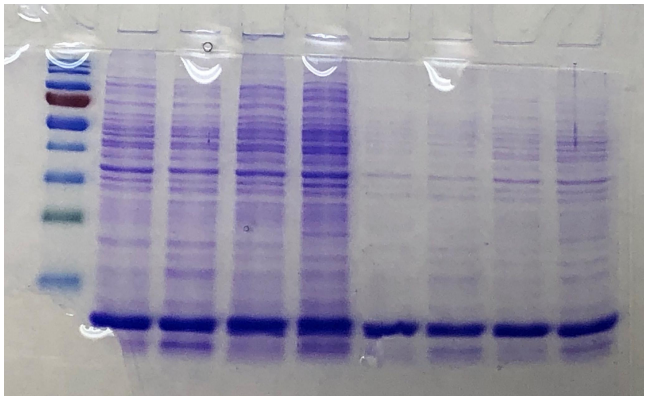
- Prepared the purification samples from yesterday and ran on four 12% SDS-PAGE gels. We were (finally) able to see a band at ~21 kDa for both the 8 hour and 16 hour inductions of 'No SP' construct samples, and not for the pSB1A3 empty vector controls! Our solution after troubleshooting seems to have worked, and we were able to purify His-tagged 6GIX on the column (4th gel picture). We may need to optimize our elution steps

IMG_2663.JPG



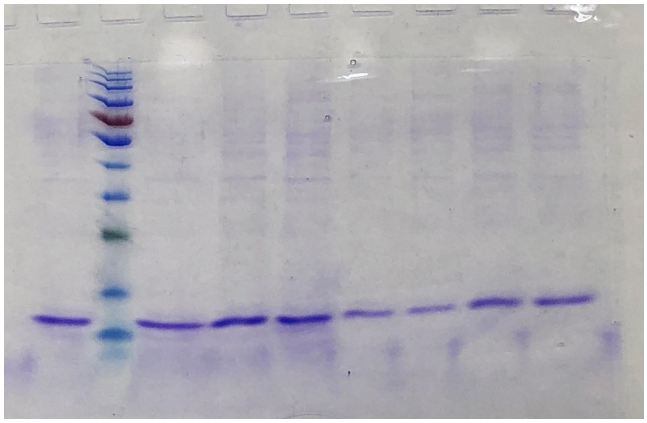
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, 8 hour induction 'No SP' construct whole cell lysate soluble fraction, 16 hour induction 'No SP' construct whole cell lysate soluble fraction, 8 hour induction pSB1A3 empty vector control whole cell lysate soluble fraction, 16 hour induction pSB1A3 empty vector control whole cell lysate soluble fraction, 8 hour induction 'No SP' construct whole cell lysate insoluble fraction, 16 hour induction 'No SP' construct whole cell lysate insoluble fraction, 8 hour induction pSB1A3 empty vector controlwhole cell lysate insoluble fraction, 16 hour induction pSB1A3 empty vector control whole cell lysate insoluble fraction

IMG_2664.JPG



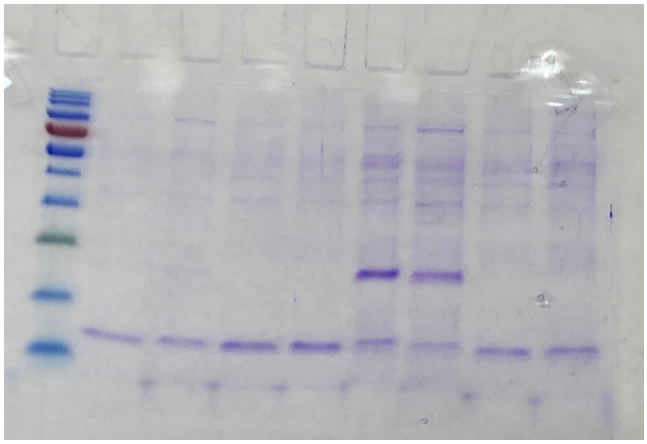
12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, 8 hour induction 'No SP' construct post-loading fraction, 16 hour induction 'No SP' construct post-loading fraction, 8 hour induction pSB1A3 empty vector control post-loading fraction, 16 hour induction pSB1A3 empty vector control post-loading fraction, 8 hour induction 'No SP' construct whole cell wash 1 fraction, 16 hour induction 'No SP' construct wash 1 fraction, 8 hour induction pSB1A3 empty vector control wash 1 fraction, 16 hour induction pSB1A3 empty vector control wash 1 fraction

IMG_2666.JPG



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, 8 hour induction 'No SP' construct wash 2 fraction, 16 hour induction 'No SP' construct wash 2 fraction, 8 hour induction pSB1A3 empty vector control wash 2 fraction, 16 hour induction pSB1A3 empty vector control wash 2 fraction, 8 hour induction 'No SP' construct wash 3 fraction, 16 hour induction 'No SP' construct wash 3 fraction, 8 hour induction pSB1A3 empty vector control wash 3 fraction, 16 hour induction pSB1A3 empty vector control wash 3 fraction

IMG_2668.JPG



12% SDS-PAGE, NEB Colour Protein Standard
Lane order (left to right): protein standard, 8 hour induction 'No SP' construct elution 1 fraction, 16 hour induction 'No SP' construct elution 1 fraction, 8 hour induction pSB1A3 empty vector control elution 1 fraction, 16 hour induction pSB1A3 empty vector control elution 1 fraction, 8 hour induction 'No SP' construct elution 2 fraction, 16 hour induction 'No SP' construct elution 2 fraction, 8 hour induction pSB1A3 empty vector control elution 2 fraction, 16 hour induction pSB1A3 empty vector control elution 2 fraction

- Recieved sequencing results that confirmed that our miniprepped stocks and newly-transformed 'No SP' construct BL21 (that we purified the protein above from) has the correct sequence and no frameshift mutation
- Repeated cPCR of 'No SP' construct new ligation and pSB1A3-BBa_K1467400 ('registry flipper'). There were some bands of the correct size for the 'No SP' construct new ligation (which is now unnecessary) but none for pSB1A3-BBa_K1467400 ('registry flipper')
- Digested and ligated our improved RFP flipper into pSB1C3 as an alternative approach for characterizing this improved part and comparing it to the old flipper pSB1A3-BBa_K1467400 ('registry flipper')

- Had an 'iGEM Road' parody video meeting to storyboard :)
- Presenters (including our own superb Sravya) worked on our presentation

WEDNESDAY, 8/28/2019

- Transformed yesterday's ligations into chemically competent DH5a and plated
- Subcultured 2:50mL in LB with Ampicilin for all signal peptide constructs (OmpA, PhoA, YcbK, TorA, MalE, DsbA), No SP, and pSB1A3 empty vector control. Induced with 1mM IPTG when OD_{600} = ~0.4 and left shaking at 25 degrees Celcius for 8 hours.
- Made glycerol stock of the good No SP construct in BL21
- Began invesigating procedures for our Bacteria night even

THURSDAY, 8/29/2019

- Pelleted and processed the cultures from yesterday placed in the fridge

FRIDAY, 8/30/2019

- End of summer lab clean-up and organization
- End of summer team meeting

Week 17 (August 26th - 30th) WEEKLY SUMMARY:

We finally gained more evidence of successful protein expression after discovering the mutation and troubleshooting accordingly, and were able to create more replicates. We are still in the process of obtaining results for the periplasmic isolations from the signal peptide constructs, and will continue to work towards these in the weeks to come. We also started preparing for the comparison of our improved RFP flipper to the registry flipper (pSB1A3-BBa_K1467400) by attempting to clone the parts into the same type of plasmid (pSB1A3 or pSB1C3).

MONDAY, 9/2/2019

- Labour Day (no iGEM)
- Wrote CMDRT grant application

WEDNESDAY, 9/4/2019

- Finished sonication of cell lysate signal peptide samples
- Loaded periplasmic samples on SDS PAGE gel and stained
- Ran cPCR of one ligation colony
- Made O/Ns (6mL) of signal peptides and no signal peptide and empty control vector samples
- Started protein purification
- Digestion and ligation of improved flipper into PSB1C3, and did an ethanol precipitation

THURSDAY, 9/5/2019

- Made new signal peptide, no signal peptide, and pSB1A3 empty vector control cultures and grew to OD_{600} = 0.4, then induced. Grew overnight at 25 degrees Celcius, shaking
- Made more LB amp liquid media and LB Chlo plates

FRIDAY, 9/6/2019

- We recieved our engineered version of 6GIX (called 'ModGIX' from here on in) from Twist Bioscience. Resuspended the DNA and digested in preparation for ligation into both pSB1C3 and pSB1A3. Set up ligation and left overnight

SATURDAY, 9/7/2019

- Attempted to gather data for our improved flipper compared to the registry flipper, but the streak plates grew too slowly
- Transformed the pSB1C3-ModGIX, pSB1A3-ModGIX, and pSB1C3-improved flipper ligations into chemically competent DH5a

Week 18 (September 2nd - 8th) WEEKLY SUMMARY:

We received our engineered version of 6GIX (called ModGIX) and set up ligations into pSB1C3 and pSB1A3. We continued making more replicates of our protein purification samples, and did periplasmic extractions from our signal peptide cultures. Additionally, we attempted to gather data for our improved flipper compared to the registry flipper, but the streak plates grew too slowly, so we will attempt this again next week.

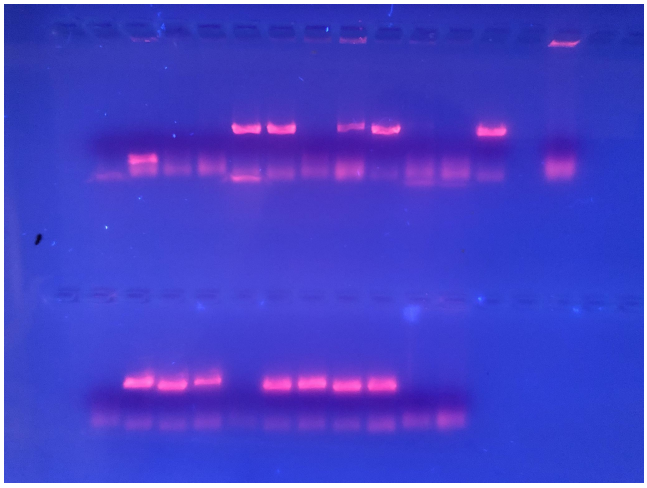
MONDAY, 9/9/2019

- Subcultured overnights for all of the signal peptide constructs, pSB1A3 empty vector control and No SP 6GIX controls. Induced when OD_{600} reached ~0.4 and grew overnight
- Streaked 2 colonies from the pSB1A3-ModGIX ligation onto new plates because satellite colonies were numerous. pSB1C3-ModGIX had no growth. pSB1C3-improved flipper had 1 colony, which will be cPCR'd tomorrow
- Prepared miniprepped pSB1C3-improved flipper (from a previous ligation) DNA for sequencing tomorrow

TUESDAY, 9/10/2019

- Processed the pSB1A3 empty vector control and No SP 6GIX cultures and placed into fridge for purification tomorrow
- Performed a periplasmic purification for each of the signal peptide cultures and the pSB1A3 empty vector control and No SP 6GIX controls.
- Ran cPCR for colonies from both streaks of pSB1A3-ModGIX. Ran on 1% agarose, but ladder didn't show up because we forgot to add the new dye to it :(

IMG_2774.jpg



1% agarose with cPCR samples for pSB1A3-ModGIX

WEDNESDAY, 9/11/2019

- Made 6ml O/Ns for colonies 6, 12 from streak 1 and 7 from streak 2 of yesterday's cPCR of pSB1A3-ModGIX

THURSDAY, 9/12/2019

- Miniprepped the overnights from yesterday
- Purified the sonicated pSB1A3 empty vector control and No SP 6GIX cultures from Tuesday

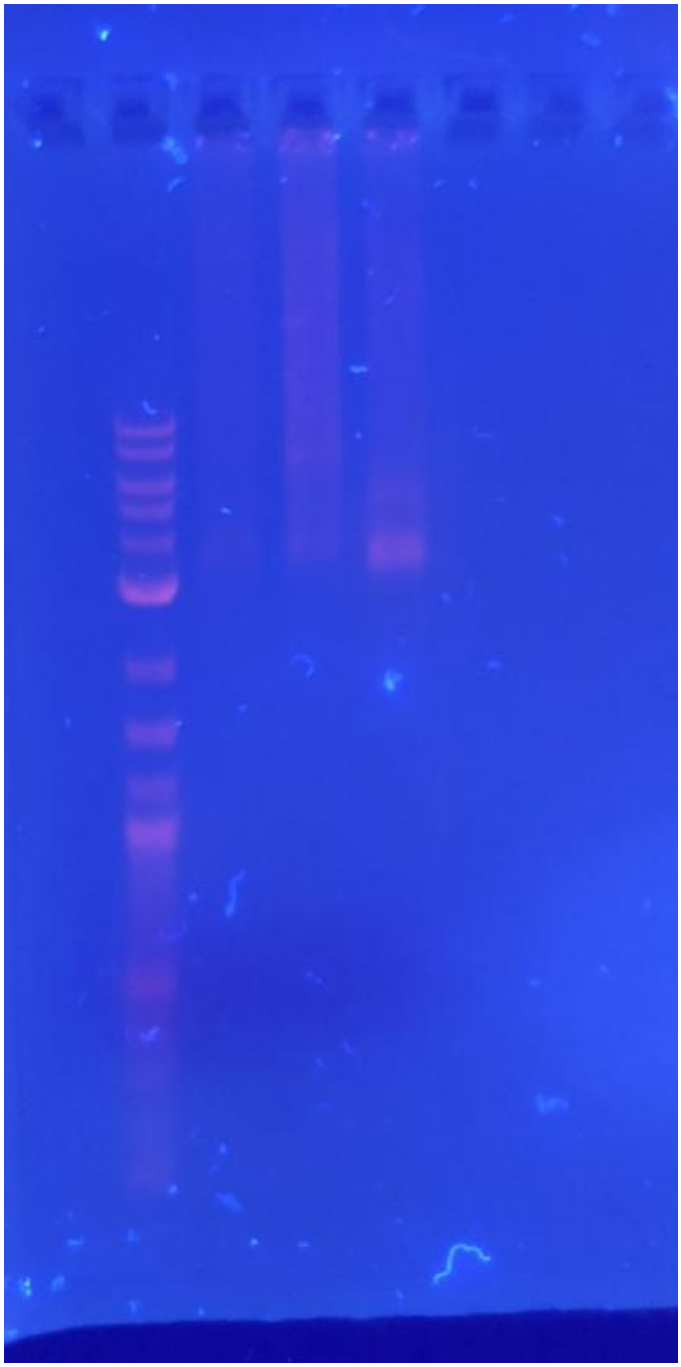
FRIDAY, 9/13/2019

- Practice presentation with Emily and Robert from FREDsense
- Finished the safety form and abstract

SATURDAY, 9/14/2019

- Performed a digest confirmation for the pSB1A3-ModGIX minipreps using BamHI and XbaI. Ran on a 1% agarose gel, but results were unclear, as the expected ~2.2kb bands were present but the expected ~600bp band was not visible

IMG_5EDDDA9BA269-1.jpeg



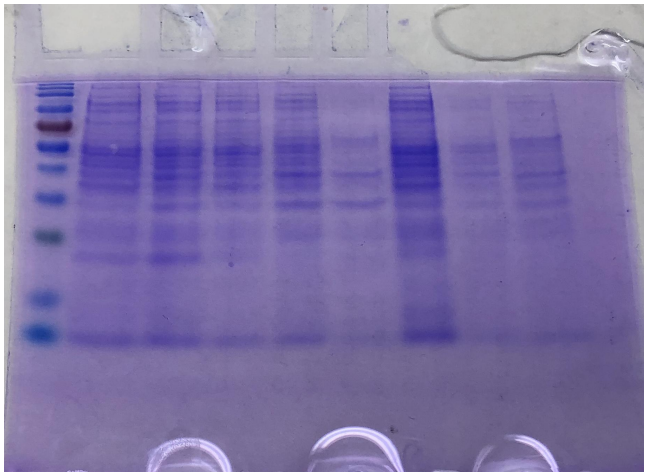
1% agarose gel, Invitrogen 1 kb+ ladder
Lane order: ladder, pSB1A3-ModGIX #6 BamHI/XbaI digest, pSB1A3-ModGIX #7 BamHI/XbaI digest, pSB1A3-ModGIX #12 BamHI/XbaI digest

- Began investigating how to characterize our improved flipper part
- Ran the periplasmic isolations of the signal peptide cultures as well as samples from the purification of the pSB1A3 empty vector control and No SP 6GIX cultures on 12% SDS-PAGE gels overnight

SUNDAY, 9/15/2019

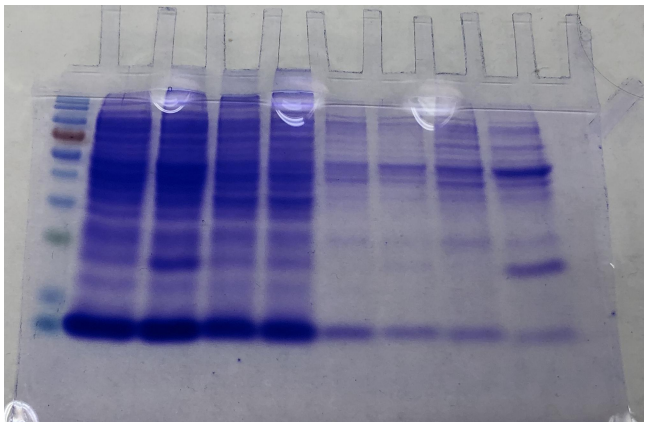
- Stained and destained the gels that were run overnight, but they were too blurry to properly visualize. Re-ran all of the samples on new gels, stained and destained as per protocol. The construct with MalE signal peptide appears to be most promising.

IMG_2831.jpg



12% SDS-PAGE gel, NEB colour protein standard
Lane order: standard, DsbA signal peptide construct, MalE signal peptide construct, OmpA signal peptide construct, PhoA signal peptide construct, YcbK signal peptide construct, TorA signal peptide construct, no signal peptide construct (control), pSB1A3 empty vector control

IMG_2834.jpg



12% SDS-PAGE gel, NEB colour protein standard
Lane order: standard, pSB1A3 empty vector control whole cell lysate, No SP 6GIX construct whole cell lysate, pSB1A3 empty vector control post-loading fraction, No SP 6GIX construct post-loading fraction, pSB1A3 empty vector control elution fraction 1, No SP 6GIX construct elution fraction 1, pSB1A3 empty vector control elution fraction 2, No SP 6GIX construct elution fraction 2

- Made overnights for pSB1C3-improved flipper
- Transformed each of the putative pSB1A3-ModGIX miniprepped samples into BL21 to get a head start while wait for sequencing confirmation

Week 19 (September 9th - 15th) WEEKLY SUMMARY:

This week we continued with creating more replicates for the periplasmic extractions from the signal peptide construct cultures. As was evident in previous weeks, the construct with MalE seems to be the most promising in secreting out protein. Additionally, we continued attempting to ligate the ModGIX construct into pSB1A3, and purified more 6GIX protein from the no signal peptide construct.

MONDAY, 9/16/2019

- Made a glycerol stock for pSB1C3-improved flipper
- Made overnights for pSB1A3-ModGIX #12 in BL21
- Prepared miniprepped DNA from pSB1A3-ModGIX #12 for sequencing

TUESDAY, 9/17/2019

- Subcultured all of the signal peptide constructs, two of each of the No SP 6GIX construct and pSB1A3 empty vector control, and one ModGIX construct. Induced when OD_{600} = 0.4
- Made a streak plate for all of the coloured bacteria that we have in preparation for Bacteria night

WEDNESDAY, 9/18/2019

- Processed the No SP 6GIX, ModGIX, and pSB1A3 empty vector control cultures, which will be sonicated tomorrow

THURSDAY, 9/19/2019

- Recieved sequencing results that told us that our putative ModGIX samples were actually No SP 6GIX...

FRIDAY, 9/20/2019

- Repeated the ModGIX digestions and ligations
- Drove to Edmonton for aGEM!

SATURDAY, 9/21/2019

- Presented at aGEM and made lots of new friends! We brought home the Best Value Proposition, Best Modelling, and Top Performance awards!
- Transformed the pSB1A3-ModGIX ligations into ccDH5a
- Nimaya presented for Falling Walls and kicked butt!

SUNDAY, 9/22/2019

- Participated in workshops at aGEM

Week 20 (September 16th - 22nd) WEEKLY SUMMARY:

This week we sent our miniprep of the ModGIX-pSB1A3 to sequencing, and discovered that it was actually our no signal peptide construct. We also processed cultures of the no signal peptide and control, which will be purified next week. Besides this, we began preparing for one of our events, Bacteria Night by making a streak plate with the colored bacteria that we have.

MONDAY, 9/23/2019

- STUFF HAPPENED THIS WEEK BUT I DIDN"T HELP I NEED YA"LL TO FILL IT IN

ASK SARA!!!

TUESDAY, 9/24/2019

- dd

WEDNESDAY, 9/25/2019

- dd

THURSDAY, 9/26/2019

- d

FRIDAY, 9/27/2019

- Ran periplasmic extractions on SDS gel, stained and destained
-

SATURDAY, 9/28/2019

- d

SUNDAY, 9/29/2019

- d

Week 21 (September 23rd - 29th) WEEKLY SUMMARY:

Stuff

MONDAY, 9/30/2019

- Miniprepped ModGIX to send to sequencing
- Made SDS Gel and ran periplasmic extractions on them
-

WEDNESDAY, 10/2/2019

- Ran test transformations for the chemically competent cells that were made

THURSDAY, 10/3/2019

- Transformed pSB1C3-ModGIX #1 and #2 (which were supposedly sent to sequencing earlier in the week) into ccBL21
- Processed and sonicated No SP 6GIX construct and pSB1A3 empty vector control samples, then purified them with 2 extra elution steps

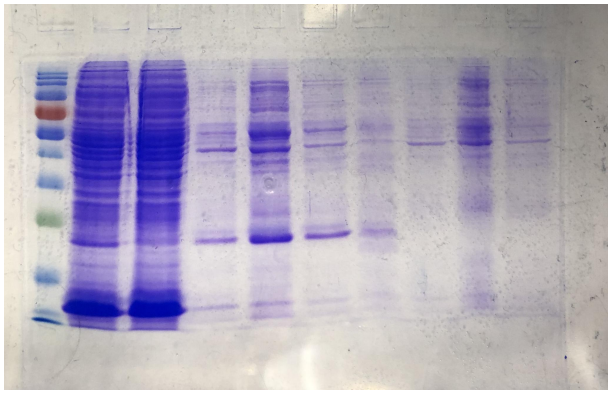
FRIDAY, 10/4/2019

- Presented for Emily and Robert of FREDsense and got lots of useful feedback
- Finished our 'iGEM Road' parody video and submitted it to iGEM HQ
- Ran periplasmic extractions for signal peptide constructs on a 12% SDS-PAGE gel and transferred to a PVDF membrane overnight in preparation for Western blotting
- Made overnights for all of the following:
    - pSB1A3 empty vector control
    - pSB1A3-No SP 6GIX
    - pSB1A3-DsbA 6GIX construct
    - pSB1A3-MalE 6GIX construct
    - pSB1A3-PhoA 6GIX construct
    - pSB1A3-OmpA 6GIX construct
    - pSB1A3-YcbK 6GIX construct
    - pSB1A3-TorA 6GIX construct
    - pSB1A3-ModGIX construct (#1)
    - pSB1A3-ModGIX construct (#2)
    - pSB1C3-BBa_K1467100
    - pSB1C3-BBa_K1467200
    - pSB1C3-BBa_K1467300

SATURDAY, 10/5/2019

- Performed Western blot for the signal peptide gel as per protocol using anti-His Tag MAb as primary antibody and anti-mouse IgG conjugated with HRP as secondary antibody. Imaged blot on gel doc, but only an indistinct, fuzzy band was present for the MalE construct. We will need to troubleshoot
- Made glycerol stocks for pSB1C3-BBa_K1467100, pSB1C3-BBa_K1467200, and pSB1C3-BBa_K1467300 in DH5a
- Prepared samples from Thursday's purification and ran them on two gels; one for staining and one for blotting. The blotting gel was transferred to a PVDF membrane overnight. The stained gel is below.

IMG_3081.jpg



12% SDS-PAGE gel, NEB colour protein standard
Lane order: standard, No SP 6GIX construct whole cell lysate, pSB1A3 empty vector control whole cell lysate, No SP 6GIX construct elution fraction 1, No SP 6GIX construct elution fraction 2, No SP 6GIX construct elution fraction 3, No SP 6GIX construct elution fraction 4, pSB1A3 empty vector control elution fraction 1, pSB1A3 empty vector control elution fraction 2, pSB1A3 empty vector control elution fraction 3

- Subcultured and induced cultures of the following:
  - pSB1A3 empty vector control
  - pSB1A3-No SP 6GIX
  - pSB1A3-DsbA 6GIX construct
  - pSB1A3-MalE 6GIX construct
  - pSB1A3-PhoA 6GIX construct
  - pSB1A3-OmpA 6GIX construct
  - pSB1A3-YcbK 6GIX construct
  - pSB1A3-TorA 6GIX construct
  - pSB1A3-ModGIX construct (#1)
  - pSB1A3-ModGIX construct (#2)

SUNDAY, 10/6/2019

- Placed the overnight western blot membrane transfer into blocking solution and left in the fridge
- Performed periplasmic extractions for each of the signal peptide cultures. This time, all 50ml of culture was processed. Placed samples into the freezer
- Began processing the No SP 6GIX, ModGIX, and pSB1A3 empty vector control cultures. Left pellets in freezer for further processing
- Made more ampicillan stocks, restocked lab consumables, and cleaned the lab

Week 22 (September 30th - October 6th) WEEKLY SUMMARY:

We attempted our first western blot for the 6GIX protein from the periplasmic extractions and the no signal peptide cultures, but will need to troubleshoot in the weeks to come. Additionally, we continued purifying more 6GIX protein and continued gathering replicates for the periplasmic extractions from the signal peptide constructs. Finally, we took the time to restock lab consumables and clean the lab.

MONDAY, 10/7/2019

- Made more LB+amp plates

- Sonicated the No SP 6GIX, ModGIX, and pSB1A3 empty vector control samples and placed into freezer
- Ran periplasmic extraction samples from yesterday on a 12% SDS-PAGE gel and stained. Results showed no difference between any samples or the controls.
- Made more 10X SDS-PAGE running buffer

TUESDAY, 10/8/2019

- Purifed the No SP 6GIX, ModGIX, and pSB1A3 empty vector control samples
- Made more resuspension, wash, and elution buffers for purifications
- Created registry pages for all of our parts and the other subgroups. Added and annotated sequences for each part

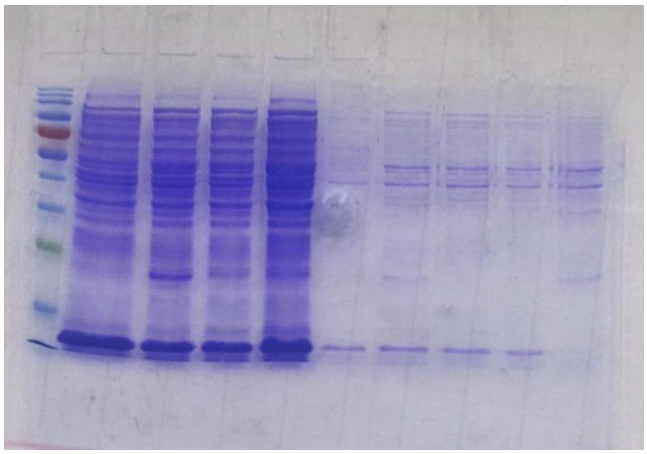
WEDNESDAY, 10/9/2019

- Finished adding sequences to all registry pages for all of our parts and annotated
- Re-ran periplasmic extraction samples from Sunday on a 12% SDS-PAGE gel and stained overnight
- Ran purification samples from Monday on a 12% SDS-PAGE gel, but forgot half the samples and left in water overnight without fixing in methanol...

FRIDAY, 10/11/2019

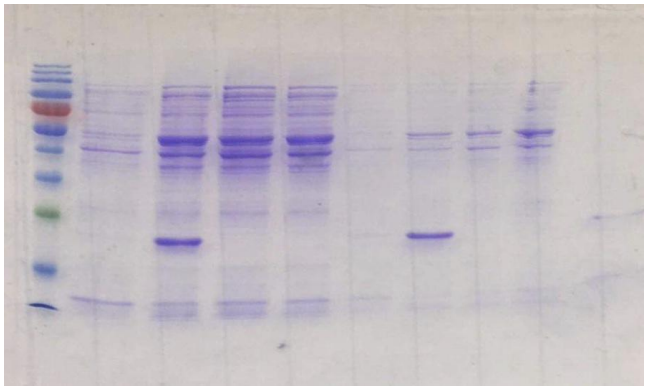
- Had to re-run the purification gels on 12% SDS-PAGE gel. Results show that No SP 6GIX was successfully purified, but a band of the correct size was not present for either ModGIX sample

IMG_3143.jpg



12% SDS-PAGE gel, NEB colour protein standard
Lane order: standard, pSB1A3 empty vector control whole cell lysate, No SP 6GIX construct whole cell lysate, ModGIX construct #1 whole cell lysate, ModGIX construct #2 whole cell lysate, pSB1A3 empty vector control elution fraction 1, No SP 6GIX elution fraction 1, ModGIX construct #1 elution fraction 1, ModGIX construct #2 elution fraction 1, sample run for other subgroup

IMG_3144.jpg



12% SDS-PAGE gel, NEB colour protein standard
Lane order: standard, pSB1A3 empty vector control elution fraction 2, No SP 6GIX construct elution fraction 2, ModGIX construct #1 elution fraction 2, ModGIX construct #2 elution fraction 2, pSB1A3 empty vector control elution fraction 3, No SP 6GIX elution fraction 3, ModGIX construct #1 elution fraction 3, ModGIX construct #2 elution fraction 3, sample run for other subgroup

- Ran another gel for a Western blot using the new purification samples. Transferred to PVDF membrane overnight

- Made overnights for pSB1C3-BBa_K1467100, pSB1C3-BBa_K1467200, pSB1C3-BBa_K1467300, pSB1C3-BBa_K1467400, and pSB1C3-improved flipper in DH5a

SATURDAY, 10/12/2019

- Miniprepped the overnights from yesterday
- Transferred the membrane for the Western blot into blocking solution and placed into the fridge

SUNDAY, 10/13/2019

- Worked on wiki content!

Week 23 (October 10th - 13th) WEEKLY SUMMARY:

Similar to previous weeks, we continued purifying more protein to be used in the emulsion experiments, as well as created more replicates for out periplasmic extractions. Though we received confirmation in the presence of the 6GIX protein from the no signal peptide cultures, there was no confirmation that the ModGIX protein was being produced. We will continued to troubleshoot protein expression of ModGIX in the future. We also miniprepped the RFP-flipper parts from the registry: pSB1C3-BBa_K1467100, pSB1C3-BBa_K1467200, pSB1C3-BBa_K1467300, and pSB1C3-BBa_K1467400 as preparation for further characterization of these parts.

MONDAY, 10/14/2019

- Thanksgiving!

TUESDAY, 10/15/2019

- cPCR of colonies from various transformations of ModGIX-pSB1A3
- Made O/Ns of 10 colonies from the plates based on cPCR results and sent samples for sequencing

WEDNESDAY, 10/16/2019

- Transformed ModGIX-pSB1A3 into BL21

THURSDAY, 10/17/2019

- Helped Seb make emulsions
- Received sequencing results for ModGIX saying it wasn't ModGIX

FRIDAY, 10/18/2019

- Induced 6 50mL no signal peptide subcultures with 50uL 1M IPTG for 8 hr induction at 37 C and took OD measurements hourly after 6 hrs of induction until 8 hrs
- Counted colonies for the RFP-Flipper parts from the registry and our improved Flipper part

SATURDAY, 10/19/2019

- Wiki writing and coding
- protein purification of the 6 No Signal peptide cultures from last day
- Finished the western blot of 6GIX which confirmed the presence of our protein

T--Calgary--blat.png

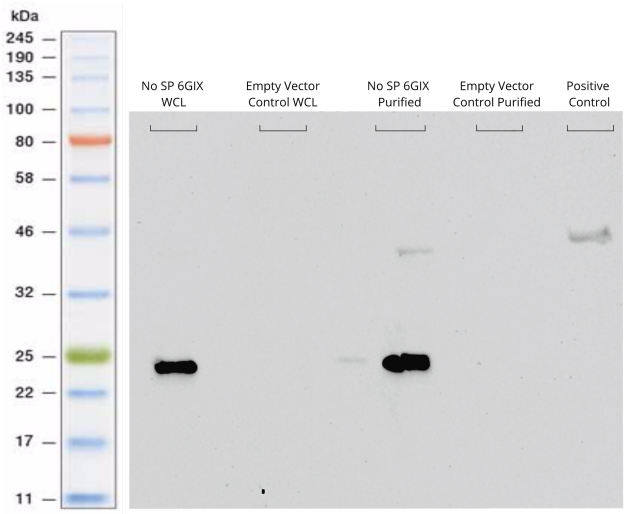


Fig. Western blot showing whole cell lysate and elutions after purification of no signal peptide 6GIX and empty vector control.

Week 23 (October 10th - 13th) WEEKLY SUMMARY:

The last week before wiki freeze, we primarily focused on parts characterization, and purifying more 6GIX protein to obtain more results. We also completed a western blot which confirmed the presence of the 6GIX protein. Finally, we spent our time writing up wiki content.