Lab Notebook

June 7, 2017—Preparing LB Agar Plates
Members: Ethan, Teresa, Monica, Farynna
Supervisor: Rochelin

- Prepared three batches of LB agar plates following protocols:
  - first batch with ampicillin and IPTG for plating cells with the gvp plasmid
  - second with chloramphenicol for plating cells with the BACTH plasmid
  - third with ampicillin, chPloramphenicol and IPTG for plating cells with the BACTH and gvp plasmids

Volume of Added Antibiotic/IPTG

Flask	Ampicillin (µL)	Chloramphenicol (µL)	IPTG (µL)
Ampicillin + IPTG	2400	0	600
Chloramphenicol	0	600	0
Chloramphenicol + Ampicillin + IPTG	3200	800	800

June 8, 2017—Streaking out pET11a
Members: Teresa
Supervisor: Rochelin

- Streaked cells transformed with pET11a for amplification

June 9, 2017—Picking colonies with pET11a and Incubation
Members: Ethan, Farynna
Supervisor: Rochelin

- Inoculated 6 culture tubes each with a colony of DH10B *E. coli* cells transformed with pET11a to amplify plasmids following protocols

June 10, 2017—Miniprep of overnight cultures
Members: Rafael, David, Abhi, Teresa, Farynna
Supervisor: Rochelin

- Extracted and purified pET11a plasmids from the six overnight cultures following the ThermoScientific GeneJet Plasmid Miniprep kit protocol.

June 12, 2017—Digestion of pET11a vector and Preparing LB and SOB media
Members: Rafael, David, Farynna, Ethan
Supervisor: Rochelin

- Prepared liquid LB media for cell incubation and SOB media for preparing competent cells.

- Digested pET11a with XbaI and BglII to linearize the vector for eventual Gibson Assembly reaction.
    - 19 µL of the digestion master mix is transferred to each of the three PCR tubes
    - 1 µL of each of BglII and XbaI is added to each tube

Volume for Digestion Master Mix

pET11a DNA (µL)	Milli-Q water (µL)	FastDigest Buffer (µL)
10	58	8

- Electrophoresed, gel extracted and analysed the dsDNA concentration of the digested sample.
- dsDNA concentration was too low, therefore we cannot proceed with Gibson Assembly. The low yield was likely due to the mistake of washing the sample with 700 µL of Wash Buffer twice. It is also likely that the pDNA yield from the miniprep was low due to the low copy nature of pET11a.

June 13, 2017—Digestion of pET11a vector and Preparing Competent Cells
Members: Ethan, Farynna and Monica
Supervisor: Rochelin

- Digested pET11a with BglII and XbaI

Volume of Each Reagent for Digestion

pET11a DNA (µL at 18.3 ng/µL)	Milli-Q water (µL)	BglII (µL)	XbaI (µL)	FastDigest Buffer (µL)
10	6	1	1	2

- Digestion products were gel electrophoresed.
- Band found roughly around 5000 bp was excised and gel extracted following ThermoFisher Scientific GeneJet Gel Extraction Kit protocol, eluting in 30 µL of Elution Buffer.
- Concentration of the extraction product was 33.5 ng/µL.

June 16, 2017—Picking colonies
Members: David, Ethan, Heather, Farynna
Supervisor: Rochelin

Procedure:

Inoculation with colonies and incubation

1. Light the bunsen burner to keep a sterile bench.
2. Pipette 50 µL of 1000x ampicillin and 50 µL of IPTG into a 50 mL falcon tube. Run the lid of the tube through the flame a few times before opening. Prepare four falcon tubes with the same volume of these reagants.
3. Pour 50 mL of LB media from the stock into each of the four tubes and shake.

4. While keeping all lids at a 45° angle, pour approximately 4 mL of media into 48 labeled culture tubes. 24 of the culture tubes are labeled “mix”, while the other 24 are labeled “sep”. To observe sterile technique, run the lid of the falcon tube through the flame a few times before opening.
5. Using a pipette tip, pick a single colony from the “mix” plate and drop the tip into one of the “mix” culture tube, while observing sterile technique. Repeat for the other 23 “mix” culture tubes. To observe sterile technique, before picking a pipette tip, run your hand through the flame a few times. Repeat this to inoculate the 24 “sep” culture tubes with colonies from the “sep” plate.
6. Incubate all tubes at 37°C overnight in the incubator.

June 17, 2017—Miniprep, Digestion and Gel Electrophoresis of overnight cultures
Members: Ethan, Deanna, Farynna
Supervisor: Rochelin

- Miniprep of overnight cultures to extract plasmids that potentially contains the gvp cluster
- Conducted diagnostic digests on the miniprep samples using BglII and XbaI
  - 15 µL of the digestion master mix is transferred to each of the twelve PCR tubes
  - 0.5 µL of each of BglII and XbaI is added to each tube
  - 3 µL of miniprep samples from the “sep” group

Volume for Digestion Master Mix

Milli-Q Water (µL)	FastDigest Buffer (µL)
182	13

  - Repeated for diagnostic digests of the “mix” group samples
- Diagnostic digest products were gel electrophoresed with linearized pET11a loaded into one well as control
- Bands were unclear. Eight samples will be diagnostically digested a second time.
- Second attempt at diagnostic digests using XhoI and KpnI instead of BglII and XbaI
  - 17 µL of the digestion master mix is transferred to each of the eight PCR tubes
  - Pipette 3 µL of selected DNA samples into PCR tubes

Volume for Digestion Master Mix

Milli-Q Water (µL)	FastDigest Buffer (µL)	KpnI (µL)	XhoI (µL)
126	9	4.5	4.5

- Diagnostic digest products were gel electrophoresed

<u>Monday June 19th 2017</u>
<u>Interlab</u>
Chemical Tranformation of DH5α
Members: David, Ethan, Farynna, Teresa, and Rochelin
Plating Interlab devices
Members: Ethan, Farynna, and Rochelin
Plated on chloramphenicol plates and incubated at 37C overnight.

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Digestion and gel extraction of pET11a plasmid

Members: Ethan, Farynna, and Rochelin

- pET11a was digested with BglII and XbaI.
- Set up two digestions.
- Refer to Rochelin's digestion method in Protocals
-

	Fast Digest Buffer (µl)	ddH2O (µl)	DNA (µl)	BglII (µl)	XbaI (µl)
Digestion 1	6	0	48	3	3
Digestion 2	6	23	25	3	3

- Both were incubated in PCR machine at
  - 37C for 16 hours
  - 85C for 10 min
  - 4C for infinity

Electroporation and Plasting of DH10B and BTH101 each with mScarlet, mRuby, and meGFP

Members: Ethan, Farynna, and Rochelin

- Different *E. coli* strains were compared to see levels of expression and growth.
- mScarlet was in pBAD plasmid
- mRuby was in pBAD plasmid
- meGFP was in plac psB1C3
- In each cuvette, there was 50 µl of competent cells (DH10B OR BTH101) and 0.5 µl of plasmid
- Electroporated cells were plated and incubated at 37C overnight.
-

	DH10B transformed with...	BTH101 transformed with...	Antibiotic Plate Type
Plate 1	mRuby	mRuby	Ampicillin
Plate 2	mScarlet	mScarlet	Ampicillin
Plate 3	meGFP	meGFP	Chloramphenicol

Results:

- Refer to rochelin's computer file

Growth Curves

Members: Ethan, Farynna, and Rochelin

- Growth curves were performed for modeling
- Growth was compared between BTH101, DH10B, DH5α, and MG1655 cya^-.
- Plated onto SOB plates (Using sterile needle tips).
- Incubated overnight at 37C.
- MG1655 cya^- and BTH101 both don't have indogenous adenylate cyclase.

Results:

- Refer to later

Tuesday, June 20th 2017

BB-SYS

Gel electrophoresis and extraction of digested PET11a performed June 19th

Members: David, Teresa, Raf, and Monica

- Lane 1: 7µl of DNA ladder
- Lane 2: 60µl of PET11a digested twice
- Lane 3: 120µl of PET11a digested once
- Lane 2 and 3 were extracted and purified. Lane 3 was digested a second time:
    - 48µl of DNA
    - 6µl Green FastDigest Buffer
    - 3µl of BglII
    - 3µl of XbaI
- 37C for 3 hours, 70C for 15 min, and 4C for infinity.

Results:

- Gel electrophoresis results are seen in the lab notebook

Diagnostic Digest of PET11a for Gibson Assembly

Members: Heather

- Determine if pET11a is able to be cut by BglII and XbaI. i.e If the restriction enzymes are active
    - 15µl of ddH2O
    - 3µl of DNA
    - 2µl of fast digest buffer
    - 1µl of BglII
    - 1µl of XbaI
- Lane 1: Undigested DNA (control)
- Lane 2: DNA digested with BglII
- Lane 3: DNA digested with XbaI
- Lane 4: DNA previously double digested.
    - (Double digestion previously done on June 19th)
- For reactions without restriction enzymes, replace volume with ddH2O

Results:

- Is found in the lab notebook

Gel Electrophoresis, Extraction, and transformation of Gibson Assembly Product with DH10B

Members: Ethan, Farynna, and Rochelin

- Note that this is Gibson Assembly Attempt number 4
- Gel electrophoresis of product (Gene+pET11a)
- Gel extraction and purification of product
- Product DNA was transformed with DH10B strain and plated on ampicillin and IPTG plates. This was then incubated at 37C overnight.

Results:

- Image of the gel electrophoresis is found in lab notebook

Interlab

DH5α Transformed with Positive Control

- DH5alpha was then plated onto chloramphenicol plates and incubated at 37C.

June 21st, 2017

BB-SYS

Digestion of pSB1C3 with EcoRI and XbaI

Members: Farynna, Teresa, and Rochelin

Aim: Digest pSB1C3 containing a BioBrick with mRFP in Kit 1, well 23P with EcoRI and XbaI to prepare for the insertion of BACTH gblock.

Diagram: Available in lab notebook

Materials:

- 10 µl of pSB1C3 with RFP (Kit 1, Well 23P)
- 2.5 µl each of EcoRI and XbaI
- 30 µl of ddH2O
- 5µl of CutSmart Buffer

Procedure:

- Incubated in PCR machine…
    - 37C for 2 hours, 70C for 15 min, and 4C for infinity.

Observations:

- Sample was run on a gel.
- Available in lab notebook

June 22nd, 2017

Interlab

Transformation and plating of DH5α Bacteria with the needed Interlab DNA

Members: Teresa, Ethan, Farynna, and Heather

Aim: To transform DH5α strain for the interlab study (kit 6)

Procedure:

- Electroporation was performed for all test devices and the positive and negative controls
- Plating of transformed DH5α was done on chloramphenicol plates
- Plated were incubated at 37C overnight
- Test devices 1 and 6 as well as negative control arched during electroporation. Regardless, they were plated.

Diagram:

- Image of plating methods are seen in the lab notebook.

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Inoculation of DH10B cells with Plac and eGFP

Members: Ethan, Teresa, Farynna, and Heather

Aim: Inoculate DH10B cells in order to replicate pSB1C3 for cloning of BACTH

Procedure:

- Colonies were selected from the plate transformed on June 19th with DH10B and BTH101 transformed with pSB1C3 plac+eGFP

- 50µl of chloramphenicol was added to a falcon tube (50x antibiotic)
- 50 ml of LB was added
- Transfer ~4 mL of LB+chloramphenicol to 6 tubes
- Using a pipette tip, pick a single colony from the plate and is inoculated.
- A total of 6 tubes were incubated at 37C overnight.

June 23rd, 2017

BB-SYS

Miniprep of pET11a and Plac + eGFP

Members: Ethan, Teresa, Farynna, Heather, Rochelin, and Abdullah

Aim: To separate pET11a for gibson assembly and to separate pSB1C3 out to clone in BACTH

Procedure: As listed in the Qiagen Miniprep Kit

Results:

- Nanodrop results for pET-11a showed relatively low concentrations: ~70ng/µl
- Nanodrop results for pSB1C3 revealed high concentrations: ~350ng/µl

June 24th, 2017

BB-SYS

Digestion of pSB1C3 and BACTH

Members: Farynna, Teresa, Deanna, and Rochelin

Aim: Digest pSB1C3 and BACTH in order for insertion of BACTH into pSB1C3.

Procedure:

- Digest pSB1C3 with EcoRI and SpeI.
- Digest BACTH with EcoRI and XbaI.

Gel Electrophoresis and Extraction of pSB1C3

Members: Farynna, Teresa, Deanna, and Rochelin

Aim: Gel Extract the pSB1C3 backbone which may allow appropriate ligation with the BACTH

Procedure: As listed in the gel electrophoresis and extraction protocols.

Results:

- The bands (pSB1C3 and the extracted eGFP) were seen to be approximately the same size but we went ahead and purified it and used for ligation.

Conclusions: After ligation, we may find many fluorescent proteins. I.e we did not limit down the background.

Ligation of BACTH into pSB1C3

Members: Teresa, Farynna, and Rochelin

Aim: To clone the BACTH gblock into pSB1C3

Procedure:

- 16µl of DNA insert (BACTH)
- 1µl of plasmid (pSB1C3)
- 2µl of buffer
- 1µl of DNA ligase

Conclusions:

- XbaI and SpeI are complementary overhangs and will create a destructive scarring. And thus, we may not cut at that 'speI' or 'XbaI' site again.
- Thus, if BACTH has been ligased in, it will no longer be able to be digested out. This, however, is not a big problem as we will not require to remove BACTH from pET11a.

Transformation and plating with ligation products (BACTH+pSB1C3)

Members: Rochelin and Farynna

Aim: To transform the ligated products into DH10B and plate it

Procedure:

- Electroporation was performed
- 1µl of ligation product
- 50µl of electrocompetent DH10B strain.
- Pipette 100µl of transformed cells into an ampicillin plate.
- Beads were used to spread.
- Incubate at 37C overnight.
- Only one plate was plated.
- This plate will then be analyzed and cultures will be chosen and inoculated.

Results:

- We found that there was a lot of background on the plates.

Inoculation with Gibson 5th Attempt and RF (25 colonies)

Members: Rochelin and Farynna

Aim: To inoculate and incubate colonies on both Gibson Assembly plates to eventually confirm presence of GVP

Procedure:

- 50µl of ampicillin to 50ml of LB (50x antibiotic).
- Transfer approx. 4ml of media into each tube (26)
- Label 1 tube with *Gibson 5th Attempt* while number the remaining 25 tubes from RF1 through to RF25.
- With a pipette tip, pick an isolated colony and transfer to appropriately labeled tube.
- Incubate at 37C overnight.

Digestion of pET11a with BglII and XbaI

Members: Farynna and Rochelin

Aim: To digest pET11a with BglII and XbaI for eventual insertion of GVP cluster

Procedure: As seen in procedure section (Rochelin's method)

Conclusion: This is in preparation for another gibson attempt.

June 25th, 2017

BB-SYS

Miniprep of Overnight cultures (Gibson +RF cultures)

Members: Abhi and Farynna

Aim: Extract the plasmids from the 25 RF overnight cultures and the 1 Gibson 5th attempt culture.

Procedure:

- See protocol in thermofisher manual

Diagnostic Digest of Miniprep Products

Members: Abhi and Farynna
Aim: Digest the 26 miniprep products
Procedure:

- 135µl of ddH2O
- 27µl of Fast Digest
- 8.1µl of XbaI
- 8.1µl of BglII
- 6.6µl to each PCR tube
- Add 4µl of DNA to each tube
- Incubate at 37C for 30 min, 80C for 5 min, and 4C for infinity

Conclusion:

- This will then by gel electrophoresed and extracted

Observations:

- The plasmid was cut only at BglII and therefore a gel with only one band for each well was observed.
- GVP cluster was not taken in by the plasmid.

Gel electrophoresis and extraction of pET11a (Previous)
Members: Abhi and Farynna
Aim: To extract digested pET11a
Procedure: As seen in the procedure section for lab protocols.
Conclusion: Digested plasmid will be used for another gibson assembly attempt (maybe?).
Inoculation of white colonies from BACTH and pSB1C3 plate
Members: Abhi and Farynna
Aim: To pick white colonies and inoculate LB and chloramphenicol to eventually confirm the presence of BACTH.
Procedure:

- Add 50µl of Chloramphenicol
- Add 50mL of LB
- Pick white colonies (6)
- Incubate at 37C overnight

June 26th, 2017
BB-SYS
Miniprep of BACTH and pSB1C3 White Cultures
Members: Ethan, Teresa, Abhi, and Rochelin
Aim: To extract BACTH and pSB1C3 plasmids from culture for later use and characterization
Procedure: As seen in the lab protocol.
Observation:

- After lysis and neutralization, the tubes were pelleted twice since precipitate remained floating about in the first centrifugation
- The supernatant was then pipetted rather than poured out.
- Nanodrop results:
    -

	Concentration (ng/µl)	260/280	260/230
Tube 1	141.3	1.88	2.28
Tube 2	133.7	1.87	2.29
Tube 3	106.9	1.60	1.60
Tube 4	144.4	1.87	2.28
Tube 5	142.1	1.89	2.35
Tube 6	163.1	1.89	2.32

-

PCR Gblocks and Split the vector

Members: Heather

Aim: To dilute primers and use them in PCR

Procedure: Q5

- 22.5µl of ddH2O
- 10µl of NEBuffer
- 10µl GC enhancer
- 1µl dNTPs
- 2.5µl Forward primer
- 2.5µl Reverse primer
- 1µl Template
- 0.5µl Q5
- Set the PCR machine to: 98C for 30S, (98C for 10S, 60C for 90S, 72C for 90S) repeat 34 times, 72C for 5min, and 4C for infinity.

Gel electrophoresis and extraction of the amplified G-blocks and split vectors

Members: Teresa, Ethan, and Rochelin

Aim: To observe the success of the amplification and to extract all components for preparation of the split vector Gibson Assembly.

Procedure: Load the weel at 100V, 400A, for 16 minutes.

- Two gels:
    - Gel 1:
        - DNA ladder
        - GB1
        - GB1
        - GB2
        - GB2
        - GB3
        - GB3
    - Gel 2:

- DNA ladder
- pET11a V1
- pET11a V1
- pET11a V2
- pET11a V2

Results:

- Images can be seen in the lab notebook.

Conclusion:

- Need to confirm purity and measure concentration with nanodrop tomorrow
- May need to repeat PCR for GB1 and GB3
- Will need to repeat PCR for GB2

June 27th, 2017

BB-SYS

Measuring Concentration and Purity of PCR products

Members: Farynna, Ethan, and Abdullah

Aim: To determine which PCR products are viable

Procedure: As seen in the lab protocol

Observations:

	260/280	260/230	Concentration (ng/µl)
GV1	2.43	0.04	16.3
GV2	-	-	-
GV3	2.34	0.06	25.5
V1	1.93	0.79	92.5
V2	1.94	1.31	73.0

Gradient PCR

Members: Rochelin

Aim: To determine the ideal temperature in which the primers can work at

Procedure:

- 65C-57C gradient PCR
- 16 PCRs are done total

Observations:

- A picture was taken of the gradient PCR
- Smearing was still observed.

Diagnostic digest of BACTH+pSB1C3

Members: Farynna, Teresa, and Ethan

Aim: To determine if the BACTH gblock was successfully cloned into the pSB1C3 vector (originally containing eGFP). Digest will use EcoRI and PstI and gel electrophoresis will be run to determine digestion products.

Procedure:

- Double digestion:
    - 35µl ddH2O
    - 7 µlof NEB2.1 buffer
    - 2.1µl of each enzyme (EcoRI and PstI)
    - 4µl of DNA into each 100µl tube.
    - 6.6µl of master mix into each tube
    - Incubate at 37C for 30 min, and 4C for infinity.
- Gel electrophoresis map:
    - DNA ladder
    - BACTH 1
    - BACTH 2
    - BACTH 3
    - BACTH 4
    - BACTH 5
    - BACTH 6

Observations:

- The gel showed two bands for each run
- Ladder was not able to be visualized and so the size of the bands cannot be determined

Conclusions:

- Perhaps BACTH is there since the cultures did not flouresce.
- We won't be doing diagnostic digest again.
- To avoid having a bad DNA ladder, we shouldn't turn the gel electrophoresis machine on/off.

PCR products GB1 and GB2 Diagnostic Gel electrophoresis

Members: Farynna, Teresa, Ethan, and Abdullah

Aim: To determine the results of the PCR reaction for the second attempt of GB1 and GB2 through a diagnostic gel.

Procedure:

- Gel electrophoresis protocol
- Map
-

1-1	1-2	1-3	1-4	1-5	1-6	1-7	DNA ladder
2-1	2-2	2-3	2-4	2-5	2-6	2-7	DNA ladder

Observations

- A very clear DNA ladder was visualized
- Everything else was very smeared

<u>June 28th, 2017</u>

<u>BB-SYS</u>

Pick Colonies on the Gibson Assembly Plate

Members: Heather and Deanna
Aim: to Pick colonies to inoculate and grow. There is only two colonies that grew on the plate so we;re going to hope that they're the ones and inoculate them.
Procedure:

- Picking colonies protocol.
- Incubate at 37C overnight
- Made a stock solution of LB with ampicillin and IPTG:
    - Added 50 ml of LB, 50 µl of ampicillin and 50 µl of IPTG.

Run four Gibsons using NEB/Home brew

Members: Heather, Deanna, Teresa, Farynna, and Abdullah
Aim: To perform 4 different Gibson Assemblies. Full vector+NEB, full vector+homebrew, Split vector+NEB, and Split vector+Homebrew.
Procedure:

- Homebrew (from Landon)
    - 1µl of DNA vector (0.5+0.5 for split vector)
    - 0.5µl of insert (0.5G1+0.5G2+0.5G3)
    - Gibson Assembly master mix are all in aliquot
- NEB
    - 1µl of DNA vector (0.5+0.5 for split vector)
    - 1.5µl of DNA insert (0.5G1+0.5G2+0.5G3)
    - 2.5µl GA master mix
- Incubate for 50C for 1-4 hours
- By the end, we will have four tubes:
    - Homebrew full vector
    - Homebrew split vector
    - NEB full vector
    - NEB split vector
- For tubes with full length vector: after one hour incubate at 50C, add <0.5µl of DpnI and incubate at 37C.

Overlap PCR

Members: Deanna and Heather
Aim: To piece together the 3 gblocks and the pET11A vector
Procedure:

- 27µl of ddH2O
- 10µl Q5 Buffer
- 10µl of GC enhancer
- 1µl pf dNTP
- 0.5µl of each of the gBlocks 1,2, and 3.
- 0.5µl of Q5
- Incubate at…
    - 98C for 50s
    - (98C for 10s, 61C for 30s, 72C for 3min and 10s) 15 times
    - 72C for 10 min

        - 4C for infinity
    - Add 2.5 µl of each of the forward and reverse primers
    - Incubate at:
        - 98C for 50s
        - (98C for 10s, 61C for 30s, 72C for 3min and 10s) 15 times
        - 72C for 10 min
        - 4C for infinity

Transformation of pSB1C3 with FP into DH10B

Members: Teresa and Farynna

Aim: To transform DH10B with pSB1C3 that has an RFP and GFP for eventual cloning of BACTH

Procedure:

- Transformation was done by electroporation and was spread on chloramphenicol plates using beads.
- The plate was incubated at 37C overnight.

Diagnostic Digest of Minipreped White Colonies from BACTH+pSB1C3

Members: Rochelin

Aim: To confrim the presence of BACTH fragment in the pSB1C3 vector

Procedure:

- 3µl DNA
- 2µl of NEB 2.1 buffer
- 0.6 µl of EcoRI
- 0.6µl of PstI
- 14.8µl of ddH2O
- Incubate at 37C for 30min

Results:

- Gels is found in the lab notebook page 44

Transform the Gibson products into DH10B strain

Members: Rochelin

Aim: Transform the 4 different Gibson products (split vector under NEB and Homebrew protocol and full vector under NEB and Homebrew protocol) into DH10B.

Procedure:

- Dilute Gibson products with 5µl of ddH2O
- Add 4µl of diluted products to competent DH10B cells
- Transfer to cuvettes
- Transformation through electroporation is performed
- Spread on ampicillin plates using beads
- Incubated at 37C overnight

Transformation of BACTH and Plac+eGFP+Parab+lacI into BTH101

Members: Rochelin and Farynna

Aim: Test if BACTH can modulate eGFP expression

Procedure:

- Transform using electroporation protocol
- Plated 100µl onto chloramphenicol plates and spread using beads

- Incubated at 37C overnight

pET11a vector full PCR (one fragment)

Members: Rochelin

Aim: PCR the vector so that it can be used for Gibson

Procedure:

- 85µl of ddH2O
- 22µl of HFbuffer
- 2µl of dNTPs
- 2µl DMSO
- 0.3µl of each 100µM primers
- 0.5µl of template
- 1µl Phusion
- Run PCR (gradient)
    - 95C for 1min
    - 98C for 15s
    - 55/65C for 30s (ran at 63C position)
    - 72C for 6min (1min/kb)
    - Repeat above 35 times
    - 72C for 10 min
- A gel electrophoresis was performed. It appeared to be smeary but 3 distinct bands were still seen. ~5-6 Kbp band was seen and so it was excised and gel extracted

June 29th 2017

BB-SYS

Miniprep DH10B to check for presence of Gibson Assembly products.

Members: Ethan, Teresa, and Farynna

Aim: Extract the plasmid to check for the presence of gvp cluster

Procedure:

- Refer to the miniprep protocol in lab protocol

Results:

- DNA purity and concentration:
-

	260/280	Concentration (ng/µl)	260/230
Colony 1	1.90	96.7	1.73
Colony 2	1.90	129.0	1.84

Diagnostic digest of pET11a and GVP (Gibson assembly number 5)

Members: Ethan, Teresa, Farynna, and Rochelin

Aim: XhoI and SacI are going to be used for digestion. Expecting ~8kbp and ~2kbp.

Procedure:

- Since we don't have a high-copy plasmid…
- 5µl DNA

- 2µl fast digest buffer
- 0.5µl of each enzyme (SacI and XhoI)
- 12µl of ddH2O
- Incubate at 37C for 30 min
- Lane 1: DNA ladder
- Lane 2: First digestion
- Lane 3: Second digestion

Observations:

- Lane 2: two bands are seen above the 5000 bp marker
- Labe 3: two bands are close to the 3000 bp marker. One is above and the other is below.
    - Cannot see the presence of vector (pET11a) so we're runnin a colony PCR and digesting with XhoI and KpnI.

Colony PCR on Gibson Fifth Attempt

Members: Ethan and Farynna

Aim: To confirm the presence of GVP cluster and pET11a. Conducting a Q5 PCR that amplifies a segment of sequence from the vector to the GVP insert.

Procedure:

- 22.5µl of ddH2O
- 10µl of buffer
- 10µl of GC enhancer
- 1µl dNTPs
- 2.5µl of each primer (forward/reverse)
- 1µl of template
- 0.5µl Q5 DNA polymerase
- All of the above is for each tube of sample
- Incubate for…
    - 98C for 30s
    - (98C for 10s, 59C for 30s, 72C for 3min) repeat 30 times
    - 72C for 10 min
    - 4C for infinity

Diagnostic Digest of Gibson Fifth with XhoI and KpnI

Members: Ethan and Farynna

Aim: To confirm the presence of the GVP cluster and pET11a by digesting the miniprep products with XhoI and KpnI.

Procedure:

- 6µl of Fast digest
- 36µl of ddH2O
- 1.5µl of each enzymes (KpnI and XhoI)
- Above is for the master mix
- 5µl of DNA
- Incubate at 37C for 30min, 80C for 2min, and 4C for infinity.

June 30th, 2017

BB-SYS

Miniprep of Gibson Assembly Split Vector Homebrew 1-6

Members: Ethan and Rochelin

AIm: to purify the plasmid of split vector Gibson Assembly attempt 5.

Procedure: Thermofisher plasmid miniprep standard protocol with 6 cultures

Diagnostic Digestion of Gibson 6th with XhoI and KpnI

Members: Ethan and Farynna

Aims: To confirm the presence of GVP in pET11a after split vector assembly by digestion of miniprep products with KpnI and XhoI.

Procedure:

- Mastermix:
    - 14µl of Fast Digest buffer
    - 3.5µl of each enzyme
    - 84µl of ddH2O
    - 15µl of mastermix distributed to 100µl tubes
    - 5µl of 6 DNA samples loaded into the 6 tubes (50µl reactions)
    - Incubate at 37C for 30min, deactivate at 80C for 2min, hold at 4C for infinity.

Conclusion: run digest products on agarose gels.

Gel electrophoresis of PCR products and Gibson Fifth and Sixth

Members: Ethan, Farynna, and Rochelin

Aim: To confirm the presence of the gvp insert and the pET11a vector

Procedure:

- Gel electrophoresis protocol.
- Lane 1: Diagnostic Digestion (XhoI/KpnI) gibson 5th attempt
- Lane 2: PsF gvpR
- Lane 3: PsF gvpR
- Lane 4: Diagnostic Digestion (XhoI/KpnI) Gibson 5th attempt
- Lane 5: PsR gvpF
- Lane 6: PsR gvpF
- Lane 7: Homebrew split vector Gibson 6th number 1
- Lane 8: Homebrew split vector Gibson 6th number 2
- Lane 9: Homebrew split vector Gibson 6th number 3
- Lane 10: Homebrew split vector Gibson 6th number 4
- Lane 11: Homebrew split vector Gibson 6th number 5
- Lane 12: Homebrew split vector Gibson 6th number 6
- Lane 13: DNA ladder

Miniprep of DH10B cultures transformed with pSB1C3 + RFP and pSB1C3 + GFP

Members: Ethan and Farynna

Aim: To extract the plasmid used for eventual cloning of BACTH into pSB1C3

Procedure: Miniprep protocol.

Conclusions: To be used for cloning of BACTH.

July 4, 2017

gBlock Amplification

Aim: To amplify each gvp gBlock for eventual Gibson Assembly via PCR

Members: Ethan, Rochelin, Farynna

- Created a Master Mix scaled to 9 reactions with 3 different mixes for each gBlock
  - 207 μL of ddH_2O
  - 90 μL of buffer
  - 90 μL of GC enhancer
  - 9 μL of dNTPs
  - 22.5 μL each of forward primer/reverse primer
  - 4.5 μL of gBlock template
  - 4.5 μL of Q5 DNA polymerase
- Incubation:
  - 98°C for 3 minutes
  - 98°C for 15 seconds
  - 58/65°C for 30 seconds
  - 72°C for 2 minutes
  - Repeat the above 3 for 30x
  - 72°C for 8 minutes
  - Hold at 12°C

Vector Amplification

Aim: To amplify the pET11a vector for eventual Gibson Assembly via PCR

Members: Ethan, Rochelin, Farynna

- Mix:
  - 85 μL of ddH_2O
  - 22 μL of 5x buffer
  - 2 μL of dNTPs
  - 2 μL of DMSO
  - 0.3 μL each of forward primer/reverse primer (concentrated)
  - 0.5 μL of pET11a vector template
  - 1 μL of Phusion DNA polymerase
- Incubation:
  - 95°C for 1 minute
  - 98°C for 15 seconds
  - 55/65°C for 30 seconds (tube is placed at the 63°C position)
  - 72°C for 6 minutes
  - Repeat the above 3 for 30x
  - 72°C for 10 minutes
  - Hold at 12°C

Gel Electrophoresis

Aim: To perform diagnostic gels on PCR products (gBlock Amplification and Vector Amplification)

Members: Ethan, Rochelin, Farynna

- Followed "Gel Electrophoresis" protocol
  - 10 µL of DNA loading dye is added to each sample
  - Full volume of PCR products were loaded into the wells
- Gel 1:

DNA ladder	1.1	1.2	1.3	1.4	1.5	1.6	1.7
DNA ladder	2.1	2.2	2.3	2.4	2.5	2.6	2.7

- Gel 2:

DNA ladder	3.1	3.2	3.3	3.4	3.5	3.6	3.7
DNA ladder				pET11a			

- Bands for gBlocks 1 and 2 were smears. Amplification for both gBlocks are is unsuccessful.
- gBlock 3 PCR was successful

Gel Extraction + NanoDrop of gBlock 3 Amplification and pET11a Amplification

Aim: Extract the PCR product from the pET11a and gBlock 3 gel and analyze the concentration with a NanoDrop

Members: Ethan, Rochelin, Farynna

- Followed "Gel Extraction" protocol
- Followed "NanoDrop" protocol

NanoDrop Results

Sample	dsDNA Concentration (ng/µL)	260/280	260/230
GB 3	17.0	1.87	1.23
pET11a	48.5	1.87	0.60

gBlock 1 and 2 Amplification

Aim: To amplify gBlocks 1 and 2, as the first attempt resulted in smeared bands. The gBlocks will be amplified with Phusion DNA polymerase instead of Q5

Members: Ethan, Farynna

- Mix in each tube:
  - 85 µL of ddH_2O
  - 22 µL of 5x buffer
  - 2 µL of DMSO
  - 2 µL of dNTPs

- 0.3 μL each of forward primer/reverse primer
- 0.5 μL of gBlock template
- 1 μL of Phusion DNA polymerase

❖ Incubation:
- 95°C for 1 minute
- 98°C for 15 seconds
- 55/65°C for 30 seconds (tube is placed at the 63°C position)
- 72°C for 6 minutes
- Repeat the above 3 for 30x
- 72°C for 10 minutes
- Hold at 12°C

gBlock 1 and 2 Amplification
Aim: To amplify gBlocks 1 and 2 using Pfu DNA polymerase and Pfu protocol
Members: Ethan, Rcohelin, Farynna

❖ For each 50 μL reaction:
- 38 μL of ddH_2O
- 5 μL of Pfu buffer
- 1 μL of dNTPs
- 2.5 μL each of forward primer/reverse primer
- 0.5 μL of gBlock template
- 0.5 μL of Pfu DNA polymerase

❖ Incubation:
- 95°C for 3 minutes
- 95°C for 1 minute
- 55°C for 45 seconds (tube is placed at the 63°C position)
- 72°C for 6 minutes
- Repeat the above 3 for 30x
- 72°C for 10 minutes
- Hold at 4°C

Streaking DH10B, BTH101, and MG1655
Aim: To streak out DH10B, BTH101 and MG1655 cells into SOB agar plates in preparation for growth curves
Members: Ethan, Rcohelin, Farynna

❖ Used needles to streak cells onto SOB plates (1 plate/cell line)
❖ Incubate plates at 37°C overnight

July 5, 2017
Gel Electrophoresis of Pfu and Phusion Amplification of gBlocks 1 and 2
Aim: To confirm successful amplifications of the gBlocks by looking at the fragment sizes
Members: Ethan, Rochelin, Farynna

❖ Followed “Gel Electrophoresis” protocol

- Gel:
  - Lane 1: Pfu gBlock 2
  - Lane 2: Pfu gBlock 1
  - Lane 3: Ladder
  - Lane 4: Phusion gBlock 2
  - Lane 5: Phusion gBlock 1
- All Phusion and Pfu bands were smears
- Amplification is unsuccessful.

Gradient PCR of gBlocks 1 and 2

Aim: Increase concentration of gBlocks 1 and 2; extend stocks of gBlocks

Members: Rochelin

- Mix:
  - 85 µL of ddH_2O
  - 22 µL of 5x HF Buffer
  - 3 µL of DMSO
  - 2 µL of dNTPs
  - 0.3 µL of P_F/P_R (concentrated)
  - 0.5 µL of template
  - 0.5 µL of Phusion
- Aliquot into 8 PCR tubes
- Incubate:
  - 95°C for 2 minutes
  - 98°C for 15 seconds
  - 60/72°C for 30 seconds
  - 72°C for 4 minutes
  - Repeat the above 3 for 35x
  - 72°C for 10 minutes
  - Hold at 4°C
- Gel electrophoresed the PCR products following the "Gel Electroporation" protocol
- Bands are smears thus amplification was not successful
- Insert gel picture here

Gibson Assembly the 7th of gvp cluster into pET11a

Aim: Assembly of gvp gene cluster in pET11a

Members: Rochelin

- Making an equimolar mix of gBlocks and pET11a
- gBlock 1: 10 ng/µL → 3.60 µL
- gBlock 2: 10 ng/µL → 4 µL
- gBlock 3: 17 ng/µL → 1.87 µL
- pET11a: 48.5 ng/µL → 2 µL
- Combine 2.5 µLof the DNA mixture and 2.5 µL HiFi master mix
- Incubate for 2 hours at 50°C

July 6, 2017

Growth Curves of BTH101, MG1655 and DH10B strains of *E. coli*

Aim: To determine growth rates of various *E. coli* strains**.**

Members: Heather, Deanna, Farynna, Ethan

Procedure:

- Prepare subcultures of *E. coli* strains in 4 mL of LB media
- Inoculate six 4 mL culture tubes for each strain with stationary phase subculture to make a 1% growth curve culture
    - Add 40 μL to each culture and put into shaker-incubator
- Time at 0h initial measurement
    - 384 well plate with A1 to A6 BTH101, A7 to A12 MG1655 and A13 to A18 DH10B and A20 as blank
    - Absorbance of 600 nm measured
- Every 30 minutes:
    - Remove cultures from shaker incubator
    - Add 100 μL of each culture according to: X1-6 is BTH101; X7-12 is MG1655; X13-18 is DH10B; X19 is blank
- Measure OD_{600} with plate reader
- Every iteration, move down a row in the 384 well plate

NEB PCR CleanUp kit of GB1, GB2, GB3, and pET11a

Aim: To concentrate pET11a PCR product and gBlocks 1, 2 and 3 for eventual GA

Members: Heather, Deanna, Ethan, Farynna

- Followed NEB PCR Cleanup Kit Protocol:
    - Add 40 μL of Binding Buffer to 20 μL of DNA sample
    - Transfer to spin column, centrifuge for 1 minute and discard flow through
    - Add 200 μL of Wash Buffer, centrifuge for 1 minute and discard flow through. Repeat this step twice.
    - Transfer column to a 1.5 μL microfuge tube
    - Elute in 7 μL of Elution Buffer, incubate for 1 minute and centrifuge for 1 minute
- Followed "NanoDrop" protocol for measuring dsDNA concentration for all samples

NanoDrop Results

Sample	dsDNA Concentration (ng/μL)	260/280	260/230
GB 1	22.9	-	-
GB 2	10.9	-	-
GB 3	97.2	-	-

GB 2	21.2	1.56	0.28
pET11a	87.3	1.82	2.12

Gibson the *8th* of gvp cluster
Aim: To piece together the gvp cluster (3 gBlocks) and pET11a via Gibson Assembly
Members: Rochelin

- Gibson Mix:
  - 2.24 μL of vector
  - 3.16 μL of gBlock 1
  - 3.84 μL of gBlock 2
  - 0.65 μL of gBlock 3
  - 0.76 μL of ddH_2O
  - 10 μL of NEB HiFi kit
- Incubation:
  - 50°C for 1 hour
  - Hold at 4°C
- Followed transformation protocol
  - Cells resuspended in 600 μL
  - Plate 100 μL of transformants

July 7, 2017
Transformation of three BioBrick parts (plac+RFP, strong promoter+medium RBS, lacI) into DH10B
Aim: To transform DH10B with 3 plasmids with different BioBrick parts to eventually assemble a construct for testing the BACTH system
Members: Ethan, Abhi, Heather, Farynna

- Followed "Transformation" protocols
  - BBa_J04450 (plac+RFP) was taken from Kit 2, 24P
  - BBa_I732100 (lacI) was taken from Kit 3, 8A
  - BBa_K608003 (strong constitutive promoter + mRBS) was taken from Kit 1, 5A
  - DNA in wells from the iGEM distribution kit was resuspended in 10 μL of ddH_2O.
  - Cells are plated in LB+chloramphenicol plates

Gel Extraction of PCR products (Sfx+BACTH+Pfx)
Aim: Gel extract the PCR products of Sfx+BACTH+Pfx
Members: Ethan, Heather, Farynna

- Followed the "Gel Extraction" protocol

Picking Colonies from Gibson 8th Attempt Plate

Aim: Picking colonies from the Gibson plate to eventually determine whether GA reaction was successful
Members: Ethan, Heather, Farynna

- ❖ Followed the "Inoculation" protocol
  - ➢ 48 colonies from the plate were picked

July 8, 2017

Miniprep of Colonies picked from GA 8th Plate and from plac+RFP, lacI, strong promoter+mRBS plates

Aim: Extract the plasmids from the GA plate colonies and from plac+RFP, lacI, strong promoter+mRBS plate colonies for eventual diagnostic digests and full digests, respectively.
MEMEbers: David, Ethan, Heather, Deanna, Teresa, Farynna

- ❖ Followed "Miniprep" protocols
- ❖ Followed "NanoDrop" protocols
  - ➢ NanoDrop analysis was repeated twice for all samples because the instrument was not blanked properly the first time

NanoDrop Results

Sample	dsDNA Concentration (ng/µL)	260/280	260/230
lacI 1	37.4	1.95	1.25
lacI 2	23.8	1.92	1.26
lacI 3	26.6	1.91	1.39
lacI 4	21.2	1.87	1.23
RFP 1	147.0	1.88	2.17
RFP 2	156.6	1.87	2.19
RFP 3	165.4	1.90	2.27
mRBS 1	21.2	1.86	1.10
mRBS 2	31.6	1.99	1.44

Digestion of Miniprep Samples of plac+RFP, lacI, strong promoter+mRBS and pSB1A3

Aim: Digest the parts for eventual ligation into pSB1A3 and 1 sample of RFP for BACTH
Members: Ethan, Teresa, Farynna

- ❖ Followed "Digestion" protocol
  - ➢ plac+RFP was digested with EcoRI-HF and SpeI
  - ➢ lacI was digested with XbaI and PstI
  - ➢ Strong promoter + medium RBS was digested with XbaI/PstI

- ➢ pSB1A3 was digested with EcoRI and PstI
- ➢ Plac + RFP for BACTH insertion was digested with EcoRI and PstI
- ➢ For samples other than pSB1A3, the volume of reagents for digestion are as follows:

DNA sample (ng/µL)	Milli-Q water (µL)	BglII (µL)	XbaI (µL)	NEB 2.1 Buffer (µL)
46	2	1	1	6

- ❖ Samples were electrophoresed following "Gel Electrophoresis" Protocol
  - ➢ Small aliquots (25 µL) from plac+RFP, pSB1A3, lacI and strong promoter + medium RBS digestion products were electrophoresed for a diagnostic gel
  - ➢ Full volume of Plac + RFP for BACTH insertion digestion product was loaded
  - ➢ Gel 1:
    - ■ Lane 1: Ladder
    - ■ Lane 2: RFP 2 (for BACTH)
    - ■ Lane 3: RFP 3 (for assembling the reporter)
    - ■ Lane 4: mRBS 1 (for assembling the reporter)
    - ■ Lane 5: lacI 1 (for assembling the reporter)
    - ■ Lane 6: pSB1A3 (for assembling the reporter)
  - ➢ Gel 2:
    - ■ Lane 1: Ladder
    - ■ Lane 2: RFP 2 (for BACTH)
    - ■ Lane 3: RFP 3 (for assembling the reporter)
- ❖ Gel 1, Lane 5: no bands were visible for lacI at ~ 1000 bp
- ❖ Gel 1, Lane 4: no bands were visible for mRBS 1 at ~56 bp possibly due to small size of the fragment
- ❖ RFP 3 and RFP 2 bands at ~1000 bp are both visible
- ❖ All other results showed successful digestion
- ❖ Digestion of RFP 3, however, was not complete unlike RFP
- ❖ Followed "Gel Extraction" protocol for RFP 2 and 3

Ligation of RFP coding + mRBS + pSB1A3

Aim: To do part 1 of 3A Assembly and ultimately create another reporter (to observe success in BACTH)

Members: Ethan, Farynna, Teresa

- ❖ Followed "Ligation" protocol

Insert some diagram refer to p77

Diagnostic Gels for lacI and mRBS

Aim: To check if RFP (B) + (C), lacI and mRBS are digested properly. The other gel is to observe if DNA was present in the mini prepped product.

Members: Ethan, Farynna, Teresa

- Followed "Gel Electrophoresis" Protocol
  - Gel 1
    - Lane 1: Ladder
    - Lane 2: lacI 1
    - Lane 3: mRBS 1
  - Gel 2
    - Lane 1: Ladder
    - Lane 2: lacI 2
    - Lane 3: lacI 3
    - Lane 4: lacI 4
    - Lane 5: mRBS 2
  - 15 µL of lacI were each taken out and all 15 µL of it was loaded into the well
- No bands were visible on Gel 1
- No bands visibly migrated on Gel 2. Faint bands are visible at the wells
- No lacI/mRBS in the first place

July 10, 2017

Digest BACTH, ligate into pSB1C3 and Transformation of Ligation Products

Aim: To insert BACTH into pSB1C3 vector. The insert is flanked by iGEM prefix and suffix

Members: Teresa, Ethan, Heather, Deanna, Farynna

- Followed "Full Double Digestion using iGEM enzymes" protocol
  - pSB1C3 vector and the pSB1C3 insert are both digested with EcoRI-HF and PstI
- Followed "Ligation" protocol
- Followed "Transformation" protocol

Picking Colonies from lacI+pSB1C3 and strong promoter+mRBS+pSB1C3 Plate

Aim: To inoculate colonies with lacI+pSB1C3 and strong promoter+mRBS+pSB1C3 into LB+chloramphenicol culture to eventually extract the BioBrick parts for assembling a reporter construct for BACTH

Members: Teresa, Ethan, Heather, Deanna, Farynna

- Followed "Inoculation" protocols
  - In addition to the 37°C overnight incubation, cultures were subject to an additional overnight incubation at room temperature, as the cultures were not very turbid after the first incubation

July 12, 2017

Picking Colonies from Pfx+BACTH+Sfx Ligation Plate

Aim: To pick colonies from Pfx+BACTH+Sfx ligation plate for eventual extraction of the plasmid

Members: Heather, Farynna

- ❖ Followed "Inoculation" protocols
  - ➢ Picked 10 colonies from the ligation plate

July 13, 2017

Miniprep of Colonies from Pfx+BACTH+Sfx Ligation and lacI and mRBS Cultures

Aim: To extract plasmid from colonies for future use

Members: Teresa, Farynna, Ethan

- ❖ Followed "Miniprep" protocols
- ❖ Followed "NanoDrop" protocols for measuring dsDNA concentration and purity

NanoDrop Results

Sample	dsDNA Concentration (ng/µL)	260/280	260/230
Ligation 1	127	1.82	2.25
Ligation 2	112.9	1.88	2.24
Ligation 3	210.8	1.87	2.31
Ligation 4	143.0	1.81	1.87
Ligation 5	117	1.84	2.12
Ligation 6	103.4	1.85	2.16
Ligation 7	125.4	1.86	2.25
Ligation 8	125.4	1.86	2.18
Ligation 9	108.8	1.86	2.08
Ligation 10	114.5	1.81	1.59
lacI 1	41.8	1.90	1.83
lacI 2	38.5	1.89	1.40
mRBS 1	45.8	2.06	2.02
mRBS 2	57.8	1.86	1.67

July 13, 2017

Picking Colonies from lacI and mRBS Plate

Aim: To pick colonies from lacI and mRBS plate to eventually extract these constructs for assembly of a fluorescence-based BACTH reporter

Members: Teresa, Farynna

- ❖ Followed "Inoculation" protocols
  - ➢ Picked 6 white colonies from the lacI plate and 6 colonies from the mRBS plate

July 14, 2017

Miniprep of mRBS and lacI (in pSB1C3 plasmid)

Aim: To isolate the DNA out for potentially a diagnostic digest and to eventually assemble a fluorescence-based reporter for the BACTH system

Members: Teresa, Farynna

- ❖ Followed "Miniprep" protocol
  - ➢ Eluted in 30 µL instead of 50 µL
- ❖ Followed "NanoDrop" protocol for measuring concentration and purity

NanoDrop Results

Sample	dsDNA Concentration (ng/µL)	260/280	260/230
lacI 1	185.5	1.89	2.31
lacI 2	158.7	1.88	2.18
lacI 3	140.7	1.89	2.24
lacI 4	160.5	1.89	2.22
lacI 5	147.6	1.89	2.25
lacI 6	160.5	1.88	2.25
mRBS 1	164.3	1.90	2.30
mRBS 2	87.2	1.85	2.13
mRBS 3	94.6	1.88	2.29
mRBS 4	75.7	1.87	2.22
mRBS 5	86.0	1.90	2.21
mRBS 6	88.0	1.90	2.30

July 17, 2017

Transformation of DH10B with CPB-38-441

Aim: Transform DH10B with CPB-38-441 to amplify the plasmid. If it is necessary to amplify the gvp A and C gBlock, it can be cloned into CPB-38-441, which is a high-copy vector.

Members: Ethan, Teresa, Farynna

- Followed "Transformation" protocol.
  - Transformants were plated on SOB plates without antibiotics.

Digestion and Ligation of BBa_J04450 and BBa_K608003 into pSB1A3

Aim: To assemble three BioBrick parts to create a fluorescence-based reporter for the BACTH system. Expression of a monomeric red fluorescence protein controlled by a wild type lac promoter with a CAP binding site (BBa_J04450) will be assembled in conjunction to the lacI gene (BBa_I732100) under a constitutive strong promoter+medium strength RBS (BBa_K608003) in pSB1A3. Assembly initially involves ligating BBa_J04450 and BBa_K608003 together following 3A Assembly specifications, and then the ligation of BBa_I732100.

Members: Heather, Farynna

- Followed "Full Double Digestion using iGEM enzymes" protocol.
  - BBa_J04450 is digested with EcoRI and SpeI
  - BBa_K608003 is digested with XbaI and PstI
  - BBa_I732100 is digested with SpeI and PstI
- A small aliquot of the digestion product was gel electrophoresed to check band sizes. A band at approximately 1000 bp for BBa_I732100 confirms successful digestion of lacI gene. No bands were visible for BBa_K608003 at approximately 56 bp, likely due to the short sequence.
- Followed "Ligation" protocol
- Followed "Transformation" protocol.
  - 2 µL of ligation product instead of 4 µL, as the transformations arched twice at plasmid volumes of 3 µL and 4 µL.
- After overnight incubation, no colonies were visible on the plate.

July 19, 2017

Streaking DH5α *E. coli* cells

Aim: To prepare electrocompetent DH5α cells for the Interlab Study. No colonies were present on first attempt at streaking, therefore we streaked cells from a new stock.

Members: Heather, Ethan, David, Teresa

- Streaked DH5α cells on five SOB plates from stock to prepare competent cells.

Transforming DH10B with CPB-38-441

Aim: First attempt at transforming DH10B with CPB-38-441 was unsuccessful. The plate showed a lawn of bacteria, likely due to the use of an SOB plate without kanamycin antibiotic. A second attempt at transforming DH10B with CPB-38-441 was performed for the amplification of the plasmid. The gvp A and C gBlock will eventually be cloned into CPB-38-441 for amplification.

Members: Heather, Ethan, David and Teresa

- Followed transformation protocol.
    - First attempt resulted in arcing, therefore transformation was repeated a second time.

Transforming DH10B with CPB plasmid

Aim: The last time it was transformed and streaked, it was done on SOB plates. This caused a lawn to grow due to contamination.

Members: Heather, Ethan, David, and Teresa.

Procedure:

- Streaking was done on Kanamycin plates
- Transformation one arced
- Transformation two plated
- Therefore we have ~2μL of CPB plasmid left.

Future notes: Check for DH10B and CPB growth. Ultimately, we may be able to amplify each gblock segment.

July 24, 2017

Transformation of DH10B and DH5α with pBAD and TurboRFP

Aim: To transform DH10B with pBAD and TurboFP to amplify the plasmid for eventual characterization of BACTH. To transform DH5**α** with TurboFP and pBAD as a control to check cell competency. Plated DH5**α** cells that aren't transformed to check cell viability.

Members: Ethan, Farynna

Procedure:

- Followed transformation protocol for DH5**α** and DH10B.
    - Streaked cells on ampicillin and 0.02% arabinose plates
- Streaked non-transformed DH5**α** cells.
- Incubated at 37C overnight.

Observations:

*Pictures were taken

- DH5**α** with TurboFP in pBAD plated on Amp/Ara plates was used as a control to check for cell competency.
    - Cells were found to be competent.
    - DH5**α** expressed some TruboFP- very light pink was observed.
- DH5**α** plated on SOB plates
    - Cells were found viable.
    - The first streak showed the majority of colonies and growth
- DH10B with TurboFP in pBAD plated on Amp/Ara plates
    - Was successfully amplified.
    - Showed strong expression of TurboFP.

Preparing LB Agar Plates

Aim: To prepare plates for BACTH characterization (Amp+Ara +Chloram.), growth curves (regular LB), gBlock amplification in CPB-38-441 (Kan), and additional chloramphenicol plates.

Members: David, Ethan, Heather, and Farynna.

Procedure:

- Followed protocols for preparing LB Agar
-

Plate	Concentration
Ara + Amp + Chlr	Arabinose - 0.02%, Ampicillin - 4x, Chloramphenicol - 1x
Chlr	Chloramphenicol - 1x
Kan	Kanamycin - 1x
LB	No antibiotic

July 25, 2017

Miniprep DH10B and CPB plasmid

Aim: To collect and extract the CPB plasmid for amplification

Members: Teresa

Procedure: Follow thermofisher protocol.

- Only one culture was done

Observations:

- Nanodrop results reveal:
  - Concentration: 216.2 ng/μL
  - 260/280: 1.86
  - 260/230: 2.09

July 26, 2017

Interlab Study

Aim: To transform and plate test devices with DH10B and DH5α through electroporation

Members: Teresa, Deanna, Farynna, and Ethan

Procedure:

- Using iGEM's kit plate 7 interlab…
  - (+) control - well 21B
  - (-) control - well 21 D
  - TD1 - 21F
  - TD2 - 21H
  - TD3 - 21J
  - TD4 - 21L
  - TD5 - 21M
  - TD6 - 21P
- Used Rochelin's transformation protocol
- Plated on Chloramphenicol plates

Observations:

-

DH10B	DH5α
Arcing occurred with test devices 2 and 4	No arcing occurred
Low values (<4.0) occurred with test devices 1 and 3.	
We repeated 1-4 Test devices	

- Nothing grew on the plates therefore experiment will be repeated.

Picking DH10B (Amp/Ara plates with TurboFP) for inoculation and characterization of BACTH
Aim: To grow DH10B with TurboFP in pBAD for characterization.
Members: Farynna and Teresa
Procedure:

- Follow inoculation procedure
- Culture: Amp (1x) +Ara (0.02%)
- Plate: Amp/Ara DH10B TurboFP
- Inoculated 4 tubes
- Incubate at 37C overnight.

Miniprep of TurboFP DH10B cultures for BACTH characterization
Aim: To create DNA stock for future transformations
Members: Farynna
Procedure:

- Thermofisher Geneset Miniprep Protocol
- 4 samples of DH10B and TurboRFP were miniprepped
- Concentration measure with nanodrop.

Observations:

Sample	**Concentration (ng/µL)**	**260/280**	**280/230**
1	634.3	1.90	2.32
2	635.2	1.89	2.31
3	618.6	1.89	2.27
4	561.9	1.83	2.35

Conclusions: Use in co-transformation of BACTH characterization

Double Transformation of BTH101, MG1655, and DH10B with TurboRFP and BACTH
Aim: To characterize BACTH system
Members: Ethan, Farynna, and Teresa
Procedure:

- Co-transformation protocol

- Add 2ng of each plasmid to 20µL of bacteria
- Electroporate samples at 2kV
- Plate on 2 antibiotic agar plates
- Chloramphenicol + Ampicillin plates → BACTH + TurboFP (repeat for each strain)
- Chloramphenicol → BACTH +TurboFP (repeat for each strain)
- Ampicillin → BACTH + TurboFP (repeat for each strain)
- Chloramphenicol → BACTH (repeat for each strain)
- Ampicillin → TurboRFP (repeat for each strain)
- Image in notebook (page 96)

Interlab Study
Aim: To transform test devices into DH10B and DH5**α** and plating them on chloramphenicol plates
Members: Teresa and Farynna
Procedure: Transformation protocol (electroporation)

- Test device 3 in DH10B and Test device 4 in DH5**α** arced so it was redone.
- Added 2µL of DNA (interlab) to each tube.
- Plated on chloramphenicol
- Image in notebook (page 97)

Inoculation for growth curves
Aim: To inoculate 3 different strains for July 27th growth curves
Members: Heather
Procedures: View inoculation procedures

July 27, 2017
Growth Curves
Aim: To record growth over time of MG1655, DH10B, and BTH101 strains. Will be used as refrence and modelling for iGEM
Members: David, Farynna, Heather, Teresa, Ethan, Deanna, Monica, and Abhi
Procedure: At time=x…

- Four 1.5mL tubes for each strain
  - Perform a serial dilution
    - 900µL LB + 100µL culture (first tube)
    - Plate fourth tube
  - Four cuvettes set up (Blank= just LB)
    - Each cuvette filled with 600µL
    - Cuvette 1 = BTH101
    - Cuvette 2 = MG1655
    - Cuvette 3 = DH10B
    - Results are saved in computer
      - Under: iGEM Growth Curves TX)

Restrict, ligate, and transform GVP 2.0 Gblocks into DH10B

Aim: Restrict and ligate each into plasmids: pBAD (for gblocks 1 and 3 of GVP 2.0) and pET11a (for GVP 3.0) (using XhoI/KpnI)

Members: Farynna and Teresa

Procedure:

- GVP 2.0:
  - gblock 1
    - XhoI/HindIII
    - BglII/SpeI
  - Gblock 2
    - XhoI/KpnI
  - Gblock 3
    - XhoI/HindIII
- GVP 3.0
  - XbaI/BglII
- Mix for digestion:
  - 5µL of FD
  - 9µL of DNA
  - 2.5µL of each enzyme
  - 31 µL of ddH2O
    - Cycle:
      - 37C for 1 hr
      - 85C for 10 min
      - 4C for infinity
- Mix for ligation:
  - 1µL of ligase
  - 16µL of insert
  - 2µL of buffer
  - 0.75µL of pBAD/ 1µL of Landon's vector
    - *for GVP 3.0 and GVP 2.0 gBlock 1 digested with SpeI and BglII
  - 17µL of insert
  - 3µL of pET11a vector
  - 2.5µL of buffer
  - 1µL of ligase
  - 1.5µL of ddH2O
    - Cycle:
      - 22C for 20min
      - 70C for 5min
      - 4C for infinity

July 28, 2017

Restrict, ligate, and transform GVP 3.0 Gblocks and DH10B

Aim: Restrict and ligate into CPB plasmid (tinsel) for amplification

Members: Farynna, Teresa, and David
Procedure:

- ❖ Scaled down previous restriction protocol
- ❖ Insert mix (25µL reaction):
  - ➢ 2.5µL Cutsmart
  - ➢ 4.5µL GVP 3.0
  - ➢ 2.5µL BsaI enzyme
  - ➢ 15.5µL ddH2O
- ❖ Vector mix (50µL reaction):
  - ➢ 5µL Cutsmart
  - ➢ 5µL CPB vector
  - ➢ 0.5µL BsaI
  - ➢ 39.5µL ddH2O

Miniprep of RFP + pSB1A3

July 879800-908876574, 2017

August 8, 2017
Diagnostic digests of gvp 3.0 + pSB1A3
Aim: To confirm proper ligation

- Digested with EcoRI and SpeI

Members:
Sequencing of gvp3.0 + pSB1A3 2, 3, and 5

August 9, 2017
Cloning of BACTH into pSB3K3

- Digest pSB3K3 with EcoRI and SpeI
- Digest Pfx + BACTH + Sfx + pSB1C3 with EcoRI and SpeI
- Run digests on a gel
- Gel extract ~2700bp band (pSB3K3)
  - Other band ~1000bp
- Gel extract ~1700bp band (Pfx+BACTH+Sfx+pSB1C3)
  - Other band ~2000bp
- Ligate extracted fragments
- Transform ligation product into DH10B

Aim: To produce a pSB3K3+BACTH construct
Members:
Transformation of interlab plasmids into DH5a

- Transformation (Chemical/electroporation?) of amplified Interlab constructs
- Incubation in recovery medium
- Plate transformation products
- 18+hr incubation

Aim:

Members:

August 11, 2017

Sequencing of gvp 2.0 gblock 2 + Landon's vector (

Co-transformation of BACTH plasmid and pBAD + TurboRFP

- Transform DH10B, BTH101 and MG1655 with eGFP+pSB1C3 construct and the BACTH+pSB1C3
- Plate transformation products
- Pick colonies
- Pellet down cells via centrifugation
- Resuspend in ddH2O
- Transform all samples with TurboRFP+pBAD
- Transform a new set of samples with TurboRFP+pBAD ONLY ?
    - For comparison purposes
- Check for fluorescence

Transformation of Interlab plasmids (pSB1C3/test devices) into DH5a

- Transform DH5a with amplified test devices (Interlab constructs)
    - Via electroporation
- Plate samples
- 1hr incubation in soft media @37C

August 19, 2017

Cloning of BACTH FP reporter into pBAD and pSB1A3

- pBAD for amplification
- pSB1A3 for expression

Cloning into pBAD:

- Digest gBlock with XhoI and HindIII
- Extract digested fragments
- Ligate into pBAD
- Transform ligations and plate on Amp/Ara plates

Cloning into pSB1A3

- Digest gBlock and RFP+pSB1A3 with EcoRI and SpeI
- Run the RFP+ pSB1A3 digestion on a gel
    - Extract ~2kbp band
- Ligate gBlock into pSB1A3
- Transform ligation and plate on Amp/IPTG plates

Transformation of MG1655 and BTH101 competent cells with BACTH FP reporter

- Transform MG1655 and BTH101 cells with BACTH-FP reporter cloned into pSB1A3

August 21, 2017

Miniprep of BACTH FP Reporter+pBAD and GVP 2.0 gBlock 3+pBAD cultures

Aim: To extract the plasmids and eventually confirm the successful insertion of the BACTH FP Reporter or GVP 2.0 gBlock 3 into pBAD through a diagnostic digest

Members: Ethan

- ❖ Followed Miniprep Protocol
- ❖ Followed NanoDrop Protocol

August 22, 2017

Diagnostic Digest of Extracted BACTH FP Reporter+pBAD and GVP 2.0 gBlock 3+pBAD plasmids

Aim: To confirm the successful insertion of the BACTH FP Reporter or GVP 2.0 gBlock 3 into pBAD and select promising samples to be sent for sequencing

Members: Farynna

- ❖ Six of each of the BACTH FP Reporter+pBAD and GVP 2.0 gBlock 3+pBAD samples were digested with XhoI and HindIII following diagnostic digest protocol
- ❖ Expected band sizes of complete digests of BACTH FP Reporter+pBAD are at ~2500 bp and ~4100 bp
  - ➢ Corresponds to the bands seen on the gel except for sample 2
    - ■ A second diagnostic digest was performed on sample 2 with BglII and HindIII. If the BACTH FP Reporter insert was successfully cloned, two bands should be seen on the gel: one at ~1000 bp and another at ~4200 bp. If the picked colony was background then only one band should be see at ~4200 bp.
      - Only 1 band was seen above the ~4000 bp marker, thus this sample likely does not contain the correct insert
- ❖ Expected band sizes of complete digests of and GVP 2.0 gBlock 3+pBAD are at ~2700 bp and ~4100 bp
  - ➢ Corresponds to the bands seen on the gel for all 6 samples

Transformation of eCFP+pSB1A2 and BACTH FP Reporter+pBAD samples into DH10B

Aim: To transform DH10B with eCFP+pSB1A2 and the five extracted samples of BACTH FP Reporter+pBAD plasmids for eventual cloning of BACTH FP Reporter into pSB1A2 for expression

Members: Farynna

- ❖ Followed transformation protocols
- ❖ eCFP+pSB1A2 plasmid was taken from the iGEM Distribution Kit well 23F
- ❖ Transformants for both were plated on ampicillin and arabinose plates

August 23, 2017

Picking Colonies from Transformation Plates of eCFP+pSB1A2 and BACTH FP Reporter+pBAD

Aim: To pick transformants from the plate and prepare for eventual cloning of the BACTH FP reporter gBlock into pSB1A2 for expression
Members: Farynna

- ❖ Followed Inoculation Protocol
- ❖ eCFP+pSB1A2eCFP+pSB1A2 transformants are cultured in 1x ampicillin and 0.2% arabinose
  - ➢ 6 colonies were picked from the plate
- ❖ BACTH FP reporter transformants are cultured in 1x ampicillin and 0.2% arabinose
  - ➢ 2 colonies for each variant were picked from the plate for a total of 10 colonies

August 24, 2017
Miniprep of pSB1A2+eCFP and BACTH FP Reporter+pBAD
Aim: To prepare for eventual cloning of the BACTH FP reporter gBlock into pSB1A2 for expression
Members: Farynna

- ❖ Followed ThermoFisher GeneJet Miniprep Protocol
- ❖ Samples were later assessed for purity and dsDNA concentration following "NanoDrop" Protocol

Cloning of BACTH FP Reporter and GVP 2.0 gBlock 3 into pBAD
Aim: Sequencing results of the second attempt at cloning GVP 2.0 gBlock 3 into pBAD and the first attempt of cloning BACTH FP reporter gBlock into pBAD were unsuccessful, therefore it must be repeated. To clone the BACTH FP reporter gBlock and GVP 2.0 gBlock 3 into pBAD for amplification
Members: Farynna

- ❖ Extracted digested gBlocks were used for ligation
- ❖ Followed Ligation Protocol
- ❖ Plated the ligation product of BACTH FP reporter gBlock + pBAD on ampicillin+IPTG plate
- ❖ Plated the ligation product of GVP 2.0 gBlock 3 + pBAD on ampicillin+arabinose plate

August 25, 2017
Characterization of the commercial BACTH system

- Set up commercial BACTH system
  - **Purpose:** to compare the commercial BACTH system to our modified BACTH system. This serves to increase system chracterization.
- Set up:
  - Transform commercial plasmids into cya- strains
  - Pour plates required for BACTH
  - Prepare B-galactosidase assay
- Testing:
  - BACTH-FP reporter
  - GVP reporter
  - Lac operon Reporter
  -
  -

August 28th, 2017

Transformation of Interlab devices into DH5α
Aim: To transform **DH5α** with miniprepped interlab plasmids
Procedure:

- Followed protocols for electroporation
- Added 1uL of miniprepped plasmid
- Resuspended in 1mL of SOC media and incubated for 1 hour after electroporation before plating
- Transformants are streaked on Chloramphenical plates

August 29th, 2017

Transformation of pSBIA3+GVP3.0 into DH10B
Aim: To transform four pSBIA3+GVP3.0 constructs and PSBIA3+RFP(negative control) into DH10B to eventually perform on buoyancy assay
Members: Ethan, Farynna

Procedure:

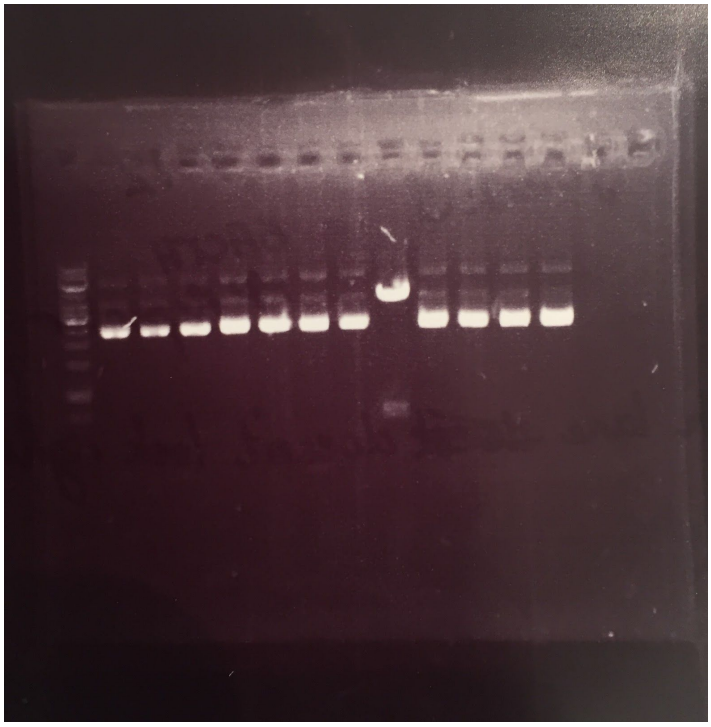
- Followed protocols for electroporation
- 1uL of plasmid
- Resuspended in 1mL of LB media and incubated for 30 minutes
- Transformants streaked on Amp/Ara plates

August 30th, 2017

Transformation of BACTH + pSBIC3 and eGFP (control) + psBIC3 into DH10B, BTH101 and MG1655.
Aim: To begin co-transformation of BACTH+pSBIC3 and TurboRFP + pSpBad for DH10B, BTH101 and MG1655 strains. Control: co-transformation of eGFP+pSBIC3 and TurboRFP+pBAD
Procedure:

- Followed electroporation/transformation protocol
- 1uL of plasmid
- Transformants streaked on chloramphenicol plates

August 31st, 2017



September 1st, 2017

Interlab: Calibration Protocols

OD600 Reference Point

	Max 100%	**H2O**
1	0.033	0.029
2	0.035	0.032
3	0.035	0.029
4	0.044	0.030

	Max 100%	**H2O**
1	0.042	0.035
2	0.043	0.031

3	0.043	0.025
4	0.050	0.035

	Max 100%	**H2O**
1	0.042	0.035
2	0.043	0.031
3	0.043	0.035
4	0.050	0.035

September 4th, 2017

Aim: Extract DNA
Procedure: Thermofisher Plasmid Miniprep Protocol
Observation:

Sample	**Concentration (ug/uL)**	**260/280**	**260/230**
Gvp 3.0 2-1	98.8	1.88	2.12
Gvp 3.0 2-2	66.0	1.88	2.07
Gvp 3.0 2-3	142.8	1.88	2.14
Gvp 3.0 3-1	255.2	1.87	2.25
Gvp 3.0 3-2	109.4	1.88	2.11
Gvp 3.0 3-3	266.3	1.87	2.25
Gvp 3.0 4-1	110.9	1.84	1.59
Gvp 3.0 4-2	93.3	1.85	1.94
Gvp 3.0 4-3	290.8	1.87	2.15
Gvp 3.0 5-1	153.2	1.88	2.13
Gvp 3.0 5-2	152.8	1.88	2.14
Gvp 3.0 5-3	229.6	1.88	2.27

gBlock 1	261.2	1.88	2.28
gBlock 2	272.9	1.86	2.28
gBlock 3	271.1	1.88	2.32
gBlock 4	280.2	1.87	2.28
gBlock 5	282.1	1.87	2.27
gBlock 6	222.0	1.87	2.26

BACTH FP 1	175.8	1.89	2.21
BACTH FP 2	204.2	1.85	2.00
BACTH FP 3	193.9	1.88	2.25
BACTH FP 4	188.8	1.88	2.21
BACTH FP 5	196.3	1.88	2.24
BACTH FP 6	183.4	1.89	2.19

September 5th, 2017

Inoculation of BTH101 Transformed with BACTH and eGFP
Aim: Assay, Innoculation into microcentrifuge tubes
Procedure:

- 2.2mL microcentrifuge
- 0.5mM IPTG
- 600uL LB
- 100ug/1mL chloramphenicol ~1x
- 1uL subculture

September 9th, 2017

Making Agar Plates containing Chloramphenicol and IPTG

- Prepared three batches of LB agar plates following protocols:
  - first batch with ampicillin and IPTG for plating cells with the gvp plasmid
  - second with chloramphenicol for plating cells with the BACTH plasmid
  - third with ampicillin, chPloramphenicol and IPTG for plating cells with the BACTH and gvp plasmids

Summary for September and October:

For the remaining 2 months, we started to collect our data and finalize our results. Rest of the month was spent practicing and preparing for the aGEM presentation, which was held on September 23rd in Edmonton, AB. After this participation, we continued to incorporate changes into the project as suggested by different judges at aGEM.

In the months of October, several things were done:

- Made another batch of plates to replicate all our results and take pictures
- Continue our collaboration with IISc to share and discuss data
- Work towards Wiki Freeze
- Continue progression towards non-wet-lab things, i.e. interviews, collaborations, modelling, and submitting required parts to iGEM.