Mutagenesis Notebook

Workflow

1. Designing primers and creating copies of DNA using PCR
    a. Using expression vector
2. Transform into E. coli strain that expresses well (i.e. WK6 or BL21)
3. Confirm sequences of colonies
4. Confirm transformation and purify proteins
5. Quantify binding affinity
    a. Using OCTET Assay

Cloning and Confirmation of CBD Anchor Binder

M01 - 6/24/19

People: Natalie, Denise, Grace, Shean Fu, Quoc, Brian

Media Prep
Things to make:

- LB
- LB+Amp Plates
- SOC

Mixture recipes can be found on Google Drive
Be sure to autoclave media before use

- Prepared 20% glucose stock
- Prepare 1000x (1 g amp/10mL H20) stock of Amp in autoclaved Milli-Q water, then create aliquots of 10x amp
    - Each aliquot should be 30 uL
    - https://www.goldbio.com/documents/1021/Ampicillin+Stock+Solution.pdf - stock solution of Amp, because working solution is in uL or ug (so 1000x)
    - 400 uL in 200 mL, so 500 uL of 10xb in 250mL

Additional

- Obtain primers for CBD anchor binder sequencing from Shoukai

M02 - 6/25/19

People: Jerry, Malia, Denise, Grace, Chenxing

Transformation

- CBD Anchor Binder plasmids located in 4C fridge, top shelf in door, labeled CA-14
    - DO NOT USE ALL OF IT. There is less than 5uL in the tube, only use 2uL for this transformation, so we still have some left over in case these transformations fail.
- Transform into NEB Competent Cells,
    - Use tubes from -80C Freezer, in a cardboard/paper box from NEB (Should contain around 5 tubes of cells and 1 tube of control DNA. If the box contains multiple tubes with the number 70 on them, that's the wrong box.)
- After transformation, be sure to plate cultures onto LB+Ampicillin Plates
    - Pre-Warm plates!

<u>Procedure:</u>

- Thawed NEB Competent Cells on ice for 25 minutes
- Put 2uL of the CBD Anchor Binder plasmids and 50uL of the NEB Competent Cells into centrifuge tube
- Incubate mixture on ice for 25 minutes
- Heat shock the tip of the mixture for 45 seconds
- Put tube on ice for 5 minutes
- Put 500uL of SOC media into tube
- Put in shaker for 45 min at 250 rpm and 47 degrees celsius
- Put 11 agar plates in the incubator until condensation is not visible
    - Labeled AB - Plate number - Agar Plates
- Spread 50uL of the mixture on each agar plate
- Place in incubator overnight at 37 degree celsius

Mutagenesis

- Dilution of primers
- Stored in -20C Freezer in iGEM Primers box

M03 - 6/26/19

People: Simran, Grace, Vera, Elgene, Quoc, Malia, Ellen, Tom, Shean Fu

Colony Picking

- Pick 20 individual colonies to grow up
- Grow in ~5mL +Amp media
    - Need 100 mL of LB in total, therefore 200 uL of amp.
- Be sure to label, let them grow overnight
- Labeled test tubes as : Day - Plate # - Colony #
    - Currently in the shaker outside J505 at 37 degrees @ 150rpm.
    - Plate numbers written on lids

Inoculate Cultures

- Inoculate 500 mL of LB media with 10 mL starter culture and grow in 37°C shaker.
- Measure the OD600 every hour, then every 15-20 minutes when the OD gets above 0.2.
- When the OD600 reaches 0.35-0.4, immediately put the cells on ice. Chill the culture for 20-30 minutes, swirling occasionally to ensure even cooling. Place centrifuge bottles on ice at this time.
    - OD600 = 0.051 2:25 pm
    - OD600 = 0.077 3:05 pm
    - OD600 = 0.339 4:15 pm

Make Turbo Competent Cells

- Make Competent Cells
- Use Glycerol Stock #58

Make LB Media:

- Make two 1-L portions
    - 10g NaCl, 10g Tryptone, 5g yeast extract
    - Add milliQ H20 to 1L

Make 1M MgCl2

- 20.350g MgCl2 6H2O
- 80 mL di water

M04 - 6/27/19

People: Brian, Aniruddh, Ellen, Shean Fu, Juliana, Jay
Plan: Miniprep CA14 samples and process for sequencing

Miniprep: for selected colonies of CA14 (CBD Anchor-Binder)

- All but 10, 11, 16, 20 do not have pellet.
    - Do mini prep on the blue ones.

		Concentrations:	Renamed:
1.	M03 - 05 - 01	3.2 ng/ul	M04-01
2.	M03 - 01 - 01	2.7 ng/ul	M04-02
3.	M03 - 10 - 02	1.7 ng/ul	M04-03
4.	M03 - 08 - 02	1.5 ng/ul	M04-04
5.	M03 - 08 - 01		
6.	M03 - 02 - 02	3.0 ng/ul	M04-05
7.	M03 - 06 - 01	2.9 ng/ul	M04-06
8.	M03 - 10 - 01		
9.	M03 - 07 - 01?		
10.	M03 - 04 - 02	5.4 ng/ul	M04-07
11.	M03 - 011- 02	26.2 ng/ul	M04-08
12.	M03 - 01 - 02		
13.	M04 - 04 - 01		
14.	M03 - 06 - 02		
15.	M03 - 02 - 01		
16.	M03 - 03 - 01	2.0 ng/ul	M04-09
17.	M03 - 07 - 02		
18.	M03 - 03 - 02		
19.	M03 - 011 - 01		
20.	M03 - 05 - 07	3.2 ng/ul	M04-10

Preparing for M05 Miniprep:

- Cell Cultures grown up from M02 CBD Plates
- Grown in LB+Amp
- Labels:
    - M05-01
    - M05-02
    - M05-03
    - M05-04
    - M05-05
    - M05-06
    - M05-07
    - M05-08
- Left in J505 Shaker at 250 RPM at 37C

M05 - 6/28/19

People: Varun, Simran, Jay, Juliana, Jerry
Plan: Miniprep new cultures

Media Preparation:
- 1L of LB for Amp and Chlor Plates
- Made 4 LB+Chlor Plates and 21 LB + Amp plates.
    - Storing the 21 LB + Amp plates in the cold room, 1 LB + Chlor plate in the 4C fridge.

Practice transformation: using PSB1C3-MRFP
- Incubating 3 practice transformation plates with LB + Chlor (PSB1C3 + MRFP)

Miniprep:
- Plates grown from M04: Only M04-08 Grew. Stored in 4C fridge
    - Miniprep put on hold
- Grew up new cultures for M06 using new colony from original plates and colonies from M04-08
- Naming::
    - M05-11 = M04-08
    - M05-12 = new colony
- Left in J505 shaker at 37C at 250RPM

If this does not grow, it might be an issue with ampicillin stock.

M06 - 6/29/19

People: Natalie, Varun, Brian, Chenxing, Jay

Practice transformation results:
VS: 27 positive colonies, from big tube of cells
SS: 4 positive colonies, from PCR tube of cells
JN: 4 positive colonies, from PCR tube of cells
Hopefully perform a miniprep!!

Miniprep:
Only M05-11 grew, running miniprep on that.
Tube 1:
- Label: M05-11 6/29/2019
- Concentration: low
- Start transformation again

Transformation

- MO6-11: transformation sample in centrifuge tube
- Plates labeled:
    - MO6-11-01
    - MO6-11-02
    - MO6-11-03
    - MO6-11-04
    - MO6-11-05
- Stored in 37 degree incubator around 2:00p.m.

Broth Culture:

- Inoculated new broth cultures from previous transformation
- Also inoculated a new broth from the only colony that worked last time (colony 11)
- Put in 37 degree shaker around 1:00 p.m.
- Cultures Naming:

M06 #1	CBD Anchor E. coli colony #1
M06 #2	CBD Anchor E. coli colony #2
M06 #3	CBD Anchor E. coli colony #3
M06 #4	CBD Anchor E. coli colony #4
M06 #5	CBD Anchor E. coli colony #5
M06 #6	CBD Anchor E. coli colony #6
M06 #7	CBD Anchor E. coli colony #7
M06 #8	CBD Anchor E. coli colony #8
M06 #9	CBD Anchor E. coli colony #9
M06 #11	CBD Anchor E.coli colony #11 from previous day

M07 - 6/30/19

People: Shean Fu, Simran, Brian, Ani

Practice transformation results:

Highlighted cultures grew and the rest did not

M06 #1	CBD Anchor E. coli colony #1
M06 #2	CBD Anchor E. coli colony #2
M06 #3	CBD Anchor E. coli colony #3
M06 #4	CBD Anchor E. coli colony #4
M06 #5	CBD Anchor E. coli colony #5
M06 #6	CBD Anchor E. coli colony #6
M06 #7	CBD Anchor E. coli colony #7
M06 #8	CBD Anchor E. coli colony #8
M06 #9	CBD Anchor E. coli colony #9
M06 #11	CBD Anchor E.coli colony #11 from previous day

- Pick M06 - 11 for growth
- Colony labels:

M07-11-01	CBD Anchor E. coli colony #1
M07-11-02	CBD Anchor E. coli colony #2
M07-11-03	CBD Anchor E. coli colony #3
M07-11-04	CBD Anchor E. coli colony #4
M07-11-05	CBD Anchor E. coli colony #5
M07-11-06	CBD Anchor E. coli colony #6
M07-11-07	CBD Anchor E. coli colony #7
M07-11-08	CBD Anchor E. coli colony #8

M07-11-09	CBD Anchor E. coli colony #9
M07-11-10	CBD Anchor E. coli colony #10

*No colonies picked from plate 1

- Plate labels:
  M07-
  - 11-02-01
  - 11-02-02
  - 11-03-01
  - 11-03-02
  - 11-04-01
  - 11-04-02
  - 11-05-02
  - 11-05-01
  - 11-06-01
  - 11-06-02

Nanodrop Data:

Sample Labels	DNA concentration (ng/uL)
M07-01 6/30 VS, AS, TC. CBD Anchor - from colony 6	73 ng/uL
M07-02 6/30 VS, AS, TC. CBD Anchor - from colony 11	48 ng/uL

M08 - 7/1/19

People: Natalie, Grace, Simran, Shean Fu

Culture Growth Results
Grew well:

- M07-11-02-02
- M07-11-04-01
- M07-11-03-02
- M07-11-05-02

Grew bad

- M07-11-06-01
- M07-11-05-01
- M07-11-03-01
- M07-11-02-01
- M07-11-04-02
- M07-11-06-02

Miniprep
Selected highlighted samples

Nanodrop data for DNA Concentration:

M07-11-02-01	n/a
M07-11-02-02 (M08-01)	66.5 ng/uL
M07-11-03-01	n/a
M07-11-03-02 (M08-02)	65.1 ng/uL
M07-11-04-01 (M08-03)	66.1 ng/uL
M07-11-04-02	n/a
M07-11-05-01	n/a
M07-11-05-02 (M08-04)	64.9 ng/uL
M07-11-06-01	n/a
M07-11-06-02	n/a

Samples sent for sequencing
Green: Sequencing Good
Red: Sequencing Bad

Sample Name	Name for Sequencing
M07.1	KA1
M07.2	KA2
M07-11-02-02 (M08-01)	KA3
M07-11-03-02 (M08-02)	KA4
M07-11-04-01 (M08-03)	KA5
M07-11-05-02 (M08-04)	KA6

M09 - 7/2/19

People: Natalie, Grace,Chenxing, Shean Fu
Mutagenesis Part 1: PCR

- Use M08-01 for mutagenesis, renamed to M09 - 01
- Reaction components:

Components	**25 µl RXN**	**FINAL CONC.**
Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µl	1X
10 µM Forward Primer	1.25 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	0.5 µM
Template DNA (1–25 ng/µl)	0.4 µl	1-25 ng
Nuclease-free water	9.6 µl	

Create new PCR file names q5mutagen -> muta

- PCR Settings for Thermocycler:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25 Cycles	98°C	10 seconds
	62°C*	20 seconds
	72°C	4500 bp so 125 seconds
Final Extension	72°C	2 minutes
Hold	4	

Making Media:

- Made 750mL of LB Broth
    - Autoclaved, but about 160mL spilled out
- Added 590uL of amp to the mixture
- Made ~25ish plates and put them into the cold room

Mutagenesis Part 2: Digestion

- Digestion components:

Components	VOLUME	FINAL CONC.
PCR Product	1 µl	
2X KLD Reaction Buffer	5 µl	1X
10X KLD Enzyme Mix	1 µl	1X

Nuclease-free Water	3 µl	

- Mix well by pipetting up and down and incubate at room temperature for 5 minutes.
- Run digestion cycle. Product will be used for ligation followed by transformation.

Mutagenesis Part 3: Transformation

- Protocol:
  1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice.
  2. Add 5 µl of the KLD mix from Step II to the tube of thawed cells. Carefully flick the tube 4-5 times to mix. Do not vortex.
  3. Place the mixture on ice for 30 minutes.
  4. Heat shock at 42°C for 30 seconds.
  5. Place on ice for 5 minutes.
  6. Pipette 950 µl of room temperature SOC into the mixture.
  7. Incubate at 37°C for 60 minutes with shaking (250 rpm).
  8. Mix the cells thoroughly by flicking the tube and inverting, then spread 50-100 µl onto a selection plate and incubate overnight at 37°C. It may be necessary (particularly for simple substitution and deletion experiments) to make a 10- to 40-fold dilution of the transformation mix in SOC prior to plating, to avoid a lawn of colonies
- Serial dilutions- 3 plates for each dilution
  - 10 fold - 50ul cells into 450ul SOC
  - 20 fold - 20ul cells into 380ul SOC
  - 30 fold - 10ul cells into 290ul SOC
  - 40 fold- 10ul cells into 390ul SOC
- Plate labels and their dilution:

M09-01	10x dilution
M09-02	10x dilution
M09-03	10x dilution
M09-04	20x dilution
M09-05	20x dilution
M09-06	20x dilution
M09-07	30x dilution
M09-08	30x dilution
M09-09	30x dilution

M09-10	40x dilution
M09-11	40x dilution
M09-12	40x dilution
M09-13	Original sample
M09-14	Original sample

M10 - 7/3/19

People - Varun, Grace
Plan: Picking Colonies

- Picked 13 colonies from M09 - 13
    - 10 plates called M10 - 01 to 10
- 3 colonies picked from plate M09-14:
    - Make 3 plates labeled M10 - 11 to 13
- Plates put in -4 degree celsius
- Put into Gu Lab's top shaker 37C at 250 rpm at 5:45 pm

M11 - 7/4/19

People: Jay, Varun, Aniruddh, Ellen, Tom
Plan: Miniprep all M10 samples

Miniprep

- Using Invitrogen PureLink Quick Plasmid Miniprep Kit
- Plating each sample to keep colonies
- Label same as the sample (M10-01, etc.), using 70 uL each
- Spilled sample M10-4
    - Will not plate, will not miniprep
- Sample labels and their DNA concentrations
    - M10-01 38.7 ng/ul Renamed to M11-01
    - M10-02 107.4ng/ul Renamed to M11-02
    - M10-03 51.6ng/ul Renamed to M11-03
    - M10-05 38.1ng/ul Renamed to M11-04
    - M10-06 38.5ng/ul Renamed to M11-05
    - M10-07 49.9ng/ul Renamed to M11-06
    - M10-08 69.8ng/ul Renamed to M11-07

- M10-09 80.2ng/ul Renamed to M11-08
- M10-10 75.8ng/ul Renamed to M11-09
- M10-11 70.6ng/ul Renamed to M11-10
- M10-12 92.5ng/ul Renamed to M11-11
- M10-13 97.8ng/ul Renamed to M11-12

M12 - 7/5/19

People: Quoc,chenxing, Jerry, Jay

Task Done:
Made TBI: 24 g yeast extract, 20 g tryptone, 4 ml glycerol, 900 ml di water.

- Amounts of yeast and tryptone got mistaken for one another → TBI thrown out.

Made TBII: 1L potassium phosphate solution. 23g monobasic potassium phosphate, 125g dibasic potassium phosphate.

Other results:
Plates from M11 grew well, stored in 4C fridge

M13 - 7/6/19

People: Brian, Jay, Juliana,Chenxing

Task Done:
Made TB Media: 24 g yeast extract, 20 g tryptone, 4 ml glycerol, 900 mi di water

M14 - 7/8/19

People: Jay, Brian, Shean Fu, Juliana

Task(s) Done:
Samples from miniprep sent for sequencing

M11 Plates Results:

M11-01	Bad

M11-02	Bad
M11-03	Bad
M11-04	Good
M11-05	Good
M11-06	Good
M11-07	Good
M11-08	Good
M11-09	Good
M11-10	Bad
M11-11	Good
M11-12	Bad

M15 - 7/9/19

People: Joanne, Jerry, Denise and Natalie, Malia
Plan: Transformation

Transformation:

- Choose one of the good M11 plates to transform in WK6 Bacteria
    - Ask Shoukai for WK6 strain to transform
- Also Transform DNA Sample M08-01 into different WK6
    - This will be used as a reference alongside the mutated binder for binding assay
- Procedure:
    1. Add 50 uL of WK6 strain into a microcentrifuge tube and add ~10ng of plasmid (M11 - 04 or M08 - 01) into the tube
        a. Added 0.15uL of the M08-01 to a microcentrifuge tube
        b. Added 0.26uL of the M11-04 to a microcentrifuge tube
    2. 30 minutes on ice
    3. 45 secs at 42ºC
    4. 2 mins on ice
    5. 45 mins in 450 uL SOC broth in 37ºC incubator
    6. Plate onto LB+Amp plates

Other tasks done:

- Prepare two 1L flasks for protein expression

M16 - 7/10/19

People: Grace, Varun

Inoculation:

- Pick 2 colonies from each plate
- Follow inoculation instructions from nature protocol (linked above)
- Inoculate a single colony into a 50-ml sterile Falcon tube containing 10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2. Grow the preculture overnight at 37 °C and 170 r.p.m.
    - 100 uL of 100 mM MgCl2 -> 1 mM MgCl2
- Make two tubes for each type of preculture
    - M08-01 1
    - M08-01 2
    - M11-04 1
    - M11-04 2

Other task(s) done:

- Autoclave glassware, be sure to autoclave the 2L flasks

M17 - 7/11/19

People: Jay, Malia, Ani, Brian, Shean Fu, Ellen, Elgene, Juliana
Plan: protein expression

Protein Expression:

- Inoculated protein expression cultures in 330mL TB w/ .33g glucose, 330uL 1M MgCl2, and .033g Amp
    - Used liquid cultures M08-01-2 and M11-04-2
- Left in Gu-Lab Top Shaker at 170 RPM at 37C
    - Started at 12:45PM
    - Check OD at ~5:00PM
        - OD concentration @ 5:15pm
            - M08-01 0.063
            - M11-04 0.160
        - @ 7 pm
            - M08-01 0.365
            - M11-04 0.770
            - M11-04 was spiked with 333 ul of IPTG and placed back into top incubator at 170 RPM and 28C
        - @7:48 pm

    - M08-01 0.605
    - Spiked with 333 ul of IPTG and placed back into top incubator 170 RPM 28C

Making Media:

- Making 10 mL Media for blanking: 0.01 g glucose, 0.1 uL Amp, 10 uL 1 M MgCl2, 9 mL TB and 1 mL TBII
- Made 1M solution of ~2.098 mL IPTG

M18 - 7/12/2019

People: Jay, Varun, Quoc, Chenxing, Jerry, Ellen

Plan: Protein Extraction, Protein Purification, Desalting

Protein Extraction:

- Followed steps 72 and 73 of the Nature Protein Extraction Protocol for M08-01 and M11-04
    - 1st incubation @ 10:22a lasting for 1 hour on an orbital shaker until 11:22a
    - 2nd incubation @ 11:38a lasting for 45 minutes on an orbital shaker until 12:23p
- Weight difference 9-10 g, took out 4 mL
- Centrifuged suspension for 30 min at 10,000g for 30 min - collected supernatant in two 50 mL falcon tubes

Protein Purification:

- Purification Steps:
- Placed tubes in protein purification pump (in J556): tube 1 into M08-01, tube 2 into M11-04 - pumped lysate through elution column for about 45 min @~1:20 pm until volume in falcon tubes was less than 5 mL (collection automatically stops)
- Labeled two 15 mL Falcon tubes with M08-01 and M11-04 for elution collection steps

  <u>Results:</u> (from FPLC machine in J500 cold room):
- M08-01 had very low concentration - UV second large peak not present - 1st distillation collected in 15 mL Falcon Tube
    - Luis and Shoukai suggested: inoculate culture into 10 mL TB, let grow, then inoculate into 1 L TB for quicker growth - take OD ~6-7 for quicker growth
- Need to repeat inoculation steps starting from M16 (7/10)

Repeated purification with M11-04

- Same results as M08-01
- Made new starter cultures like on M16
- Supplemental Media: 10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2.
- Tubes for Starter Cultures (from blue markings on plates):

- M08-01
- M11-04-1
- M11-04- 2
- Incubated in Gu Lab bottom shaker at 37 C and 170 rpm

M19 - 7/13/19

People: Natalie, Brian, Jerry, Juliana,Chenxing

Cultures from M18 did not grow, will re-inoculate starter cultures again tonight.

Prepared 2 900mL Solutions of TBI

Will regenerate His-Trap Columns

People: Ani, Varun, Chenxing

Made new starter cultures again:
Media: 10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2.
For 3 tubes worth of culture:

- 30 mL LB
- 0.003 g ampicillin
- 0.6 g glucose
- 0.3 mL 100mM MgCl2

Tubes (from black dots on plates)

- M08-01 1
- M11-04 1
- M11-04 2

Made new (backup) plates:

- M08-01-01
- M011-04-02
- Stored in 37’C incubator

M20 - 7/14/19

People: Ani, Brian, Shean Fu, Tom, Vera, Grace

Plan: Grow cultures in TB Media, Induce protein expression, make glycerol stocks of CBD-Anchor Binder in WK6 and of CBD-Anchor Mutant Binder in WK6

Protein Expression:

- Wrong glucose concentration, remaking the TBI solution (2 x 900 ml)
- Making 10 mL Media for blanking: 0.01 g glucose, 0.1 uL Amp, 10 uL 1 M MgCl2, 9 mL TB and 1 mL TBII
- Make two 1L of culture in top Gu shaker:
    - 1000 mL TB
    - 1 g glucose
    - 1 mL 1 M MgCl2
    - 0.1 g Amp
- Cultures grown in 1 L TB: M08-01-1, M11-04-02
- Put in at 1ish, check for OD until it reaches 0.7
  OD @ 8
    - M08-01 1 = 0.036
    - M11-04 2 = 0.721

  OD @10
    - M08-01 1 = 0.567

  OD @10:15
    - M08-01 1 = 0.697
- Plate M08-01-1 and M11-04-2

M21 - 7/15/19

People: Joanne, Grace, Brian and Shean Fu

Plan: Spin down colonies in high speed centrifuge, store pellets in -80C freezer until protein purification

Protein Purification Preparation:

- 400 mL each tube
- Centrifuge tube with S is mutant (2)
- Pellets are in large centrifuge tubes 1 and 2, placed upright in bottom left of -80C

- Regeneration of His-Traps by stripping and recharging

- Procedure
  1. Strip the resin by washing with 10 column volumes (10 mL) of stripping buffer (100 mM EDTA;
  pH 8.0).
  2. Wash the resin with 20 column volumes (20 mL) of deionized water.
  3. Recharge the water-washed cartridge by loading 2 column volumes of 100 mM NiSO4 (in deionized water). Salts of other metals (chlorides or sulfates) may also be used.
  4. Wash with 10 column volumes of deionized water and re-equilibrate with 10 column volumes of Buffer NPI-10.
  The cartridge is now ready for use. Store cartridge in 20–30% ethanol or 10–100 mM NaOH.

M22 - 7/16/19

People: Natalie, Denise, Joanne, Malia, Elgene, Chenxing, Shean Fu, Juliana

Plan: Follow Nature Protocol Steps 72-73: Extract protein from cells using TES and ¼ TES, load supernatant onto His-Trap columns, run FPLC, desalt proteins

Protein Extraction
Procedure:
- Resuspend the cell pellet of 1L of culture in 15mL of ice-cold TES
- Incubate it for at least 1h on ice on an orbital shaking platform (in room next door)
- Add 30mL of TES/4 buffer to the resuspended pellet
- Shake for 45 min on ice on an orbital shaking platform
- Centrifuge the suspension for 30 min at 10,000g at 4C and recover the supernatant as the periplasmic extract

Protein Purification:
Purify the His-tagged Nanobodies from the periplasmic extract by using IMAC according to the manufacturer's instructions. This expression and purification protocol routinely yields 1–10 mg per liter of culture with an estimated purity of ≥95%. For crystallography-grade Nanobodies, a subsequent polishing step via size-exclusion chromatography is often required.
- Column labelled iGEM is the M08 sample; acting as control

<u>Buffers to make and need to filter</u> (1L):
- Wash buffer (NPI-20)
  - 50 mM NaH2PO4 (6.90 g NaH2PO4*H20 MW 137.99 g/mol)
  - 300 mM NaCL (17.54 g NaCL MW 58.44 g/mol)
  - 20 mM imidazole (1.36 g imidazole MW 68.08 g/mol)
  - Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 um)

- Elution buffer(NPI-250)
    - 50 mM naH2PO4 (6.90 g NaH2PO4*H20 MW 137.99 g/mol)
    - 300 mM NaCL (17.54 g NaCL MW 58.44 g/mol)
    - 250 mM imidazole (17.0 g imidazole MW 68.08 g/mol)
    - Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 um)
- Results
- Completed purification but we didn't get good results for our mutant sample, therefore inoculating two new starter mutant sample cultures overnight. Took glycerol stocks 135 and 136 for the cultures.
- Media: 10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2.
- For 3 tubes worth of culture:
    - 30 mL LB
    - 0.003 g ampicillin
    - 0.6 g glucose
    - 0.3 mL 100mM MgCl2

Desalting

1x PBS + 10% glycerol + ddH2O (can prepare more) (make final concentration 1x PBS, use 10x PBS if have in stock for the dilution)

- Make 2 500mL bottles of solution
- Add to each bottle: 100 mLof 50% Glycerol, 50 mL of 10pbs bux PBS, fill with DI H20 until 500mL reached

** Use the FPLC machine for Purification and Desalting **

M23 - 7/17/19

People: Natalie, Varun, Joanne

- Put the starter cultures in the 4º fridge- labeled as M11-04-01 and M11-04-02
- New plan:
    - Measure concentrations of proteins
    - Confirm that the mutant plate is not full of false positive (miniprep to confirm that there is something), if sequencing doesn't come back good, redo transformations

M24 - 7/18/19

People: Jay, Ani, Shean Fu
Task done: Miniprep & plating

- Completed miniprep of M11-04 samples 1-6 (all of them)
- Concentrations of DNA in the M11-04 samples
  M24-01 37.6 ng/ul
  M24-02 38 ng/ul
  M24-03 38 ng/ul
  M24-04 54.6 ng/ul
  M24-05 30.2 ng/ul
  M24-06 41.6 ng/ul
- New plates were made and labelled M24-01 through M24-06

M25 - 7/19/2019

People: Tom, Jerry, Ellen, Varun, Jay

Plan: prepare samples for sequencing

Sequencing:
Amount in tubes:

- 30 uL pHis 4 primer for 6 reactions (primer tube)
- 10 uL plasmid (all others)

Sequencing tubes:

- JY 1
- JY 2 Sequence Bad
- JY 3
- JY 4
- JY 5
- JY 6
- JY pHis 4 (primer tube)

Making Media:

- 2 batches of TB Media:
- Recipe for each:
  - 24 g yeast extract,
  - 20 g tryptone,
  - 4 ml glycerol,
  - 900 mi di water

M26 - 7/20/2019

People: Vera, Natalie, Jay, Brian, Juliana

Plan: Inoculate starter cultures and prepare plates

- Used M-24-04

Picked 5 colonies from plate, tubes labeled:

- M26-01 renamed (JY-01)
- M26-02 renamed (JY-02)
- M26-03 renamed (JY-03)
- M26-04 renamed (JY-04)
- M26-05 renamed (JY-05)

Put in shaker at 230 rpm and 37 degrees

M27 - 7/21/19

People: Brian, Shean Fu, Tom, Ani, Grace

Plan: Miniprep cultures from yesterday, send for sequencing

Sample	Concentrations
• JY-01 (M27-01)	62 ng/ul
• JY-02 (M27-02)	42.7 ng/ul
• JY-03 (M27-03)	55.8 ng/ul
• JY-04 (M27-04)	67.8 ng/ul
• JY-05 (M27-05)	46.2 ng/ul

Additional sequencing tube: JY pHis 4 (primer tube)

Sequencing Results: All came back positive for the correct mutation!

M28 - 7/22/19

People: Natalie, Grace, Shean Fu, Jay

Making Media:

- 1L LB agar ~ 40 LB agar plates made

Inoculate cultures:

- 3 cultures made in LB + amp, glucose, and MgCl2
- Picked from M27-03
- Labels:
  M28-01
  M28-02
  M28-03
- Left in Gu Upper Shaker

M29 - 7/23/19

People: Natalie,Joanne, Denise,Chenxing, Malia

Protein Expression:

Grew up wk6 cells from culture M28-01

Nature Protocol Steps:

- Inoculate 330 ml of TB supplemented with 100 μg ml−1 ampicillin, 0.1% (wt/vol) glucose and 1 mM MgCl2 in a 1-liter baffled flask with 3 ml of the preculture.
- Shake the mixture at 37 °C and 170 r.p.m. until it reaches an OD600 of 0.7.
- Induce Nanobody expression with 1 mM IPTG (final concentration) and grow overnight at 170 r.p.m. and 28 °C. Alternatively, induce Nanobody expression for 4 h at 170 r.p.m. and 37 °C.

Started incubating at 10:50 am, check for OD until reaching 0.7

OD at 3pm: 0.082
OD at 5pm: 0.263
OD at 6:20pm: 0.777

Added IPTG to make final concentration 1mM

Placed in Gu lab top shaker 28ºC

M30 - 7/24/19

People: Varun, Joanne

Protein Extraction
Spun down sample, pellet is in bottom shelf of -80 freezer

Protein Purification Media Preparation
Made wash buffer and elution buffer
Recipe:

- Wash buffer (NPI-20)
    - 50 mM NaH2PO4 (6.90 g NaH2PO4*H20 MW 137.99 g/mol)
    - 300 mM NaCL (17.54 g NaCL MW 58.44 g/mol)
    - 20 mM imidazole (1.36 g imidazole MW 68.08 g/mol)
    - Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 um)

- Elution buffer(NPI-250)
    - 50 mM naH2PO4 (6.90 g NaH2PO4*H20 MW 137.99 g/mol)
    - 300 mM NaCL (17.54 g NaCL MW 58.44 g/mol)
    - 9250 mM imidazole (17.0 g imidazole MW 68.08 g/mol)
    - Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 um)

M31 - 7/25/19

People: Varun, Joanne, Quoc, Elgene, Ellen, Malia

Protein Purification
For M24-04 (TES incubation, FPLC, desalting)
Used a new column so ran a blank run through it before proceeding for protein purification.

M32 - 7/26/19

People: Varun, Jerry, Jay, Chenxing

Desalting
Protein concentration:

- M08-01 (wild type): 0.153
- M28-01 (mutant): 0.102

Both samples have contamination, so we are remaking the desalting buffer

- Got weird peaks around 220 nm

Biotinylation

Recipe:

- 4mL of desalted nanobody
- 0.5 mL of BirA mixA
- 0.5 mL of BirA mixB
- 3uL BirA enzyme

Scaled down reaction:

- Wild type: m08-01
    - 2mL of desalted nanobody
    - 0.25 mL of BirA mixA (done)
    - 0.25 mL of BirA mixB (done)
    - 1.5uL BirA enzyme (done)
- Mutant: m28-01
    - 2.5 mL of desalted nanobody
    - 0.31 mL of BirA mixA (done)
    - 0.31 mL of BirA mixB (done)
    - 1.88 uL BirA enzyme(done)
- Keep away from light until 12:07 p.m. (for one hour)

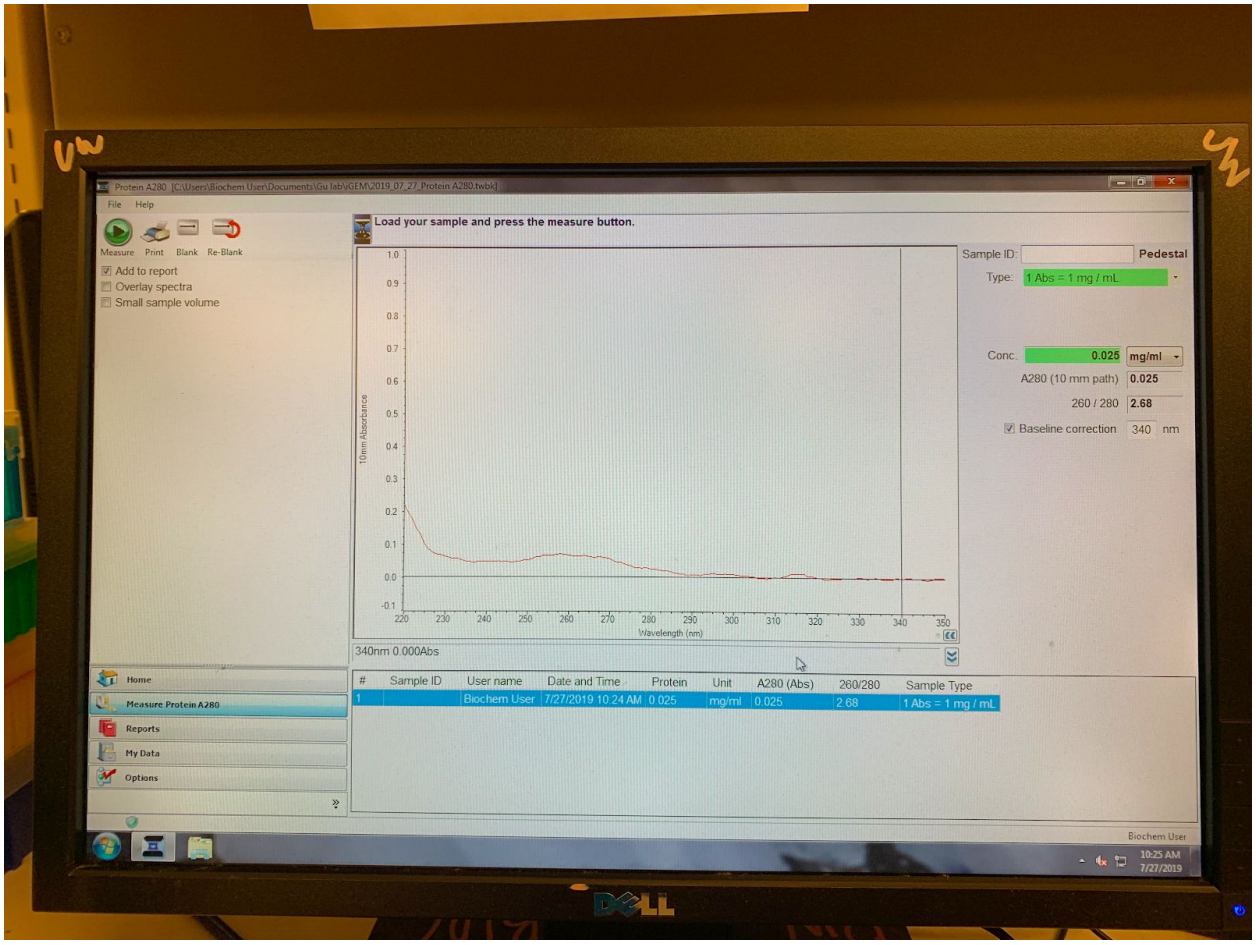
Note: When desalting, accidentally lost the wild type protein

- Mutant protein tube in bottom right of 4 C fridge in neon yellow holder

M33 - 7/27/19

People: Jay, Jerry, Natalie, Chenxing

Protein concentration:



Made TB Broth:
Made 2L of TBI: 24 g yeast extract, 12 g tryptone, 4 ml glycerol, 900 ml di water in each 2 L flask
Made 500mL of TBII: 11.55g monobasic potassium phosphate, 62.7g dibasic potassium phosphate, 500mL of water

Remaking starter culture: for mutant protein
Made new starter cultures again:
Media: 10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2.
For 3 tubes worth of culture:

- 30 mL LB
- 30 ul for ampicillin

- 0.6 g glucose
- 0.3 mL 100mM MgCl2

Taken from M07-11-02-02 (M08-01) with WK6 made on 7/9/19
Cultures Labeled:

- M33-01
- M33-02
- M33-03

Placed in shaker by J505 at 37 degree C at 170rpm

Restreaked plate with M08 w/WK6. Labeled it “M33 - Anchor Binder with WK6 (Wild Type)”

M34 - 7/28/19

People: Shean Fu, Brian, Ani, Vera, Grace
Supplemental media:
Making 10 mL Media for blanking: 0.01 g glucose, 0.1 uL Amp, 10 uL 1 M MgCl2, 9 mL TB and 1 mL TBII

Plating:
Restreaked plate with M33-01. Labeled it “M34 - Anchor Binder with WK6 (Wild Type)”

Growing starter culture: for M33 - 01
Recipe:

- 1 L of culture (labeled M34 - Anchor Binder with WK6 (Wild Type)) in top Gu shaker:
- 1000 mL TB
- 1 g glucose
- 1 mL 1 M MgCl2
- 0.1 g Amp

Put in @ 10 am, check OD until it reaches 0.7

- OD Reading 1, 2:30 pm: -0.003
- OD Reading 2, 4:30 pm: 0.110
- OD Reading 3, 6:00 pm: 0.376
- OD Reading 4, 6:30 pm: 0.545
- OD Reading 5, 6:40 pm: 0.633

M35 - 7/29/19

People: Natalie, Grace, Shean Fu

Spun down cells from culture M34 into two bottles

Bottles Labeled:
- M35-01
- M35-02

M36 - 7/30/19

People: Jay, Shean Fu, Vera, Malia

OCTET Preparation
Sample Concentrations
- WT CBD Anchor Binder: 52.9 uM
- Mutant CBD Anchor Binder: 1.76 uM

For OCTET, dilute samples with buffer to 200 nM
- For WT, dilute 265X
- For Mutant, dilute 9X

<u>Preparing Reagents for OCTET</u>
- What we need:
    - 1X PBS
    - 20% Tween-20
    - 20% BSA
- Final OCTET buffer solution: 1XPBS + 0.05% Tween-20 + 0.2% BSA
- For our OCTET, Shoukai's WT anchor binder contains 10% glycerol, while ours is 5% glycerol, so we will add glycerol to the buffer solution to make it even
- Our final OCTET Buffer Solution: 1XPBS + 0.05% Tween-20 + 0.2% BSA + 1% Glycerol
- Each well in the OCTET test plate will need 200 uL of sample+buffer
- For this experiment, we will use 8 wells: 2 for WT, 2 for Mutant, and 4 for test condition
- Flash Froze WT and Mutant Nanobody in small aliquots with liquid nitrogen
- Stored in -80C Freezer in new box labeled CBD Anchor Binders: WT and Tyr-to-Ala-32 Mutant
- Eppendorf Tubes labeled with sample name

M37 - 7/31/19

People: Varun, Joanne, Denise, Quoc

Plan:

Making BSA

- Prepare 20% BSA (25mL). Ask Shoukai if we can borrow some from the Gu Lab (we can buy BSA later on and give it back to them if need be)
    - 62.5 uL of 100xBSA with remaining 24.9375 mL of water

Research:

- Look into the OCTET Assay and how it works
- Helpful video OCTET Assay video
- Look into possible uses of a small molecule biosensor
    - Not limited to CBD detection, but CBD detection is also one thing to research
- Research submission guidelines for iGEM Biobricks for 2019
- Research Gibson Assembly

Clean up the lab

- Clean dishware, autoclave clean

Take Inventory

- Look through what's in the lab, make a new Excel Spreadsheet to take inventory of things in the lab and make note of things we are low on

M38 - 8/1/19

People: Shean Fu, Malia, Ellen, Elgene, Varun, Quoc

Plan: Run OCTET Assay for WT and Mutant CBD Anchor Binders

- Prepare plates with samples and buffers (quantities noted in M36)

OCTET Assay

Protein dilutions:

- Wild type
    - 1.3225 uL protein
    - 198.6775 uL buffer
- Mutant
    - 4.4 uL protein *WRONG CONCENTRATION*
    - 195.6 uL protein

Buffer recipe: 10mL in total

- 25 uL of 20% tween
- 100 uL of 20% BSA

- 1 mL of 10% glycerol
- PBS 8.875 mL

CBD Dilution from 300uM CBD solution in 100% methanol

- 10uM
    - 0.67uL CBD
    - 3.33 uL 100 % methanol
    - 196 uL buffer without methanol
- 25uM
    - 1.67uL CBD
    - 2.33 uL 100 % methanol
    - 196 uL buffer without methanol
- 50uM
    - 3.33uL CBD
    - 0.67 uL methanol
    - 196 uL buffer without methanol

Well lay out

	1	2	3	4	5	6	7	8	9	10	11
E	buffer	W	buffer	buffer	buffer	buffer	10uM CBD	buffer	25uM CBD	buffer	50uM CBD
F	buffer	W	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer
G	buffer	M	buffer	buffer	buffer	buffer	10uM CBD	buffer	25uM CBD	buffer	50uM CBD
H	buffer	M	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer

Results: Bad Results from M38, concentration of mutant protein was too low. Redo OCTET.

M39 - 8/2/2019

People: Varun, Jay

OCTET Preparation
Redoing Octet Assay but with fixed mutant protein concentration

Correct protein concentrations

- Wild type
    - 1.3225 uL protein
    - 198.6775 uL buffer
- Mutant
    - 22.73 uL protein
    - 177.27 uL buffer

note adding mutant straight from sample, so amounts differ from yesterday

Mutant protein amounts in wells

- 2 uL BSA
- 0.5 uL Tween
- 4 uL methanol
- 193.5 uL protein

M40 8/6/2019

People: Jay, Chenxing, Shean Fu

OCTET Assay
Buffer recipe: 12mL in total

- 30 uL of 20% tween
- 120 uL of 20% BSA
- 1.2 mL of 10% glycerol
- PBS 10.41 mL
- 240 uL 100% methOH

CBD Dilution from 300uM CBD solution in 100% methanol

- 50uM
    - 3.33uL CBD
    - 0.67 uL methanol
    - 196 uL buffer without methanol
    - For 25 uM, 12.5 uM, 6.25 uM, dilute 2x using the previous concentration

Well Layout

	1	2	3	4	5	6	7	8	9	10	11	12
E	buffer	W	buffer	buffer	buffer	6.25 uM CBD	buffer	12.5 uM CBD	buffer	25uM CBD	buffer	50uM CBD
F	buffer	W	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer
G	buffer	M	buffer	buffer	buffer	6.25 uM CBD	buffer	12.5 uM CBD	buffer	25uM CBD	buffer	50uM CBD
H	buffer	M	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer	buffer

M41 - 8/8/19

People: Jay

OCTET Analysis:
The results from the OCTET initially show that the mutation did not actually change the binding property that much. We had too low of a concentration of the mutant to say with greater certainty, so we will grow 2 1L cultures of the mutant to do protein expression, purification, biotinylation, and OCTET again.

Other tasks:
Replated M27-03

M42 - 8/9/19

People: Jay

M27 - 03 failed, replating again!

M43 - 8/10/19

People: Natalie, Brian, Jerry, Ellen, Chenxing, Vera

Inoculation

10 ml of LB supplemented with 100 µg/ml ampicillin, 2% (wt/vol) glucose and 1 mM MgCl2 (100 ul mM MgCl2).

600 ul Mg, 1.2 g glucose, 6000 ug amp, 60 mL LB

Label: M27-03 Cultures

Left on Gu's top shaker

M44 - 8/11/19

People: Simran, Grace, Shean Fu, Ani, Tom, Ellen, Vera

Protein Expression:
For 1 L of culture

- 1000 mL TB (900 mL TB1 with 100 mL TB2)
- 1 g glucose
- 1 mL 1 M MgCl2
- 0.1 g Amp (10 uL Amp)

Check OD until it reaches 0.7

- 0.122 @ 3:00 p
- 0.322 @ 5:00 p
- 0.513 @ 5:40p
- 0.546 @ 5:50p
- 0.616 @ 5:58p

Making 10 mL Media for blanking: 0.01 g glucose, 0.1 uL Amp, 10 uL 1 M MgCl2, 9 mL TB and 1 mL TBII

Inoculation:
Only one culture grew over night- M27-03-05
Used for Wk6
Plated M27-03-05
    Label- M44-01
Spiked with IPTG

M45 - 8/12/19

People: Natalie, Quoc, Grace, Shean Fu

Plating
Streaked colony from plate M44-01

- New Plate labeled M45-01

Protein Extraction
Centrifuging pellets from large WK6 cells

M46 - 8/14/19

People: Simran, Joanne

Plan:

1. Lyse cell culture for mutant CBD Anchor WK6
2. Purify proteins
3. Desalt

Desalted proteins kept in 4C fridge. There are two 15 mL falcon tubes labeled desalted protein #1 and #2. For biotinylation, use #2 because it has a higher concentration of the desalted protein.

M47 - 8/15/19

People: Shean Fu, Malia, Ani, Chenxing, Elgene

Plan: Biotinylate 1.8mL of purified protein, run overnight. We may have to regrow up cultures again since it looks like concentration is pretty low.

Biotinylation
Biotinylation solution:

~1.8-2mL protein
0.1mL Bir MixA
0.1mL Bir MixB
2uL BirA (enzyme)

Incubated at 1hr away from light at room temp and then stored in negative 4C fridge to incubate overnight. Wrapped in Tin Foil.

M48 - 8/16/19

People: Simran, Jerry

Plan: Desalt biotinylated protein, will run OCTET with samples to see if concentration is good enough/if binding is affected!

OCTET Assay:
Concentration of protein is… 0.01mg/mL, which is around 0.588uM, which is worse than last round which was 0.03mg/mL

Buffer recipe: 12mL in total

- 30 uL of 20% tween
- 120 uL of 20% BSA
- 1.2 mL of 10% glycerol
- PBS 10.41 mL
- 240 uL 100% methOH * dont add yet

Serial Dilution for CBD
50uM (800uL)

- 13.2uL CBD
- 2.68 uL methanol
- 784 uL buffer without methanol

25uM (800uL)

- 400uL 50uM
- 392uL Buffer
- 8uL MeOH

12.5uM (800uL)

- 400uL 25uM
- 392uL Buffer
- 8uL MeOH

6.25uM (800uL)

- 400uL 12.5uM
- 392uL Buffer
- 8uL MeOH

OCTET Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	WT	B	B	B	6.25	B	12.5	B	25	B	50
B	B	WT	B	B	B	B	B	B	B	B	B	B
C	B	MT	B	B	B	6.25	B	12.5	B	25	B	50
D	B	MT	B	B	B	B	B	B	B	B	B	B

M49 - 8/19/19

People: Simran, Shean Fu, Chenxing, Natalie, Joanne, Jay

Plan:
ONLY DO THIS IF WE HAVE IPTG.

1. Grow up cultures from M45-01 for protein expression
    a. Grow at least 4 test tubes of 10mL cultures. Probably want to do more in case some fail, be sure to have it include the necessary reagents alongside the LB+Amp

M50 - 8/21/2019

People: Joanne, Varun

Growing 4 1L cultures of TB from M49 cultures, were put in the shaker at 37ºC at 170 rpm at 10am. Borrowed a 1L culture flask from the Gu lab, need to return it when we're done.

OD readings:

- 2:25 pm:
    - Flask 1: 0.105
    - Flask 2: 0.079
    - Flask 3: 0.058
    - Flask 4: 0.055
- 4:00pm:
    - Flask 1: 0.162
    - Flask 2: 0.158
    - Flask 3: 0.128
    - Flask 4: 0.165
- 5:30 pm:

    - Flask 1: 0.479
    - Flask 2: 0.400
    - Flask 3: 0.356
    - Flask 5: 0.518
- 6:05 pm:
    - Flask 1: 0.647
    - Flask 2: 0.560
    - Flask 3: 0.539
    - Flask 4: 0.666
- 6:25 pm:
    - Flask 2: 0.651
    - Flask 3: 0.667

Add 0.238 g IPTG to make the final concentration of the culture 1mM.
Placed the four flasks in the Gu lab shaker.

M51 - 8/22/19

People: Jay, Elgene, Malia, Shean

Protein Expression Preparation
Spin down cells for protein expression and stored in -80C. They were very overgrown, as they have been there for over 20 hours, so this may cause problems with protein yield?

M52 - 8/26/19

People: Joanne, Natalie, Jerry, Brian

Plan: Incubate cells with TES, purify proteins, and desalt.

Other tasks done: Make more TES buffer

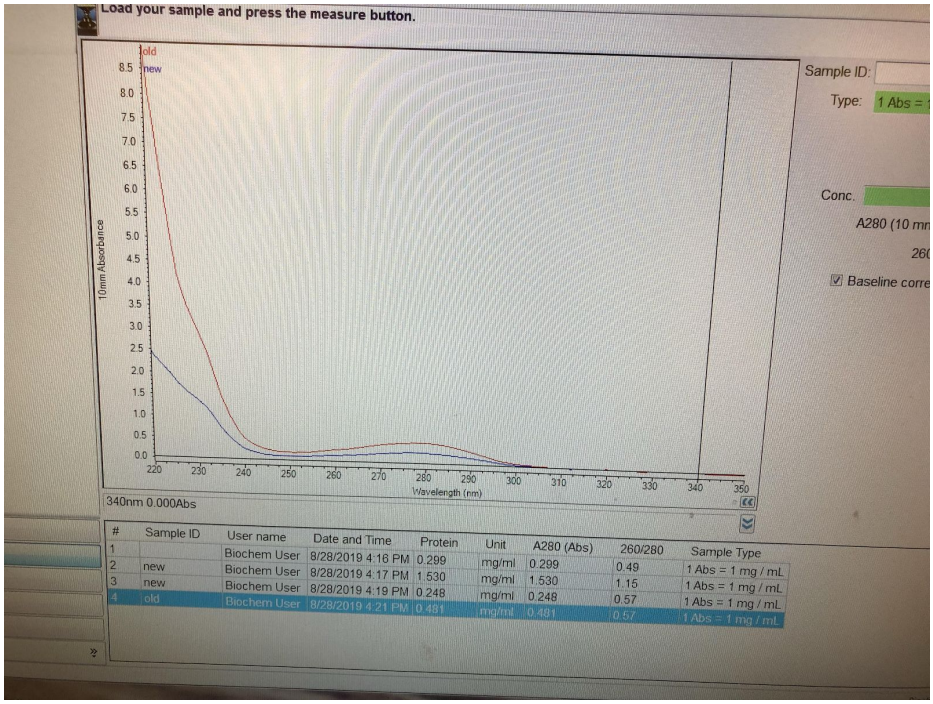
M53 - 8/28/19

People: Varun, Natalie, Malia, Shean, Elgene, Simran

Biotinylation & Desalting
Protein concentration: 0.30 mg/mL
Biotinylated and desalted mutant CA14
Typical recipe:

1. 4mL of desalted nanobody
2. 0.5 mL of BirA mixA
3. 0.5 mL of BirA mixB
4. 3uL BirA enzyme

Take out biotinylated proteins after one hour (12:53 pm)
Desalted biotinylated product concentration: 0.248 mg/mL -> 14.588uM



Stored in 1.5 mL tubes in 4C fridge (blue holder)

M54 - 8/30/19

People: Jay, Varun

Plan: Another OCTET, dilute Mut Protein 26fold for reading.

Sequencing from 8/28/19 confirmed that our colonies are expressing the mutated protein!

OCTET Assay

Buffer recipe: 12mL in total

- 30 uL of 20% tween
- 120 uL of 20% BSA
- 1.2 mL of 10% glycerol
- PBS 10.41 mL
- 240 uL 100% meOH * don't add yet

Wild type amount (52.9uM)

Need 200nM

- 1.3225 uL protein
- 198.6775 uL buffer

Mutant protein amount (26fold dilution)

- 7.69uL protein
- 192.31uL buffer

Serial Dilution for CBD

50uM (800uL)

- 13.2uL CBD (done)
- 2.68 uL methanol (done)
- 784 uL buffer without methanol (done)

25uM (800uL)

- 400uL 50uM (done)
- 392uL Buffer (done)
- 8uL MeOH (done)

12.5uM (800uL)

- 400uL 25uM (done)
- 392uL Buffer (done)
- 8uL MeOH (done)

6.25uM (800uL)

- 400uL 12.5uM (done)
- 392uL Buffer (done)
- 8uL MeOH (done)

OCTET Assay Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	WT	B	B	B	6.25	B	12.5	B	25	B	50
B	B	WT	B	B	B	B	B	B	B	B	B	B
C	B	MT	B	B	B	6.25	B	12.5	B	25	B	50
D	B	MT	B	B	B	B	B	B	B	B	B	B

Results

Poor results; the wells most likely had some form of contamination and resulted in data that was not usable. There were also discrepancies in protein concentrations, for some reason only some the mutant control well and WT test well did not have high binding, but the other two protein wells had very good concentration and binding.

M55 - 09/11/19

People: Simran, Varun, Natalie

Made buffer for OCTET

Buffer recipe: 12mL in total

- 30 uL of 20% tween
- 120 uL of 20% BSA
- 1.2 mL of 10% glycerol
- PBS 10.41 mL
- 240 uL 100% meOH * dont add yet

Buffer stored in 4C fridge

Buffer made for Octet assay- Date of assay: TBD

M56 - 09/24/19

People: Varun, Quoc, Natalie

OCTET scheduled for 4 pm, we'll start setting everything up at 2:30

Wild type amount (52.9uM)
Need 200nM

- 1.3225 uL protein
- 198.6775 uL buffer (with methanol)

Mutant protein amount (26fold dilution)

- 7.69uL protein
- 192.31uL buffer (with methanol)

Serial Dilution for CBD
50uM (800uL)

- 13.2uL CBD
- 2.68 uL methanol
- 784 uL buffer without methanol

25uM (800uL)

- 400uL 50uM
- 392uL Buffer
- 8uL MeOH

12.5uM (800uL)

- 400uL 25uM
- 392uL Buffer
- 8uL MeOH

6.25uM (800uL)

- 400uL 12.5uM
- 392uL Buffer
- 8uL MeOH

OCTET Assay Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	WT	B	B	B	6.25	B	12.5	B	25	B	50
B	B	WT	B	B	B	B	B	B	B	B	B	B
C	B	MT	B	B	B	6.25	B	12.5	B	25	B	50
D	B	MT	B	B	B	B	B	B	B	B	B	B

M57 - 10/16/19

People: Quoc

Plan: make OCTET buffer without methanol

Making Buffer:

- Buffer recipe: 12mL in total
    - 30 uL of 20% tween
    - 120 uL of 20% BSA
    - 1.2 mL of 10% glycerol
    - PBS 10.41 mL
    - 240 uL 100% meOH * don't add yet
- Falcon tube with white cap, put in 4C fridge right next to ½ TES buffer

M58 - 10/17/19

People: Jay, Varun, Natalie

Repeating the same OCTET procedure as before

Wild type amount (52.9uM)
Need 200nM

- 1.3225 uL protein
- 198.6775 uL buffer (with methanol)

Mutant protein amount (20 fold dilution)

- 10 uL protein
- 190 uL buffer (with methanol)

Serial Dilution for CBD
50uM (800uL)

- 13.2uL CBD
- 2.68 uL methanol
- 784 uL buffer without methanol

25uM (800uL)

- 400uL 50uM
- 392uL Buffer
- 8uL MeOH

12.5uM (800uL)

- 400uL 25uM
- 392uL Buffer
- 8uL MeOH

6.25uM (800uL)

- 400uL 12.5uM
- 392uL Buffer
- 8uL MeOH

OCTET Assay Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	WT	B	B	B	6.25	B	12.5	B	25	B	50
B	B	WT	B	B	B	B	B	B	B	B	B	B
C	B	MT	B	B	B	6.25	B	12.5	B	25	B	50
D	B	MT	B	B	B	B	B	B	B	B	B	B