

iGEM Lab Notebook

This **lab notebook** is for tracking our lab progress during the summer in iGEM. Not everyone will be coming in iGEM on the same days, so we need to make sure to be **VERY specific** with what we did, when (the exact times; e.g., time when we put into the incubator), and where you put equipment. Once we have the exact schedule (of when and who is coming on each day), we will set one specific person to account and update this lab notebook for that day. That person will be in charge of updating the lab notebook, and staying in communication.

Date: Month, Day, Year

Time:

Member: (who documented this lab notebook entry)

Members: (who were involved in this process)

What:

-
-

Date: June 18th, 2021

Time: 9:00-9:15 AM

Member: Seeun

Members: Seeun, Matthew, Sophia, Kay, Eva, Koharu, Kai, Annmarie, Aree, etc.

What:

- Main topic: PCR
- Amplifying the sequence that codes for our biomarker for Gibson Assembly
- Prepared PCR for HER2, Mammaglobin B, and MUC1 (3 PCR tubes each)
 - In total, we prepared $3 \times 3 = 9$ PCR tubes. Started running it at around 9:15 AM (a bit earlier though).
- Our plan: running for 4 hours for the biomarker sequences to amplify enough.

Protocols

- 1) Selected forward and reverse primers for each of our biomarkers

HER-2

Forward primer CAACCAAGTGAGGCAGGTCC

Reverse primer GGTCTCCATTGTCTAGCACGG

MUC-1

Forward primer GTGCCCTAGCAGTACCG

Reverse primer GACGTGCCCTACAAGTTGG

SCGB2A1

Forward Primer AAACTCCTGGAGGACATGGTT

Reverse Primer ACTGCTTGAATTCCCCATAGC

2) In a PCR reaction tube added the following components

- 5 μ L PCR buffer
- 5 μ L DNTP
- 10 μ L biomarker sequence
- 5 μ L forward primer
- 5 μ L reverse primer
- 2 μ L TAQ polymerase

3) Run in thermocycler for ****two hours** under Program 330 (we ran PCR for longer times to generate more sample) ****22**

Date: June 18th, 2021

Time: 1:27 PM

Member: Eva, Seeun, Kai

Members: Seeun, Eva, Kay, Sophia, Kai, Koharu, Aree, Matthew

What:

- **Gibson's Assembly**
- We designed two constructs for each biomarker (Mammaglobin, MUC1, HER2), using 2 promoters (standard T7 promoter and Anderson BBa_K2300 promoter).
- Prepared Gibson's Assembly for HER2, Mammaglobin B, and MUC1 (3 tubes each). In total, we prepared $3 \times 2 = 6$ tubes.
- Our plan: We are running it in the thermocycler for 15 minutes at 50C because 2 - 3 fragments are being assembled for each construct
- Comment: We will only be carrying out PCR and Gibson's Assembly today. Transformation will be scheduled for Monday, June 21st.
- See the [iGEM Gibson Assembly](#) document for the combinations we used.

Protocols

1) In a PCR reaction tube added the following components

- 5 μ L biomarker sequence
- 5 μ L promoter (T7 or Anderson BBa_K2300 promoter)
- 5 μ L T7 terminator
- 5 μ L [pSB1C3](#) Backbone
- 5 μ L RBS
- 5 μ L GFP
- 5 μ L Master Mix

2) Run in thermocycler for 15 minutes under Program 100

Date: June 21st, 2021

Time: 8:30 - 9:50 AM

Member: Seeun

Members: Seeun & Kay (HER-2), Sophia & Eva (MUC1), Take & Yamato (Mamma. B)

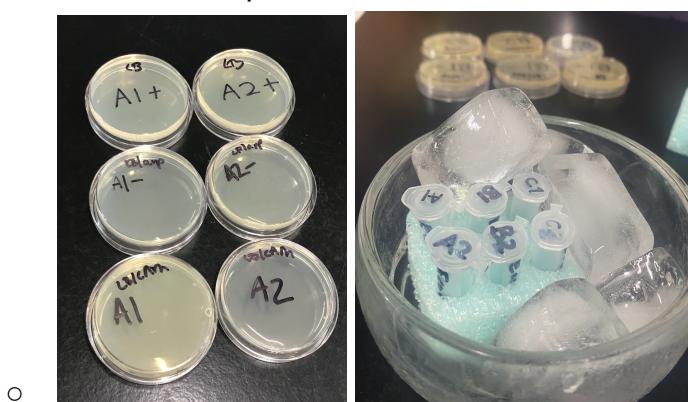
What:

- **Bacterial Transformation**

- Using our 6 plasmid constructs that we assembled through Gibson Assembly last Friday, we carried out bacterial transformation. The 6 plasmid constructs that we transformed today are highlighted in green in our [spreadsheet](#), also shown below:

MUCIN	HER-2	Mammoglobin-B			
A1	T7+T7	B1	T7+T7	C1	T7+T7
A2	Anderson+T7	B2	Anderson+T7	C2	Anderson+T7

- None of the new promoters and terminators arrived this morning (probably will come at 3PM today), so we decided to carry out the transformation of the initial 6 constructs first, then carry out Gibson Assembly for the rest of our 27 constructs.
- Ms. Crissy prepared the 100+ Agar plates over the weekend, and she also prepared the six E. Coli test tubes to be used today.
- For the transformation plates, we decided to use small agar plates (50 microliters of each dilution placed onto selection plates).
- We are doing three plates for each biomarker/promoter construct (**so total = 18 plates**), where:
 - LB = positive control
 - LB/Amp = negative control
 - LB/CAM = Experimental



- 15 minutes before step 8 (60 minutes incubation of test tubes at 37 C), we put the selection plates into the incubator as well, to warm them up to 37C. This way, warm, dry plates are easier to spread and allow for the most rapid colony formation.

- Note: C2 control (C2 + LB) might be messed up since we used the plate streaker from C1 when streaking for the C2 control.
- Final Notes: 11:00 AM → put all the plates in the incubator overnight. We placed the 6 test tubes from today into the freezer (the one next to the Refrigerator by the door to Ms. Crissy's room door) at -80C. This way, we can reuse the test tubes if needed (a lot of them were left over).

Lab Protocol:

1. Put in competent E. Coli cells (test tubes) on ice for 10 minutes.
2. Add 5µL plasmid constructs (6 of them from Friday) to each cell test tube.
3. Flick the tube 4-5 times to mix cells and DNA.
4. Place the test tubes in an ice bath for 30 minutes.
5. Heat shock at 42C for exactly 30 seconds.
6. Place in an ice bath for 5 minutes.
7. Pipette 950µL of room temperature LB Broth into the mixture.
8. Place at 37C incubator for 60 minutes, shaking vigorously.
9. Warm selection plates to 37C.
10. Mix cells thoroughly by flicking the tube and inverting.
11. Spread 50µL (used small plates) onto a selection plate.
12. Incubate overnight at 37 C.

Date: June 22nd, 2021

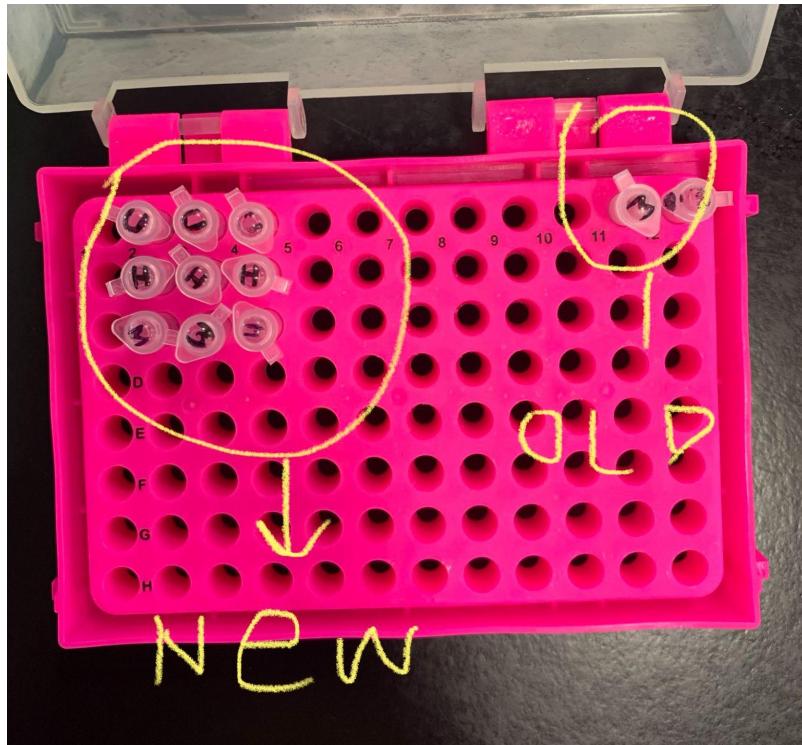
Time: Morning (before lunch; 8:30 - 11:30)

Member: Seeun

Members: Seeun, Matthew, Kay, Eva, Sophia

What:

- **Gibson Assembly**
- We finished Gibson Assembly for the rest of our 27 constructs, as the new promoters and terminators arrived this morning. See above for the protocols.
- **PCR**
- We ran out of the plasmid constructs, so we did more PCR (3 for each construct) → so in total, we made 9 more PCR test tubes. This was done after lunch (12:30 - 13:00)
- Finished PCR running at 15:20.



**Note: the new 9 PCR test tubes (3 for each biomarker) is labeled in the photo above. The 2 old biomarkers are on the top right corner.

Note: are we planning to isolate the protein only or cleaning up the plasmid itself? **Will it still have fluorescence after you purify the protein?** (measuring absorption and fluorescence) → **Simpler the better and more useful** (thinking about it as we are moving next year) → problem: do you want the GFP on the protein during purification or not? (type of protein purification method - **know what we WANT to do**)

Date: June 23rd, 2021

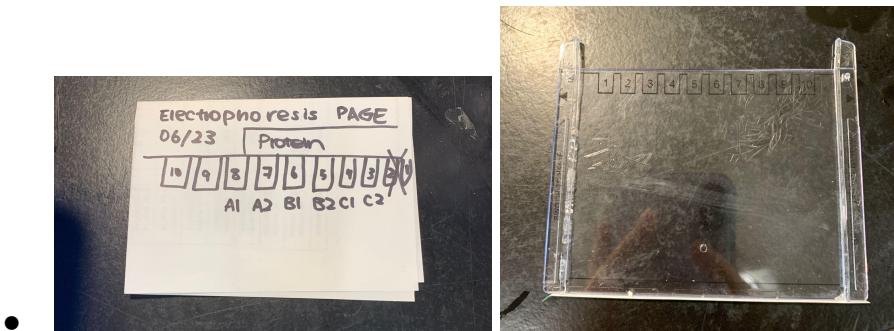
Time: the whole day

Member: Seeun

Members: Seeun & Matthew

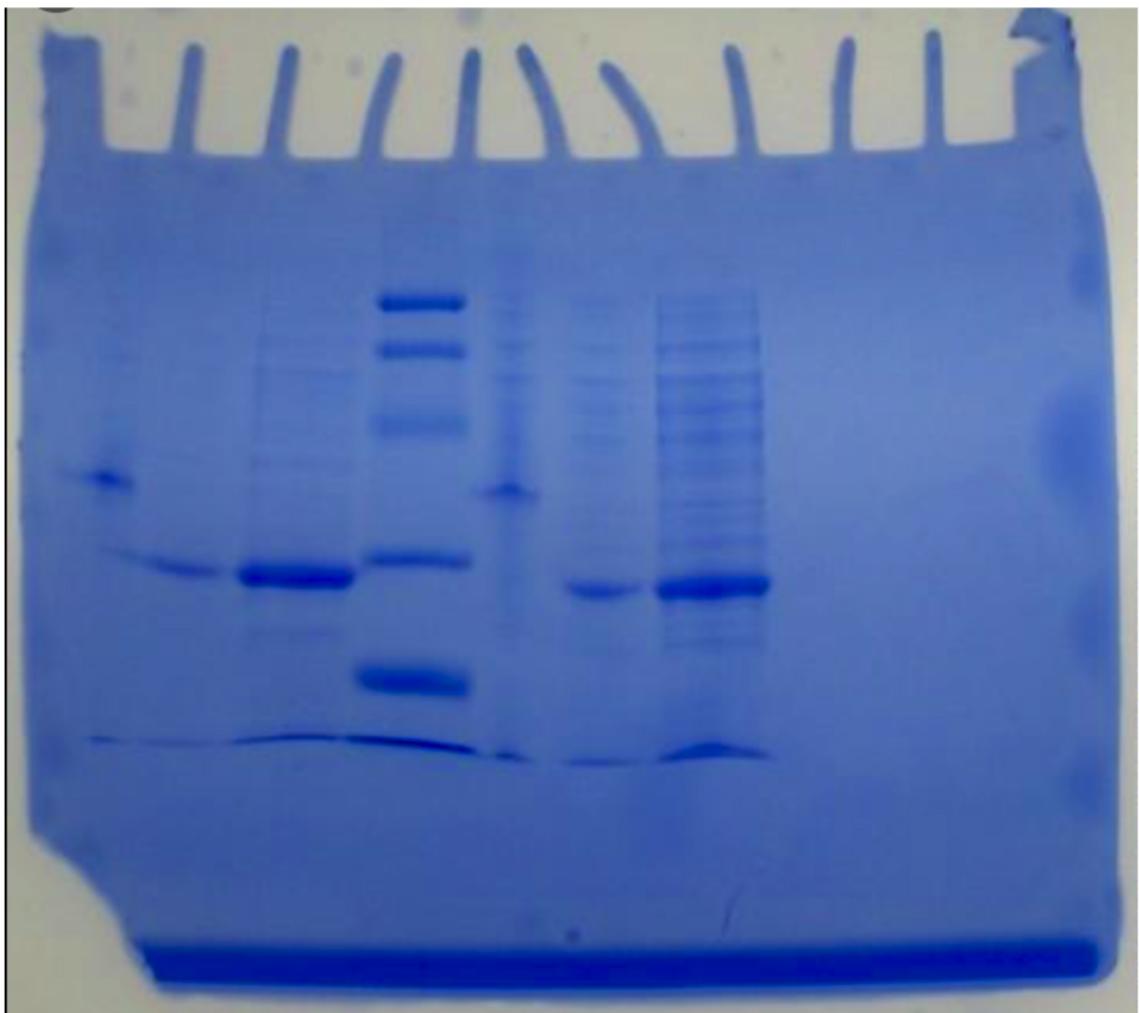
What: **Cracking/Freezing, Spin Column Protein Purification, and Electrophoresis**

- [Protocol Link](#)
- We carried out the protein purification for the first time this summer. We did two separate experiments: cracking and freezing and spin column purification.
- We did 6 test tubes in total, with A1-A2, B1-B2, and C1-C2.
- We also did PAGE electrophoresis. The results are shown below:



- Ms. Crissy derived our results for us. On the first well, we put in the dye.

10 9 8 7 6 5 4 3 2 1



Date: June 24, 2021

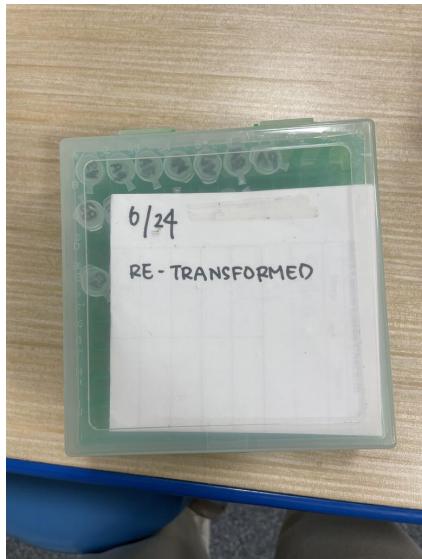
Time: 9:15

Member: Eva, Seeun and Kai

Members: Seeun, Sophia, Eva, Kai, Annmarie, Koharu, Aree

What: Transformation

- We found that the experimental (LB/CAM) plates A3 - A9, B3 - B9 and C3 - C9 were not growing or glowing (note: only C9 grew minimally and slightly glowed)
 - Since the positive controls grew, we predict that it was a problem with transformation itself, most likely heat shock (temperature too low/too high)
- Today, we are redoing the transformation for the experimental plates for A3 - A9, B3 - B9 and C3 - C9 (3 biomarkers x 7 constructs = 21 in total)
 - In order to do protein purification, we must transform today.
 - No protein purification will be done today, given that transformation requires overnight incubation.
 - We used the Gibson Assembly products (27 constructs that we completed) from last time - June 22nd.
- The cause of not glowing:
 - Transformation step
 - Given that the E. Coli on the positive LB + plates all grew, and it was only the LB/CAM plates that weren't growing and glowing, we can assume that the source of error was the transformation process of the plasmid. We can assume our heat shock steps and ice bath steps to have an error, along with the possibility of inaccurate pipetting. The plating section of transformation should be alright, as the LB + plates were still growing (only the CAM plates that were affected).
 - We also assumed the buffer solution (Matthew added TE buffer to Gibson Assembly) might have been a source of error. But talking to Ms. Crissy, the buffer would not have any effect on the Gibson Assembly, so it should be okay.
- Last notes:
 - The protocol for transformation was the same as last time
 - ~11:50-We placed all of the agar plates inside of the incubator
 - ~11:50-We placed all of the test tubes inside of the freezer
 - Some of the labeling for the agar plates is on the wrong side because the plate was wet and we couldn't write with the black marker on it
 - There was one test tube that was left on the table with no label, it looked like there was some liquid in it so it could be E. Coli
 - Now this is in an ice bath container on the table in the far corner
 - We ran out of tape



Date: June 24, 2021

Time: (spin column purification) → 1:40 PM - 2:20 PM

Member: Seeun, Sophia, Eva

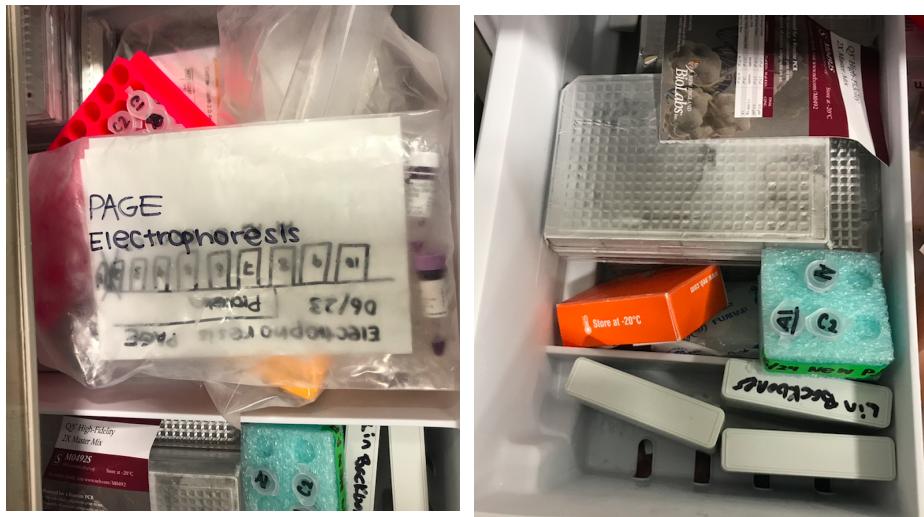
Members: Seeun, Sophia, Eva

What: Protein Purification ([Protocol Link](#))

- We repeated protein purification (Cracking and Freezing; Spin Column Purification) with two plates: A1 and C2.
- A1 - Eva and Sophia carried out purification. (so two trials in total)
- C2 - Seeun repeated. (1 trial total)
- Instead of using the yellow plastic scrapers to remove the transformed e coli, we use the metal scrapers which we sterilized with the bunsen burner.
- Since we are doing protein purification tomorrow, we left the centrifuge, vortex and bunsen burner together.
 - Froze remaining protein in -20C freezer (pictures below)
- In the end, three test tubes were purified. The exact photos of our test tubes are located below:



Where PAGE and proteins are located: (electrophoresis DNA ladder & 6x dye for PAGE Electrophoresis is also located in the plastic bag below; placed in -20 C freezer)



(PAGE stuff + 6/24 3 protein purification test tubes are placed in the -20C freezer)

Date: June 25, 2021

Time: 9:00 - 12:00

Member: Eva

Members: Eva, Kay, Sophia, Naomi

What: Freezing & Cracking, Protein Purification

We purified the proteins from plates A3, A5, A4, B8, C3, and C4. We couldn't purify any other HER2 (B) plates than B8, since they were not glowing. We decided to do these plates since they were glowing the most, and we could scrape off the most transformed colonies.

Lab Protocols for the two (freeze/cracking and protein purif) listed above.

Date: June 25, 2021

Time: 1:00 - 3:00

Member: Matthew, Eva, Kai, Annmarie

What: Transformation/Gibson Assembly

Based on the results from the morning, it was identified that B1, B2, B3, B4, B5, B6, B7, B9, A7, and C9 were not glowing.

We decided to redo Gibson Assembly and did not add the buffer this time.

We redid transformation based on the protocol above but identified that there was an error with the transformation protocol regarding how long to sit the cells on ice. We hypothesized that this error caused the results of our experiment to be flawed.

Date: June 28, 2021

Time: 9-12:30

Member: Eva, Kay, Sophia, Naomi, Seeun, Matthew

What: Freezing & Cracking, Protein Purification

We purified the proteins from plates A6, A7, A8, B3, B4, B5, B6, B7, C5, C6, C7, C8. As mentioned above, since some of the transformation did not work, we tried transformation again and most constructs did work, indicating experimental success. Upon completion of protein purification, we only have 3 constructs left.

Date: June 29, 2021

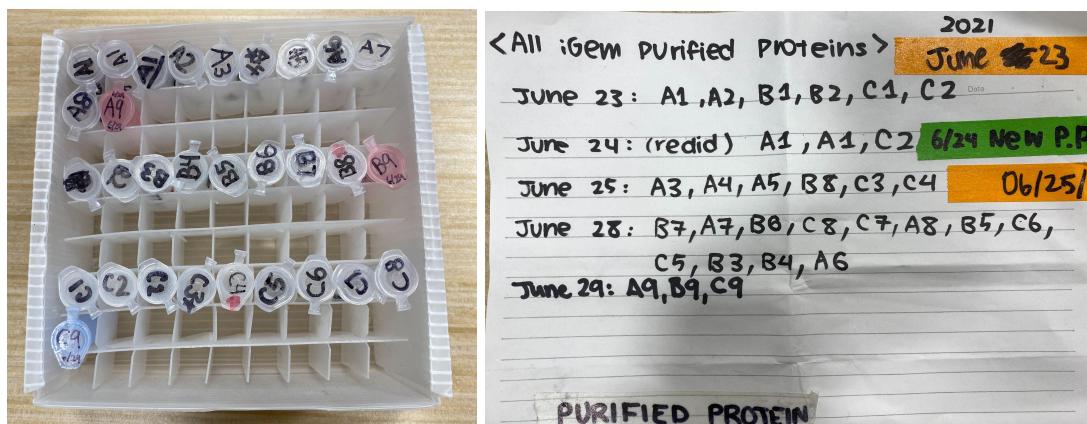
Time: 9:00-11:00, 1:00-2:00

Member: Kay

Members: Kai, Annmarie, Aree (Kay, Eva)

What: Freezing & Cracking, Protein Purification

We purified the remaining proteins from plates A9, B9, and C9. Note: C9 wasn't glowing very much. All constructs have been transformed and purified.



Date: June 29, 2021

Time: 12:40~3:00(?)

Member: Seeun

Members: Seeun & Matthew

What: Bradford Assembly + first time using 96 Well Plate that finally came in!!

- We carried out Bradford Assembly (photo of protocol shown below)

Bradford Assay-

The Bradford protein assay is a simple procedure for determination of protein concentrations in solutions that depends upon the change in absorbance in Coomassie Blue G-250 upon binding of protein (Bradford, Anal. Biochem. 72, 248, 1976)

When using a 96 well plate reader

For Standard Curve

A1 is 200ul of 1x BSA standard
Fill lanes A2-A12 with 100ul of PBS

Transfer 100ul of A1 into A2 pipette up and down to mix 3x, then using same pipette transfer 100ul into A3 repeat. After mixing A11, do not transfer to A12 (only PBS)

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	0
1	0.50	0.25	0.125	0.0625	0.0313	0.0156	0.008	0.003	0.001	0.0009	0

** If just running standard add 40ul of bradford dye to each well A1-A2 and run sample
So this brings total volume to 140 ul per well

If doing at same time as all unknown samples

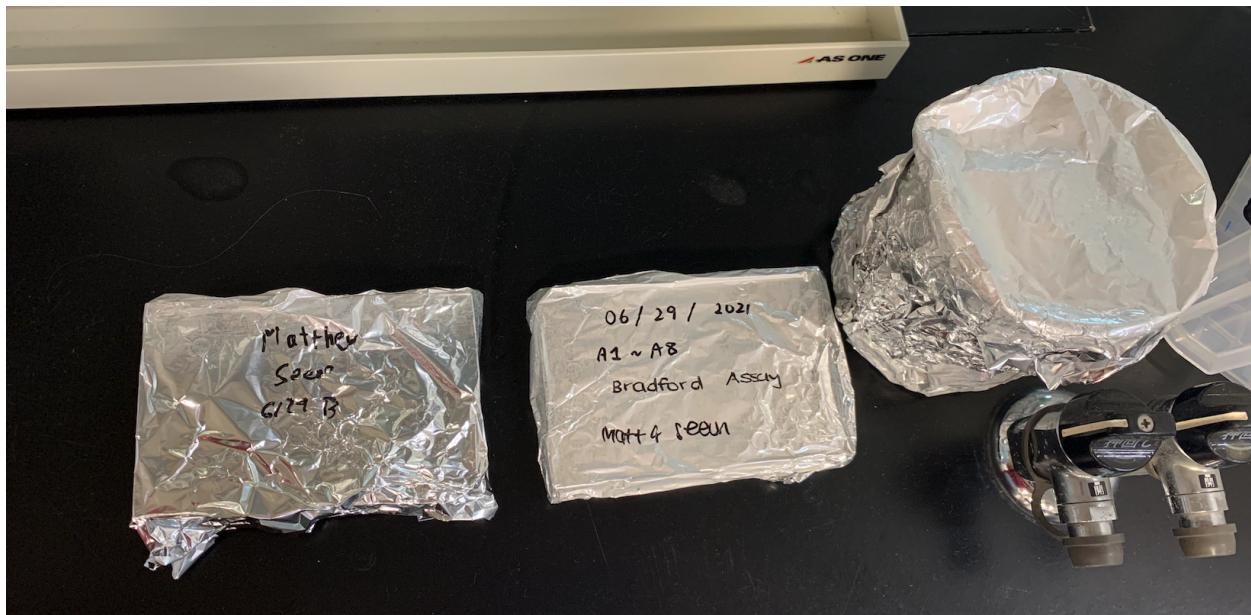
2. Pipette up to 20 μ l of unknown samples into individual wells of a 96-well plate.
3. Add 40 μ l of Bradford Reagent into all wells containing standard and/or sample.
4. Add 80ul PBS to all unknown sample wells to bring the final volume to 140 μ l.
5. Read absorbance at 595 nm without any prior incubation.

Matt U 4 Sevin
Bradford Assay → /

06/29/2021 / No. _____
(Wells) / Date _____

B	1	2	3	4	5	6	7	8	9	10	
Protein	A1	A1	A1	A2	A3	A4	A5	A6	A7	A8	
	5ul	10ul	20ul								
B	1	2	3	4	5	6	7	8	9	10	11 12
	B1	B1	B2	B2	B3	B3	B4	B4	B5	B5	B6 B7
	4.5	4.0	-	-	-	-	-	-	-	-	
C	1	2	3	4							
	B7	B7	B8	B8							

→ H12 = A12



- The Bradford Assay was **twice** in two protocols. The first time we did Bradford Assembly ([using this protocol](#)) and tested it out on the 96 well plate reader, the standard curve was not the best fit. Thus, to increase the efficiency of our standard curve data analysis (on the plate reader), we tried out [another protocol the second time](#) we carried out the Bradford Assay.
- 1st Bradford Assembly: 06/29
 - [Our protocol for the first Bradford Assembly](#)
 - We used A1 ~ A8 purified protein construct samples.

06/29/2021

<WELLS>

	B	1	2	3	4	5	6	7	8	9	10
Protein	A1	A1	A1	A2	A3	A4	A5	A6	A7	A8	
	5ul	10ul	20ul						10ul		

- Proteins A1 was pipetted into wells B1-3, with increasing concentrations of x2 ul. Thus, B1 = 5ul of A1, B2 = 10ul of A1, and B3 = 20 ul of A1.
- Proteins A2 through A8 were placed in order into wells B4 to B10.
- 2nd Bradford Assembly: 06/29
 - [Our protocol for the second time](#) (We edited the protocol a bit - added a new table - to better fit the variables for the sample concentrations. If you click on the link, you will be able to access our modified versions.)

B	1	2	3	4	5	6	7	8	9	10	11	12
	B1	B1	B2	B2	B3	B3	B4	B4	B5	B5	B6	B6
	5ul	10ul										
C	1	2	3	4								
	B7	B7	B8	B8								

- We used two wells for each protein construct, starting with B1 to B8. First well was 5ul, and the second well was 10 ul.
- In conclusion, we finished two Bradford Assemblies of all protein constructs from A1-A8 and B1-B8.

July 2, 2021

Time: 9:00 - 11:30, 1:00-3:00

Member: Kay & Sophia

Members: Kay & Sophia

What: cracking and freezing + protein purification for leftover plasmid from plates B7, B9, A9, B5, B4, B3

- We skipped the streaking and twirling step since the plasmid was already in the microreaction tube, instead we pipetted up and down and centrifuged/vortexed for 3-5 seconds to get the plasmid off the sides
- We had to repeat the freezing and thawing procedure once for B5 because the pellet was still glowing
- None of the microreaction tubes that supernatant that was glowing significantly
-

July 2, 2021

Time: 9:00-12:00

Member: Eva, Matthew, Seeun

What: Bradford Assay and well-plate reader

The Bradford Assay we had been looking at was not yielding good results so we tried an alternate protocol with the remaining Mammaglobin B constructs, and constructs A9 and B9.

In order to not waste samples, we decided to construct a perfect linear standard curve(took us two tries) before loading our samples.

We read the remaining samples and the results were more accurate and feasible.

There were some samples that had negative results from the multiple trials. We read them again to determine whether it was experimental or a construct error. The results can be found [here](#).

Summer Lab Achievements

This summer we completed Gibson Assembly, transformation, and protein purification to design an optimal biomarker producing E Coli construct that will serve as our human model for aptamer based experiments in the fall.

Autumn Lab Summary

We encountered an issue with receiving lab equipment and parts during the fall but have included all our protocols for the labs and experiments we were planning to conduct and do.

Aptamer Biomarker Binding ELISA

Protocols created by NTU and ASIJ_Tokyo

1. Prepare standards and sample dilutions in Blocking buffer.
2. Pipette 100 µL of standards (the aptamers) and samples (biomarkers) into designated wells. Incubate for 1 hour at room temperature with gentle continual shaking. (500rpm).
 - a. Would recommend starting with positive control aptamers and optimize for the concentration of the aptamer required (can use concentration from literature as a guide)
 - b. Then, pick the three best concentration to screen for other aptamers.
3. Aspirate and wash 5 times with >200 µL of Wash buffer per well. Following wash, invert and tap on absorbent paper to remove excess liquid.
4. Prepare detection antibody solution by diluting the Detection antibody in blocking buffer. For recommended antibody dilution, refer to manufacturer's instruction.
5. Add 100 µL of the detection (primary) antibody solution into each well. Incubate for 2 hours at room temperature with gentle continual shaking (500rpm)
6. Add 100 µL of TMB substrate solution to each well. Incubate plate for 30 minutes at room temperature. Refer to manufacturer's instruction for TMB substrate for more information on incubation timing.
7. Add 100 µL of Stop solution to each well.
8. Measure absorbance at 450 nm within 30 minutes of adding Stop solution.
9. Calculate Results

AU-Nanoparticle Experiments

Protocol created by Joshua E. Smith, Jorge L. Chávez, Joshua A. Hagen, and Nancy Kelley-Loughnane from Alvernia University.

- 1) Incubate the aptamer with the stock AuNP solution (10 nM) for 3-4 hr at room temperature and protect from the light. Vary the volume of AuNPs as desired to provide enough sample for the tests to be performed (2.5-7.5 ml).
- 2) Add an equal volume of 20 mM HEPES, 2 mM MgCl₂, pH 7.4 buffer and place the sample at 4 °C in the dark overnight. The aptamer-AuNP assay was in a 10 mM HEPES, 1 mM MgCl₂, pH 7.4 (assay buffer).
- 3) Determine the initial salt concentration needed to induce the assay color response by salt titration with the assay blank. Add 20 µl of methanol (blank) to 180 µl of aptamer-AuNP assay in a 96-well plate. Titrate the samples with increasing volumes of stock NaCl solution (1M) and determine the equivalence point.
- 4) Add 20 µl of biomarker diluted in methanol to 180 µl of aptamer-AuNP assay in a 96-well plate at room temperature. Immediately add the NaCl concentration determined in the previous step to initiate the assay color response(color change).
- 5) Obtain the largest color change possible by changing the NaCl concentration, and comparing the target response to the blank response(methanol). Use the NaCl concentration that provides the largest response difference.
- 6) Measure the assay response 150 seconds following NaCl addition. Analyze the absorbance at 650 nm and 530 nm using a 96-well plate reader.

