03/08/2026 - Monday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 03 Aug 2026 17:19:08 UTC
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MONDAY, 03/08/2026

Members

Hector, Katie, Vasily

Objectives

- ~~Sonicate the last two 24 hr samples from last week~~
- ~~Finish destaining the Comassie gel from last week~~
- ~~Run SDS-PAGE of the other 18 hr and 24 hr samples~~
- ~~Prepare destaining solution~~
- ~~Transform the seed constructs~~

Sonication

Sample 12-eGFP-cap-32°C-24hr and 12-eGFP-cap-37°C-24hr were sonicated following the same protocol (see 30/07/2026 - Thursday and 7.2 - Expression testing lysing)

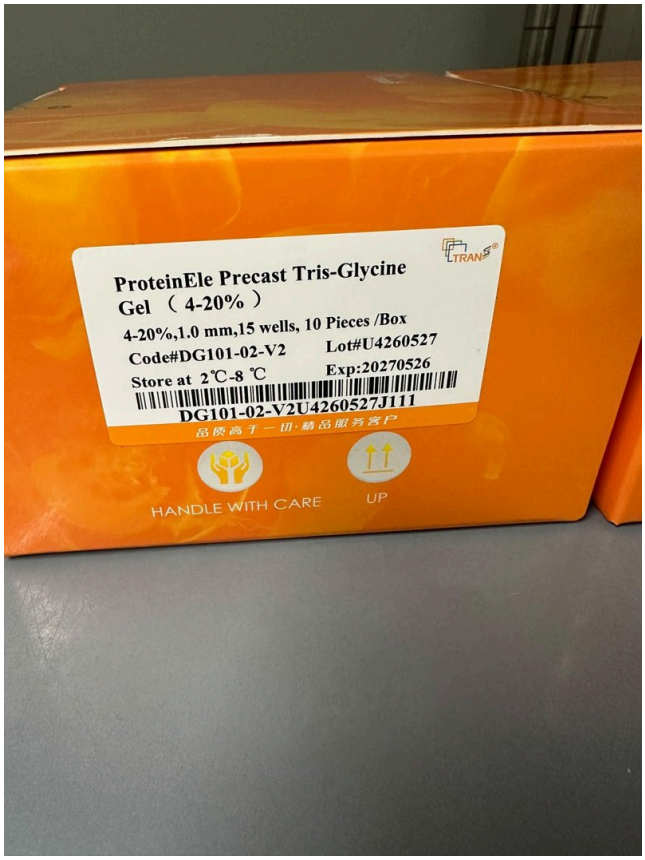
Destaining Comassie blue gel

Destaining solution was prepared with 30% methanol, 10% acetic acid, and 60% MiliQ inside a fume hood

SDS-PAGE of 18 hr samples

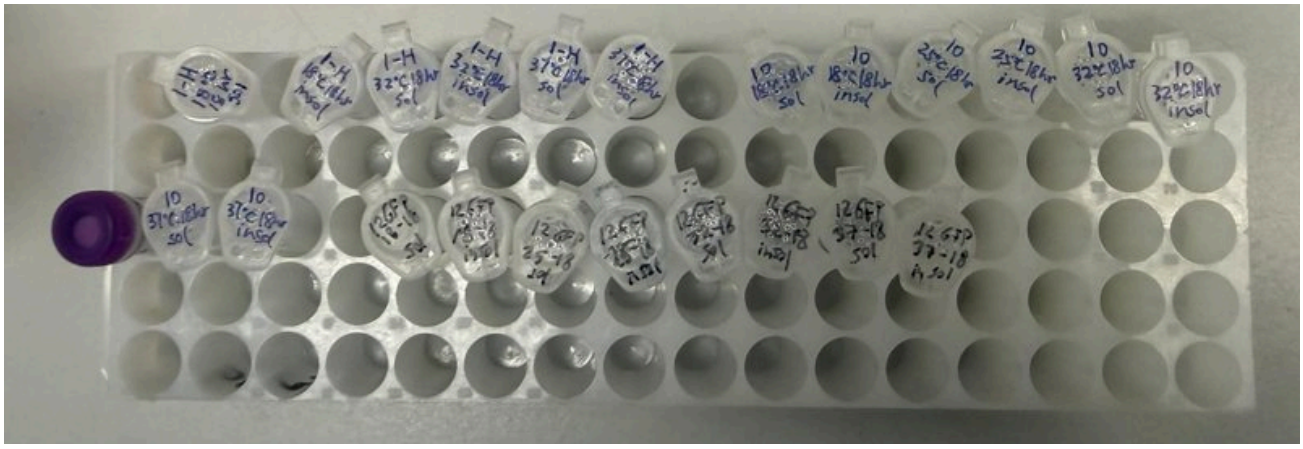
1. SDS-PAGE was performed with all of the 18 hr samples of 1-HET-s, 10-mCardinal-HET-s, and 12-eGFP-cap constructs
2. The gel used was the ProteinEle Precast Tris-Glycine Gel (4-20%) with 15 wells

2eb1a3ce-5b7b-4f07-bbf2-86736842409d.JPG



3. Samples were prepared following the same protocol (see 31/07/2026 - Friday) and 15 µl of each sample and 3 µl of the ladder were added, see the following picture for the loading order (the first row is for the first gel and the second row is for the second gel)

IMG_1014.jpeg



4. Gels were stined with Comassie blue overnight on a shaking bed

Transformation of BLR(DE3)

1. Seed, seed-eGFP were resuspended with MiliQ water to make the final concentration 10 ng/µl
2. 1.5 µl of the seed, seed-eGFP, cap, cap-eGFP were added to 45 µl of the BLR(DE3) competence cells (final DNA = 15 ng)
3. Following the transformation protocol (see 27/07/2026 - Monday)
4. 50 µl of the each sample was plated on LB+K+C plates

Pre-culture

Prepare pre-cultures for 1-HET-s, 8-cap, 10-mCardinal-HET-s, and 12-eGFP-cap (BL21(DE3) transformants), following the protocol (see 28/07/2026 - Tuesday)

04/08/2026 - Tuesday

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MONDAY, 03/08/2026

Gels - Katie comes in early to destain, run and image
Destain WT, WT-mCard & Cap-eGFP 18hr
Tomorrow morning: Cap 18hr & 24hr, Cap-eGFP 24hr
2nd round: WT 24hr, WT-mCard 24hr

Pickup MES and make 20x running buffer (protocol in Benchling need to sterile filter)

Check BLR transformation with Twist Seed, Seed-eGFP, Cap & Cap-eGFP
    If successful grow preculture in TB-10x ON

If gels come out good we can put a preculture on and send TB media for autoclave in baffled flasks (remember to book out incubators)

Members

Katie Wei, Hector Cross

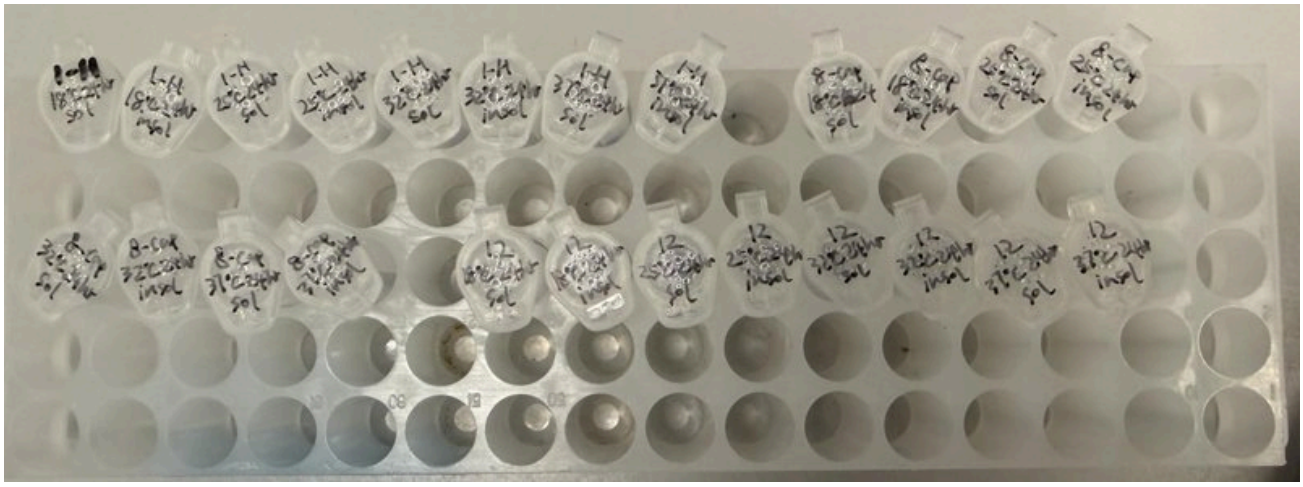
Objectives

- Destain two gels from yesterday
- Run SDS-PAGE for the reamining 24 hr samles of 1-HET-s, 8-cap, 10-mCardinal-HET-s, and 12-eGFP-cap
- Pre-culture samples of seed and cap of BLR (DE3) transformants
- Negative control culture
- Image all of the gels and label them

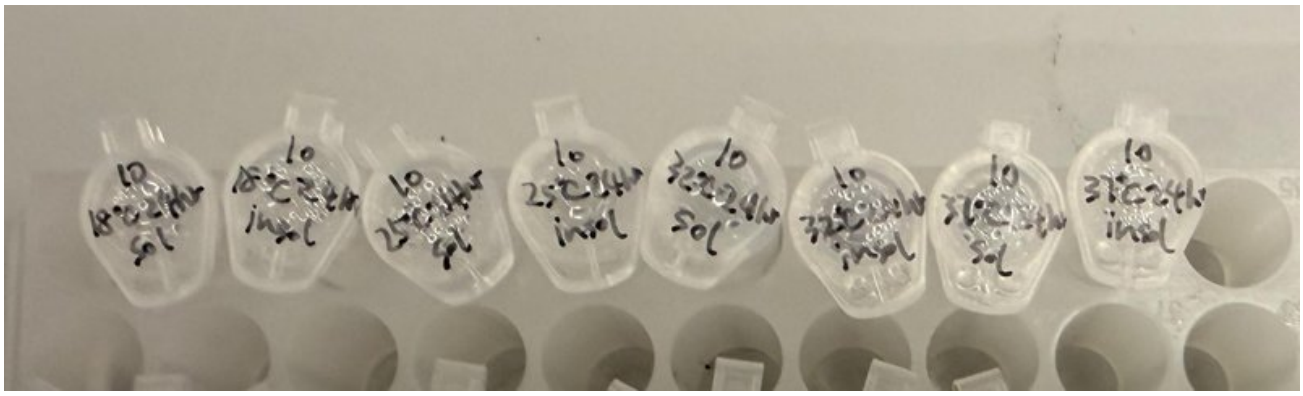
SDS-PAGE

1. SDS-PAGE was performed with 24 hr samples of 1-HET-s, 8-cap, 10-mCardinal-HET-s, and 12-eGFP-cap
2. ProteinEle Precast Tris-Glycine gel (4%-20%) was used
3. Samples were prepared following the same protocol (see 31/07/2026 - Friday) and 15 µl of each sample and 3 µl of the ladder were added, see the following picture for the loading order (three gels were run in total)

IMG_1020.jpeg



IMG_1025.jpeg



4. Gels were stined with Comassie blue and then destained with destaining solution

Negative control culture

1. The OD of precultures were measured in 1:10 dilutions and the measurements were listed below:

Table1

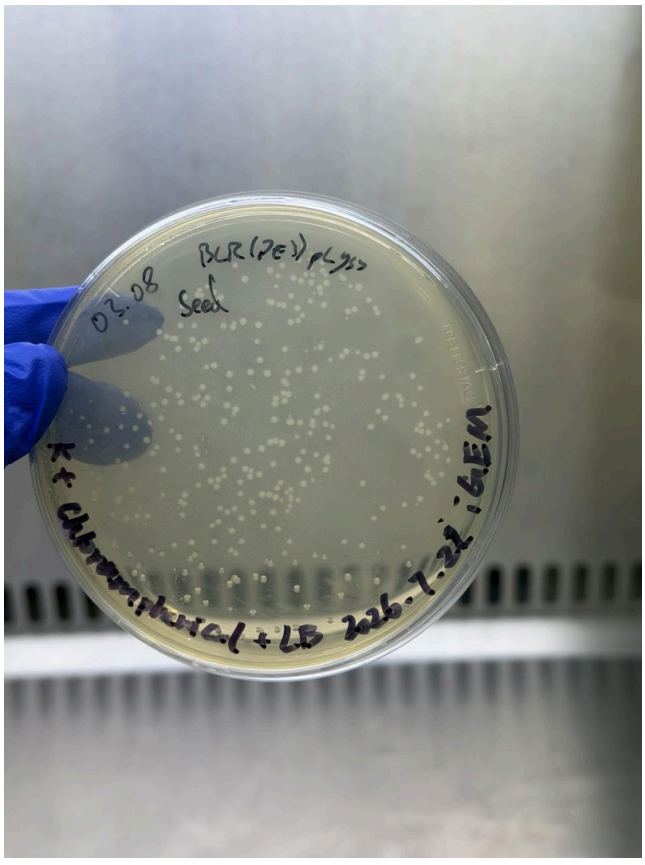
	Construct	OD600 (1:10 dilution)	Adjusted OD600
1	1-HET-s	0.812	8.12
2	8-cap	0.598	5.98
3	10-mCardinal-HET-s	0.715	7.15
4	12-eGFP-cap	0.828	8.28
5			

2. 100 ml of culture medium was prepared with 90 ml TB base, 10 ml of 10x TB salt, and 50 µl of kanamycin
3. 20 ml was transferred to each tube and the respective volume of pre-culture was added to make the starting OD600 0.05 (1-HET-s: 123 µl, 8-cap: 167 µl. 10-mCardinal-HET-s: 139 µl, 12-eGFP-cap: 120 µl)
4. Placed the tubes in 37°C shaking incubator at 220 rpm overnight

Plate results of BLR (DE3) transformants

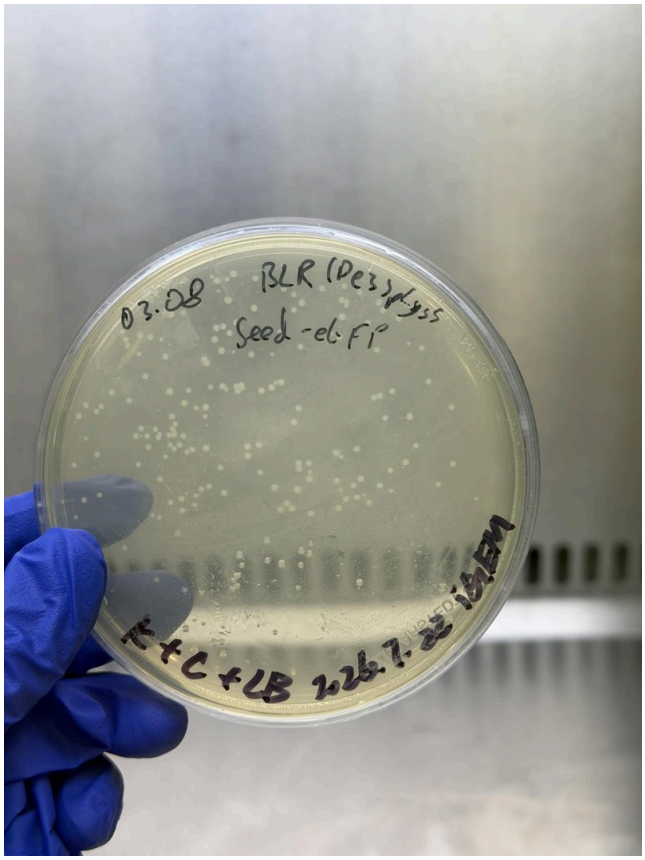
1. Seed

IMG_1047.jpeg



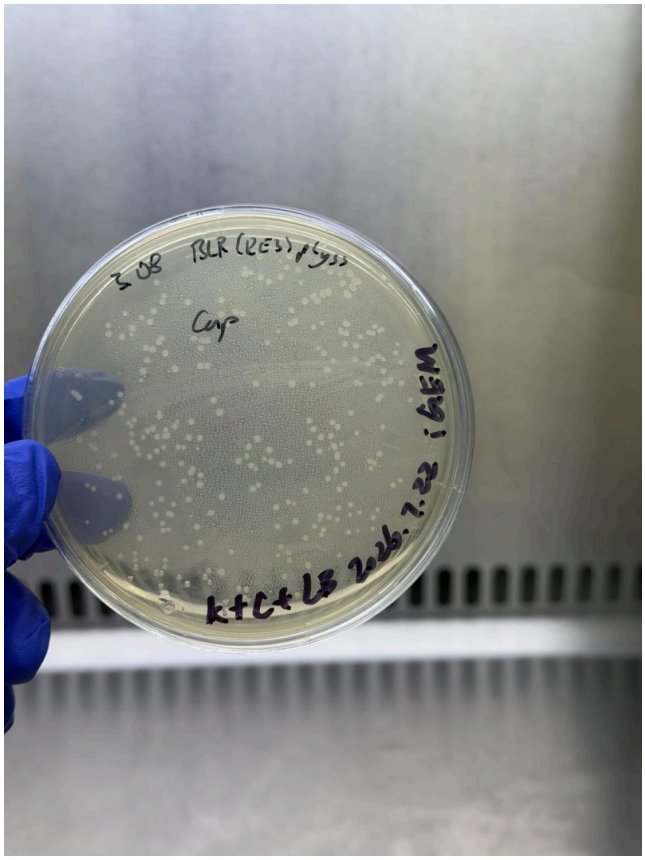
2. Seed-eGFP

IMG_1046.jpeg



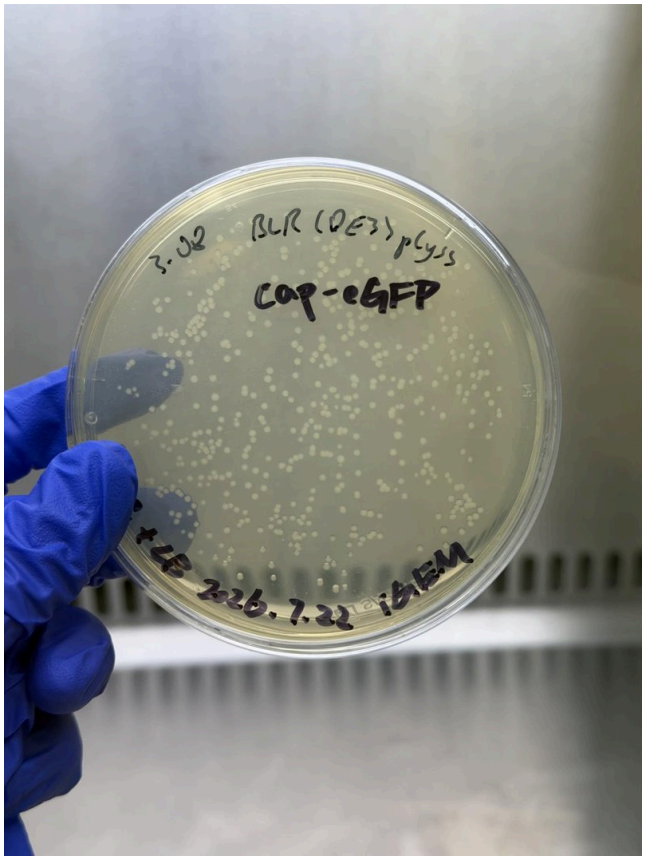
3. Cap

IMG_1045.jpeg



4. Cap-eGFP

IMG_1044.jpeg



Pre-culture of BLR (DE3) transformants

1. 22.2 ml of pre-culture medium was prepared with 20 ml TB base, 2.2 ml 10x TB salt, 11 µl kanamycin, and 11 µl chloramphenicol
2. Tranferred 5 ml of the pre-culture medium to each tube and inoculated colonies from plates (seed, seed-eGFP, cap, cap-eGFP)
3. Tubes were grown at 37°C shaking incubator at 220 rpm overnight

05/08/26 - Wednesday

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TUESDAY, 04/08/2026

Members

Katie Wei, Hector Cross

Objectives

- Destain WT-mCard 24hr
- Make 600mL of 20x MES (protocol on benchling)
- Inoculate BL21 & BLR cultures at OD=0.05 4 and take pre-induction samples (3 temps x 1 timepoint x 4 samples per construct x 8)
- Transform mutants on Kan

Expression test

1. Prepared 250 ml cultures for BL21 strains and 250 ml cultures for BLR strains
2. For BL21 culture, added 25 ml of x10 TB salt, 5 ml of x50 Autoinduction solution, 220 ml TB base, and 125 µl of kanamycin
3. For BL21 culture, added 25 ml of x10 TB salt, 5 ml of x50 Autoinduction solution, 220 ml TB base, 125 µl of kanamycin and 125 µl of chloramphenicol
4. 20 ml of the culture was transferred to each tube and respective volume of pre-culture was added to make starting OD600 = 0.05, the OD600 measurement and the volume added was listed in the following table:

Table1

	Construct	OD600 (1:10)	Adjusted OD600	Volume added (µl)
1	1-HET-s (BL21)	0.253	2.53	400
2	8-cap (BL21)	0.243	2.43	415
3	10-mCardinal-HET-s (BL21)	0.248	2.48	405
4	12-eGFP-cap (BL21)	0.205	2.05	490
5	Seed (BLR)	0.444	4.44	225
6	Seed-eGFP (BLR)	0.422	4.22	240
7	Cap (BLR)	0.487	4.87	205
8	Cap-eGFP (BLR)	0.519	5.19	195

5. All tubes were placed in 37°C 220 rmp shaking incubator and were taken out to measure the OD, the second OD measurement was listed below:

Table2

	Construct	OD600
1	1-HET-s (BL21)	0.876
2	8-cap (BL21)	1.031
3	10-mCardinal-HET-s (BL21)	0.947
4	12-eGFP-cap (BL21)	1.054
5	Seed (BLR)	0.754
6	Seed-eGFP (BLR)	0.756
7	Cap (BLR)	0.743
8	Cap-eGFP (BLR)	0.738

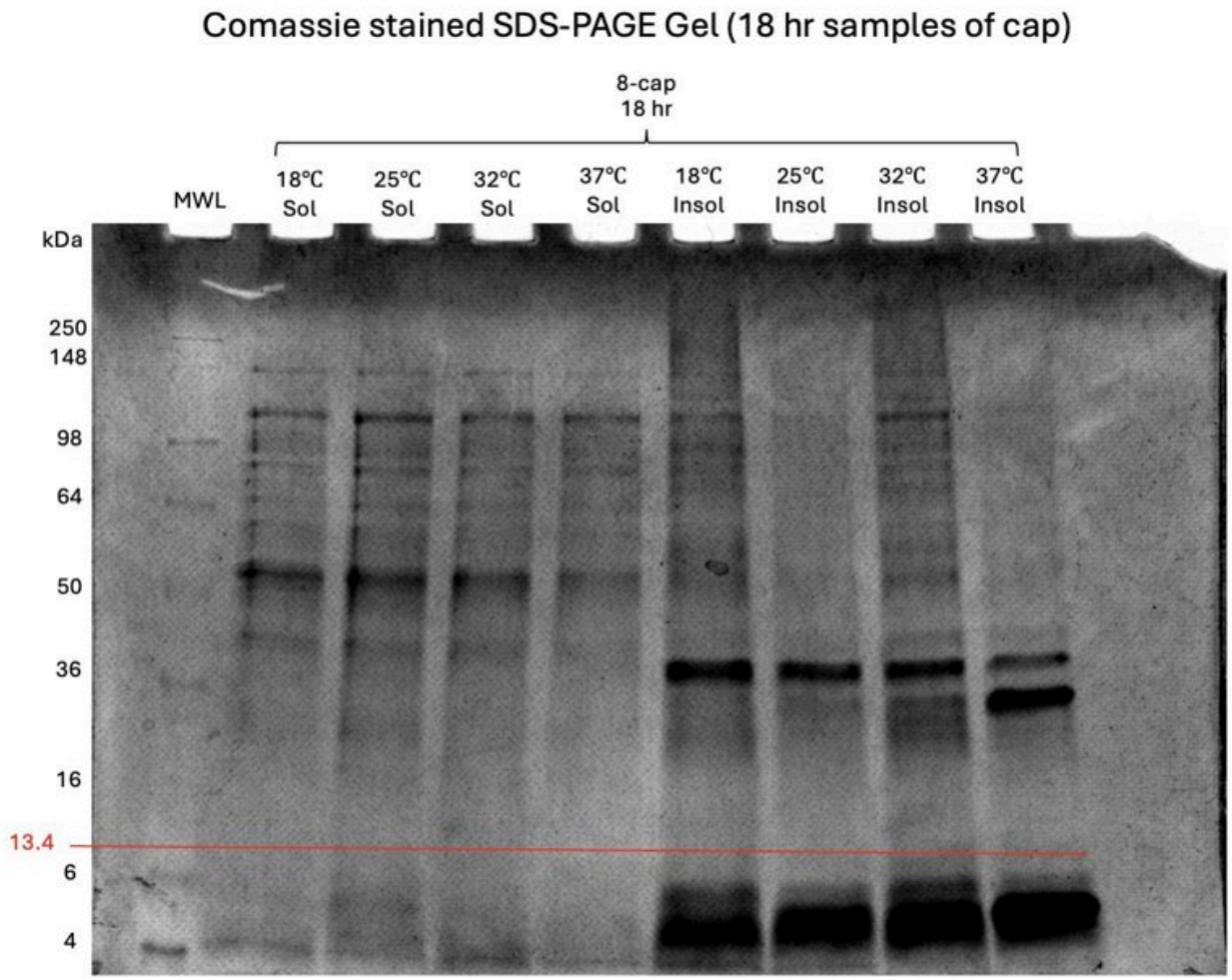
6. The tubes were switched to different expression temperatures (25°C, 32°C, 37°C) and expressed over 24 hours
7. Before switching them to expression temperatures, 1 ml of the pre-induction cultures were collected as negative control, they were centrifuged for 3 min at 12,000 rpm, the supernatants were discarded and the pellets were resuspended with 400 µl of PBS, samples were freezed at -20°C

Transformation of mutants

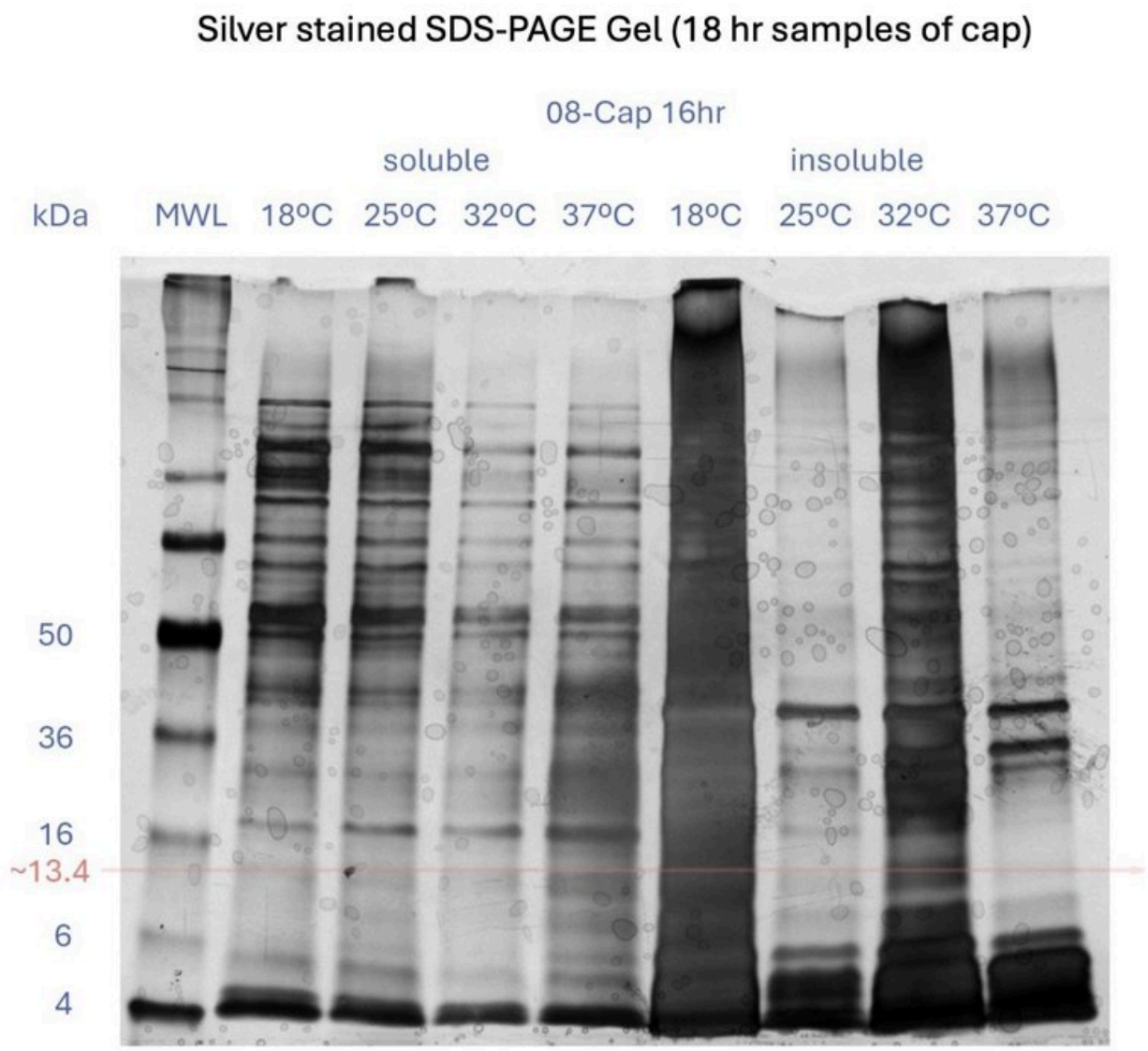
1. HET-s mutant DNAs (v1, v2, double cys) from Genscript were resuspended in 400 µl of ddH2O to make the stock concentration 10 ng/µl
2. 1.5 µl of each DNA was transferred to 50 µl of the BL21 (DE3) competent cells
3. Follows the transformation protocol (see 27/07/2026 - Monday)
4. 50 µl of the recovered cells were plated on LB+K plates and incubated overnight at 37°C

Gel image results

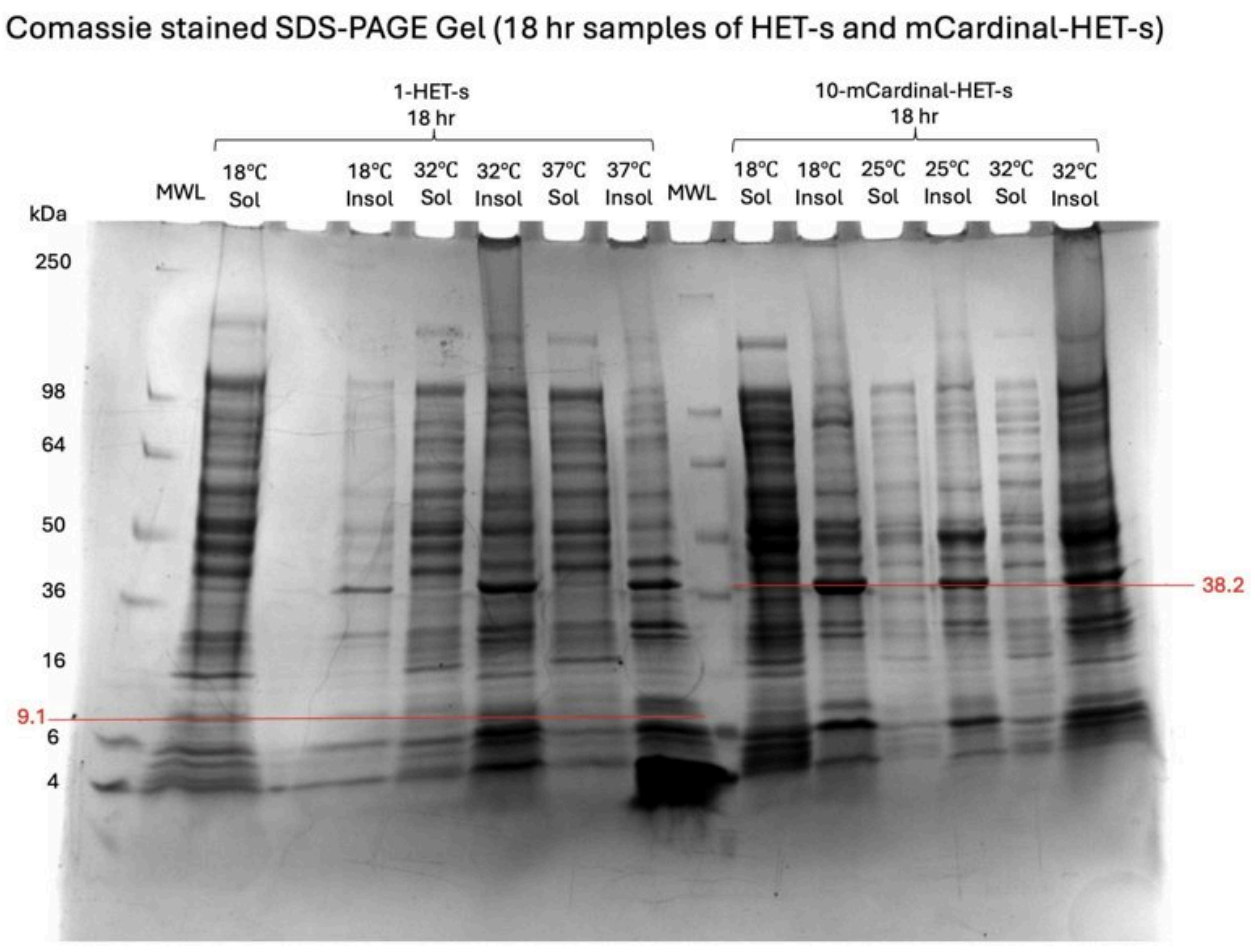
2026-07-31_18hr_cap_Comassie_stained_copy.jpeg



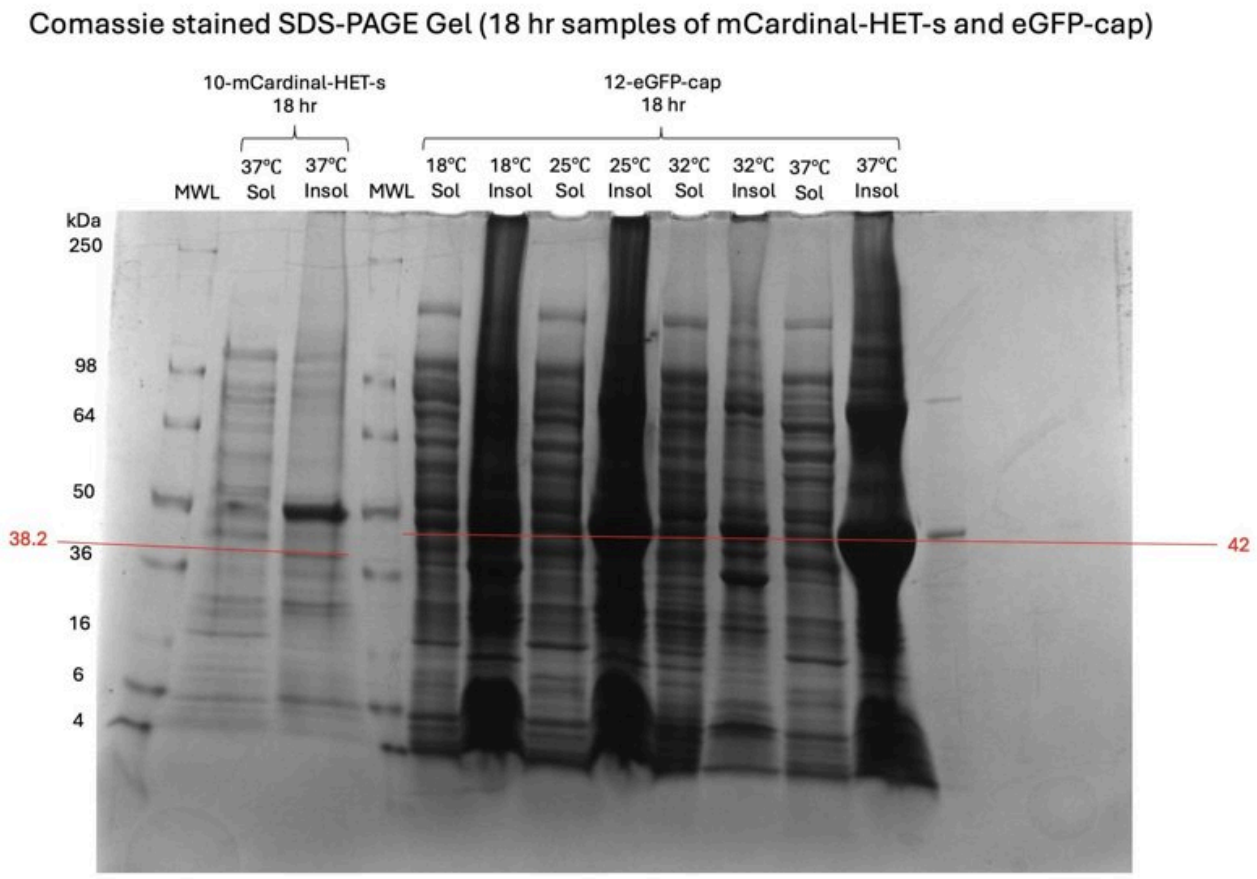
2026-07-31_18hr_cap_Silver_stained_copy.jpeg



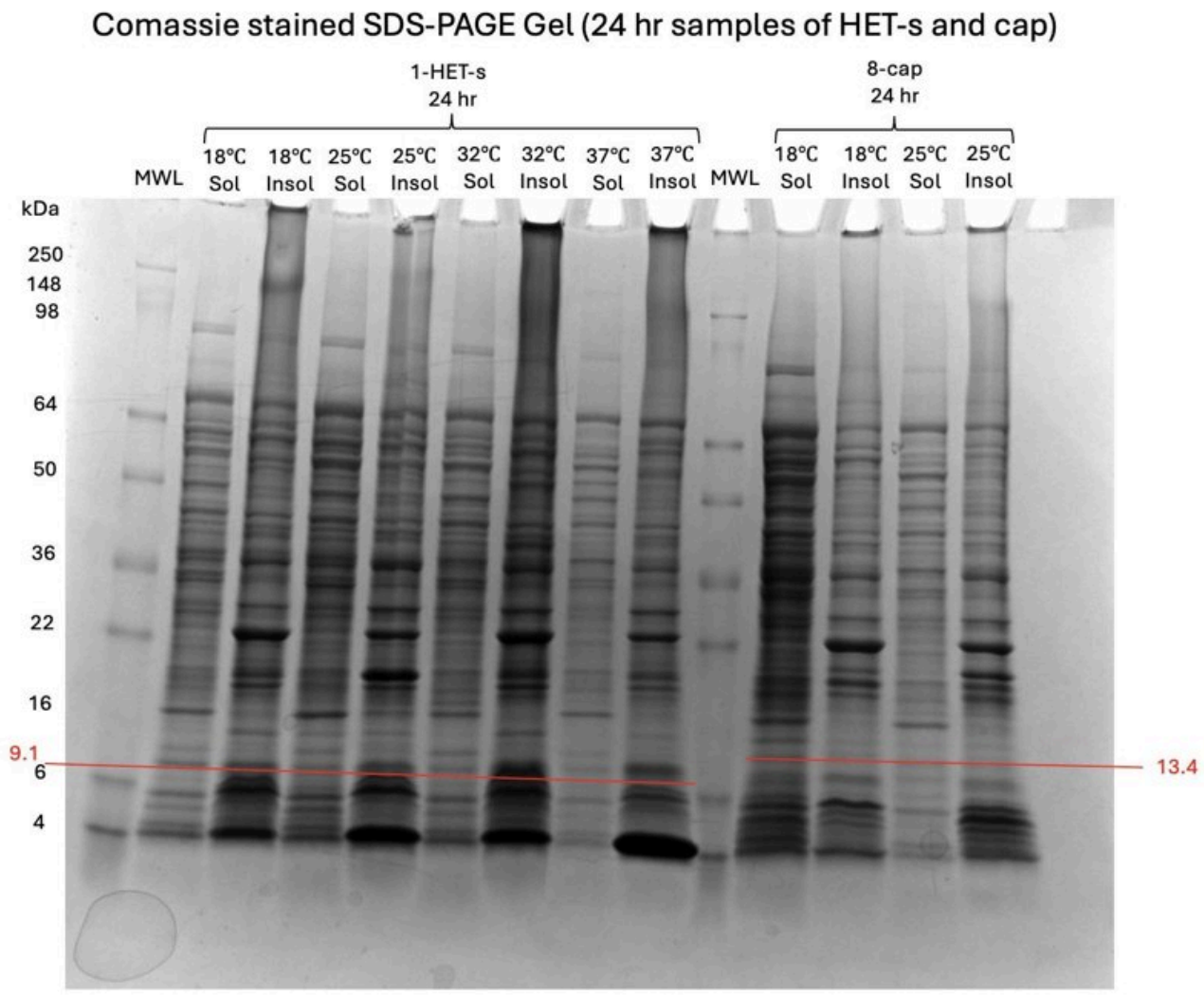
2026-08-03_18hr_HET-s_and_mCardinal-HET-s_copy.jpeg



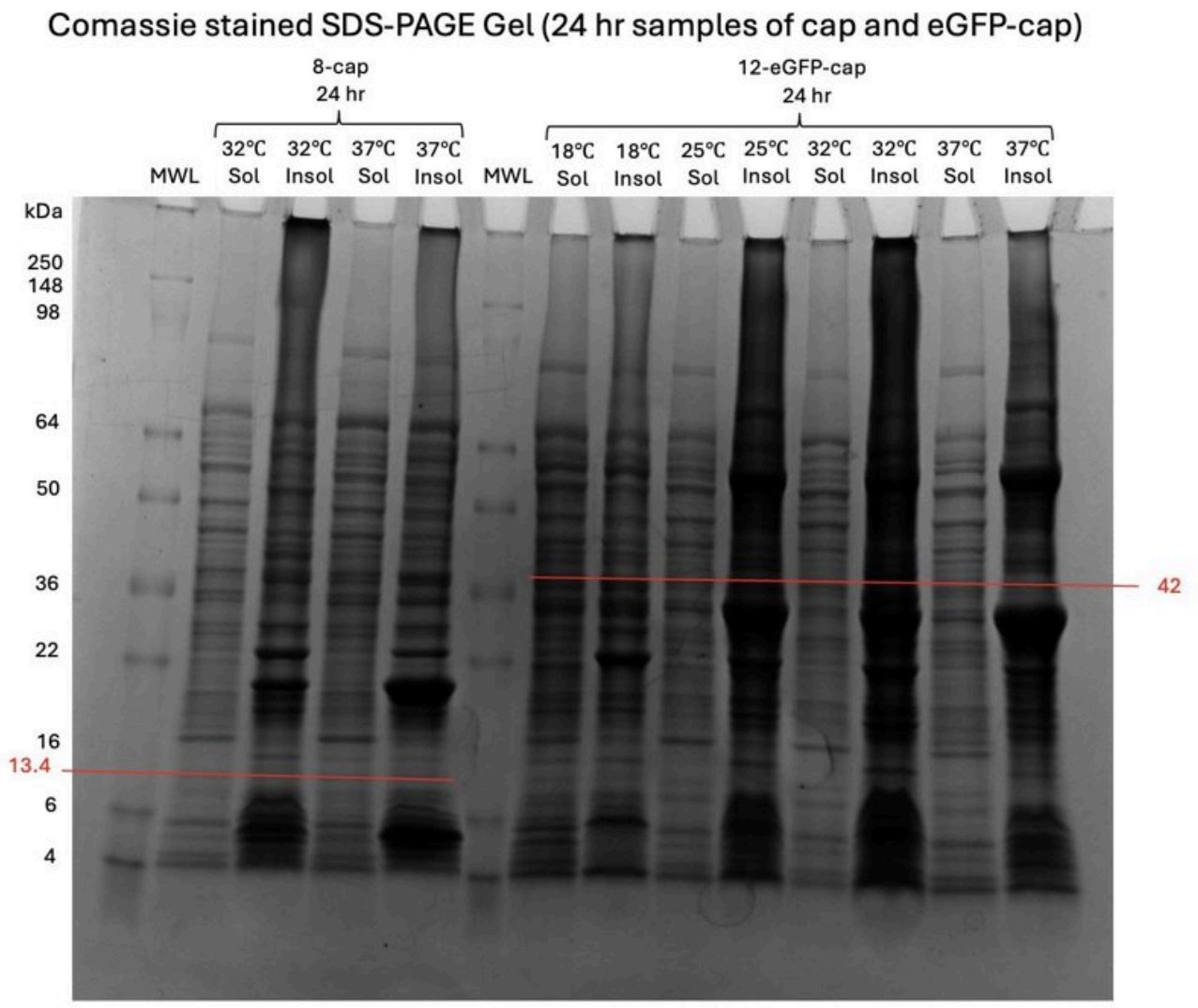
2026-08-03_18hr_mCardinal-HET-s_and_eGFP-cap_copy_2.jpeg



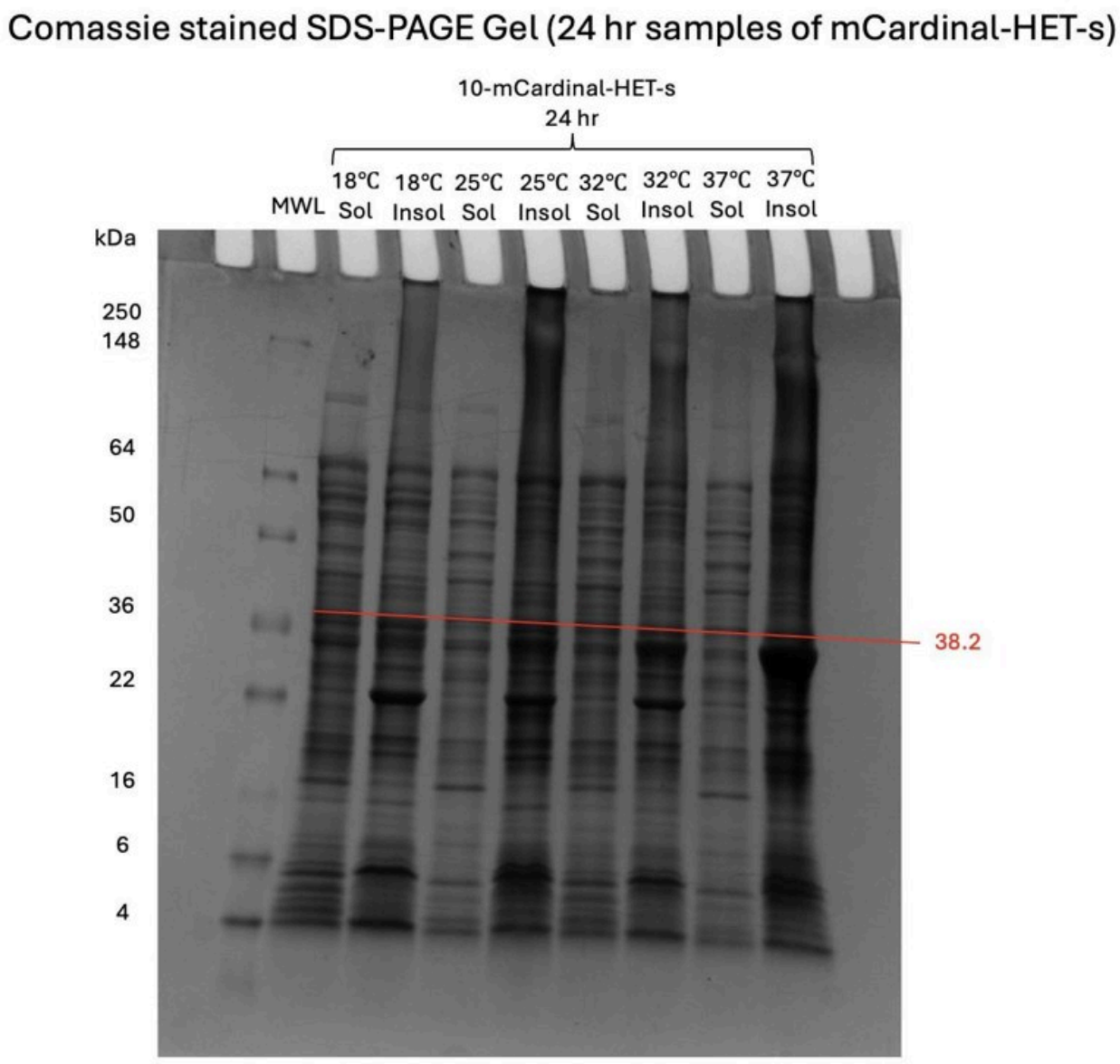
2026-08-04_24hr_HET-s_and_cap_copy.jpeg



2026-08-04_24hr_cap_and_eGFP-cap_copy.jpeg



2026-08-04_24hr_mCardinal-HET-s_copy.jpeg



06/08/26 - Thursday

Project: iGEM 2026 Shared Project
Author: Hector Cross
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WEDNESDAY, 05/08/2026

Wrap mutants

Take 24hr sample (~1pm) 1mL post induction spin down

Sonicate all the samples and separate soluble from insoluble

Make Mes 20x 20x MES-SDS Running Buffer - 300 mL

BMG Labtech plate reader training

1. Hardware
   - There is a button on the front of the plate reader, long press to switch the machine on and off
   - Purple light appears when the machine is switched on
   - When opened, there are two buttons that says 1 and 2, press 1 to open the filter columns (there are three filters, the excitation, dichroic, and emission)
   - Keep pressing button 1 to cycle between the different filter columns
   - Press button 2 to close all the filter columns
   - Short press the button on the front of the plate reader to put the plate in

PHOTO-2026-08-06-11-03-32_2.jpg

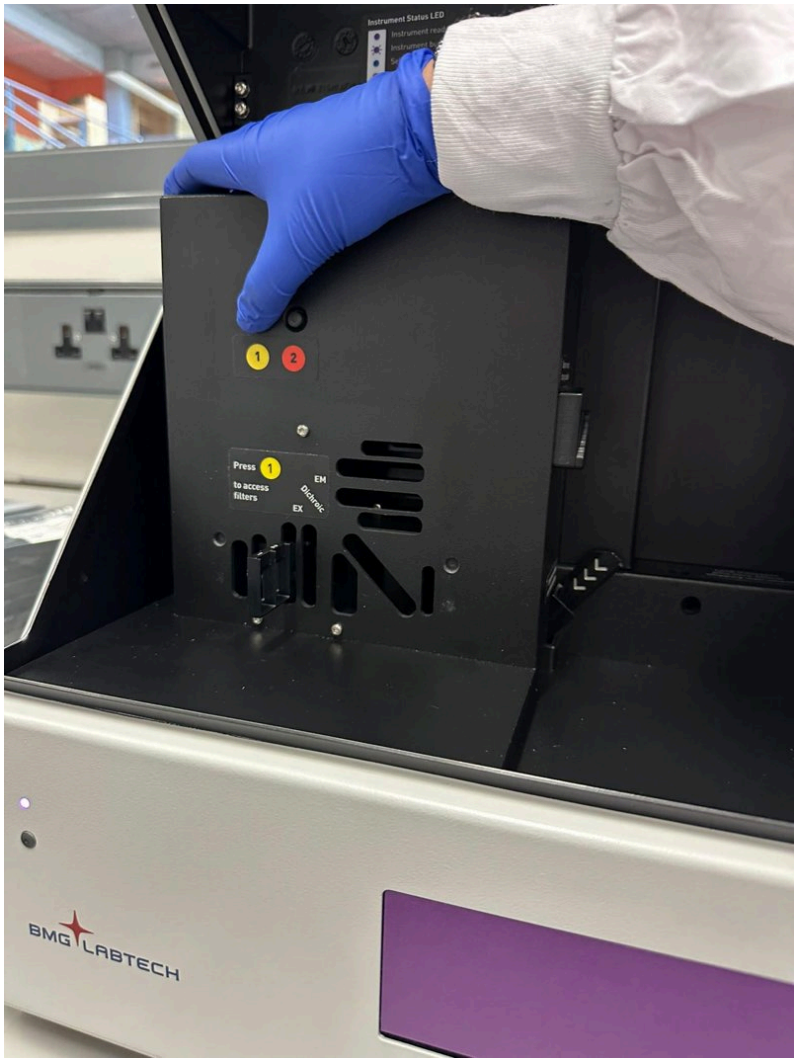
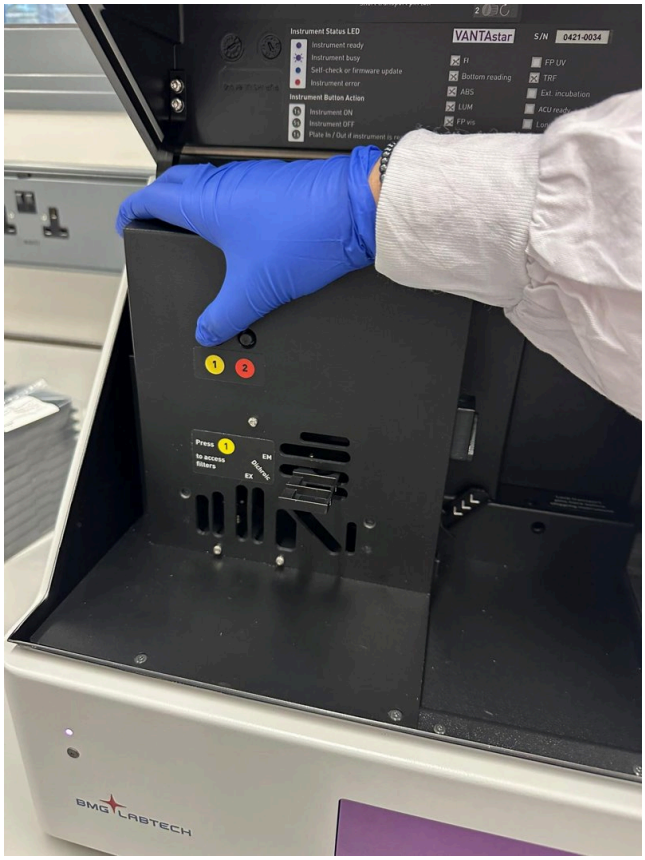


PHOTO-2026-08-06-11-03-32_3.jpg



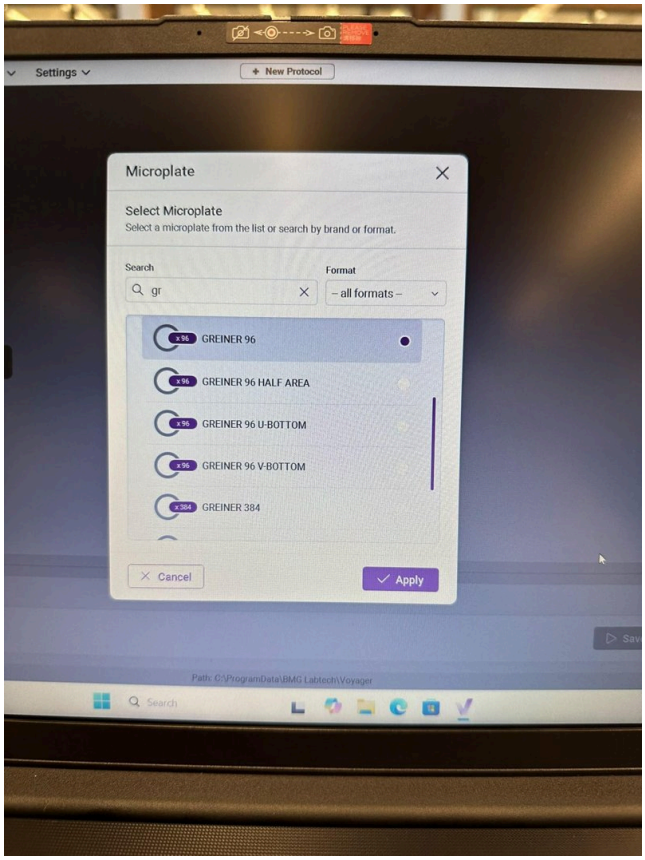
PHOTO-2026-08-06-11-03-33.jpg



2. Software Voyager

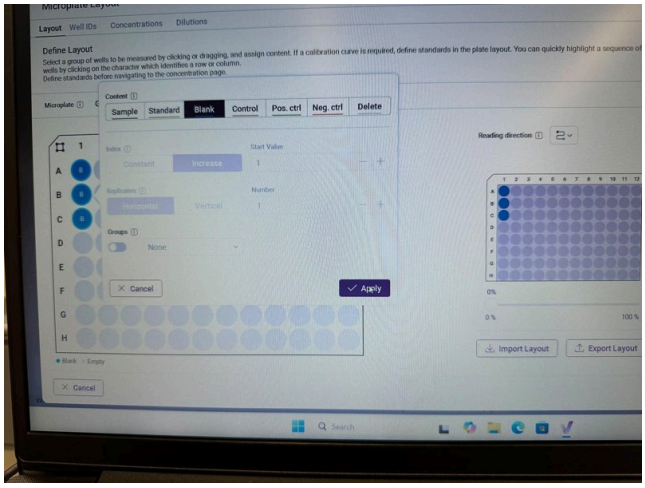
- Go to setting -> filters -> select the filter that you put in
- Add new protocol -> select the microplate you are using (this is Greiner 96 for us)

PHOTO-2026-08-06-11-03-37.jpg



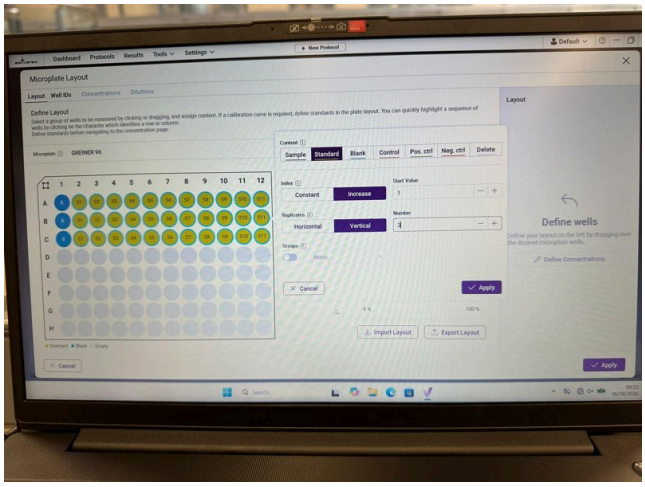
- The plate reader would read all 96 wells by default but you could also select the wells individually
- Different wells can be labelled as blanks, standards, samples etc., the machine would normalise the data with blank automatically

PHOTO-2026-08-06-11-03-38.jpg



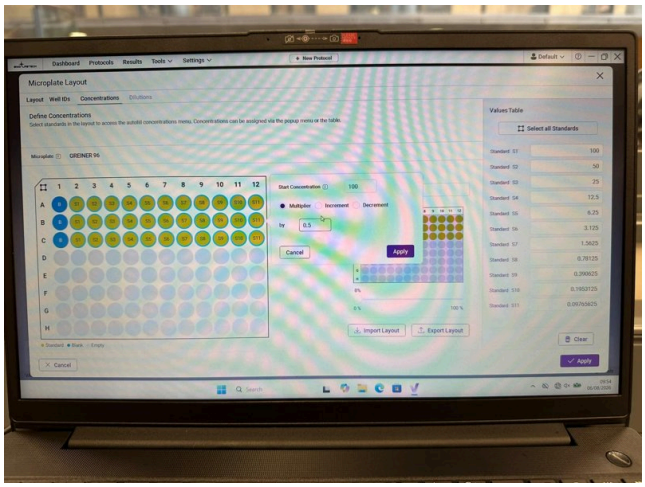
- You can label replicates by selecting the number and whether they are vertical or horizontal

PHOTO-2026-08-06-11-03-38_2.jpg



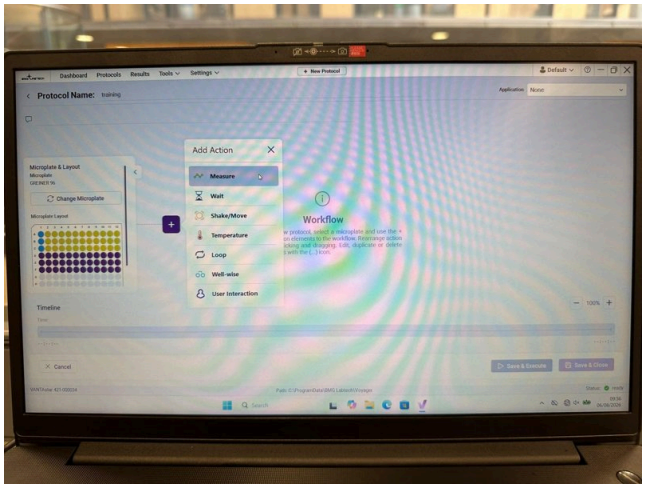
- For standards, you can fill in the starting concentration and the dilutions

PHOTO-2026-08-06-11-03-38_3.jpg



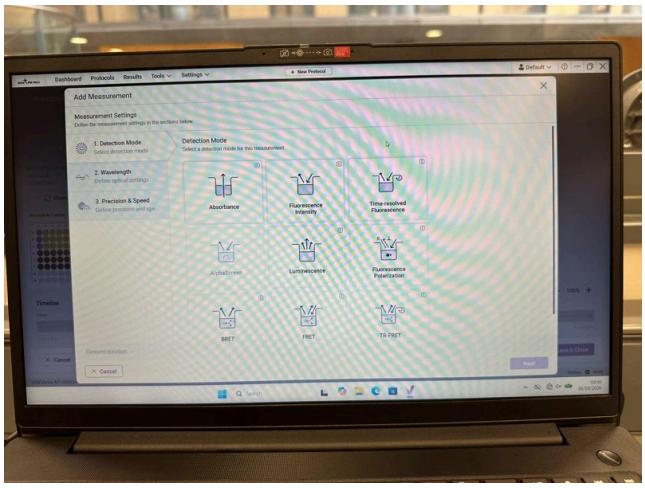
- After finishing the plate layout, you can press the plus sign to add a measurement

PHOTO-2026-08-06-11-03-39.jpg



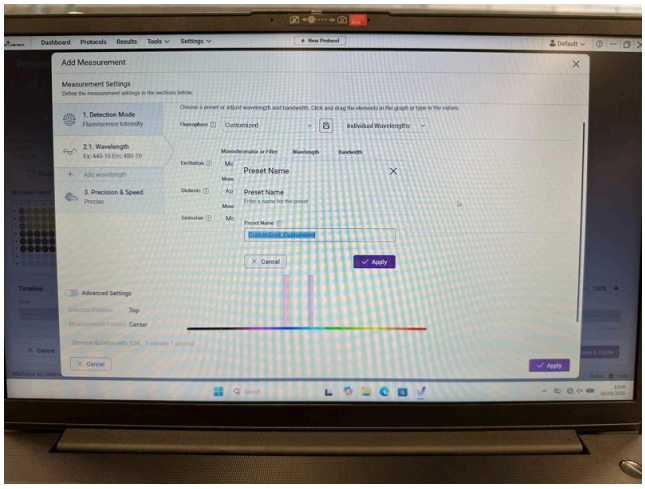
- For measurement setting, the detection mode allows you to select different types of measurements, including absorbance, fluorescence intensity and fluorescence polarisation

PHOTO-2026-08-06-11-03-39_2.jpg



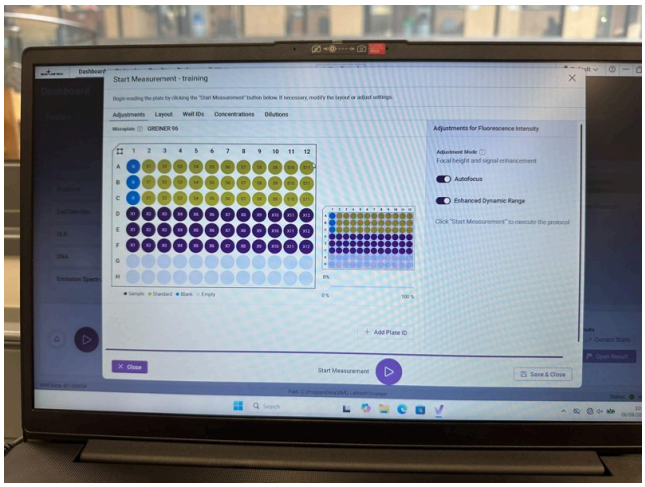
- Take fluorescence intensity for example, you can customise the excitation and emission wavelength, as well as the band width

PHOTO-2026-08-06-11-03-39_3.jpg



- The precision and speed option allows you to adjust the number of flashes (the higher number of flashes the better quality of the reading) and the setting time
- You can also use the more advanced setting options for detector position and measurement pattern
- Detector position can decide whether the machine is reading from the top or the bottom
- In terms of adjustments for fluorescence intensity, autofocus and enhanced dynamic range are on by default

PHOTO-2026-08-06-11-03-40.jpg



- For kinetic measurement, select "Loop" and input the total duration and loop duration, the machine would shake between readings

PHOTO-2026-08-06-11-03-39_4.jpg

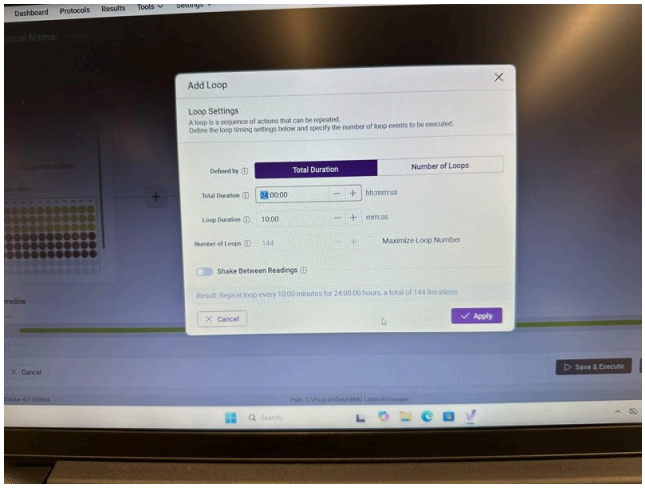
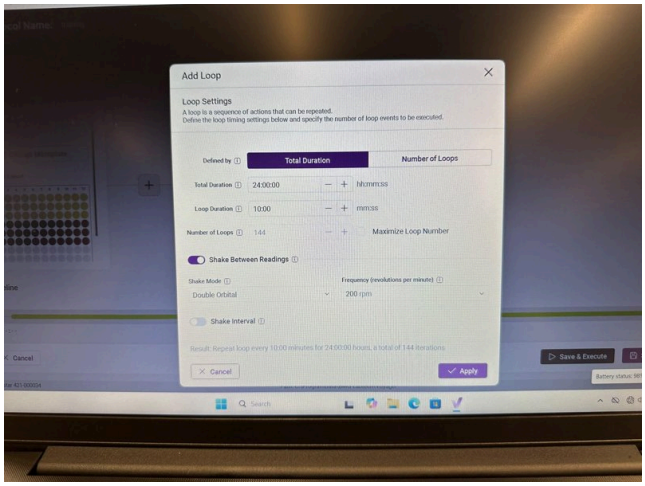
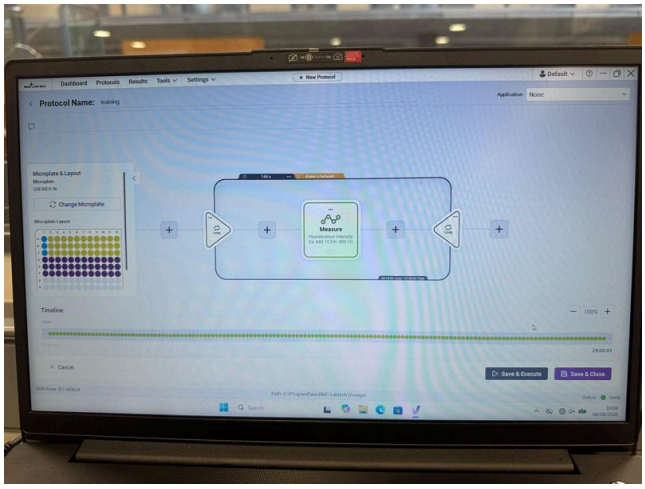


PHOTO-2026-08-06-11-03-39_5.jpg



- People usually use double orbital shaking pattern and the frequency is usually set to 200 rpm
- You need to drag the measurement into the loop

PHOTO-2026-08-06-11-03-39_6.jpg

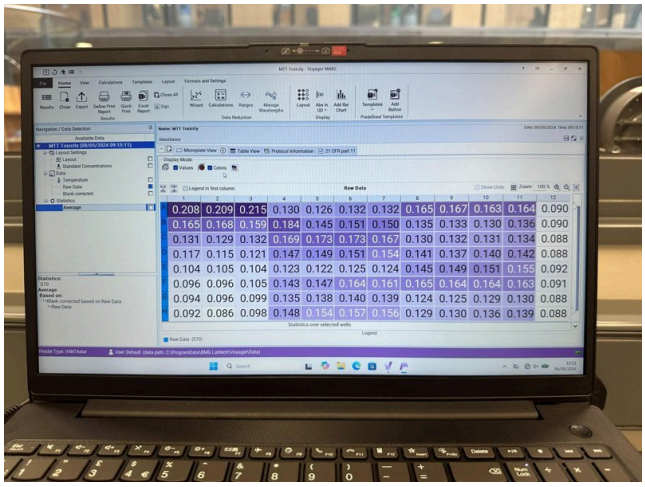


- For fluorescence polarisation measurement, a polarisation filter is needed for ThT
- Temperature can either be set in the whole environment or in each protocol (the range is from 25°C - 45°C)
- Data will be available in the result section which opens up the analysis software

3. Analysis software

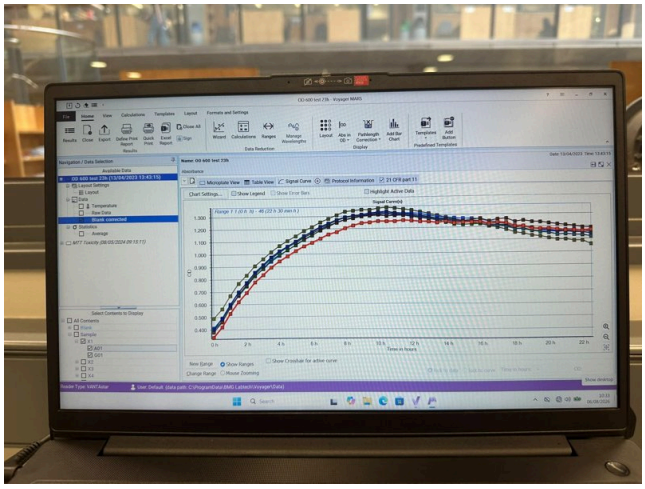
- You can see both the raw data and the blank corrected data
- Click on colour to visualise the colour intensity in each well

PHOTO-2026-08-06-11-03-40_2.jpg



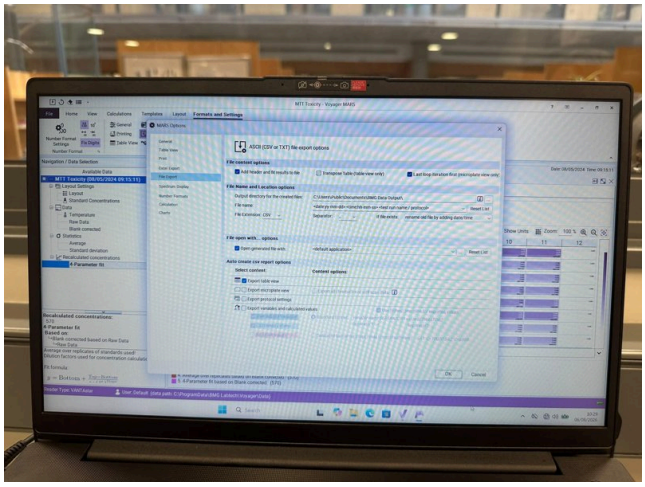
- Wizard tool can be used to plot standard curves (you can import your own equation for fitting the curve)
- F1 key opens up the help page for more information on how to use the software
- For kinetic data, you can generate kinetic curves

PHOTO-2026-08-06-11-03-40_4.jpg



- You can change the default export option in setting

PHOTO-2026-08-06-11-03-40_3.jpg



Sonication of 24 hr samples

1. 1 ml of the sample was collected and centrifuged for 3 min at x 12,000 rpm, supernatant was discovered and 400 µl PBS was added to resuspend the pellets.
2. The resuspension was then sonicated for 40 s (5 s pulse ON and 5 s pulse OFF) at amplitude 40%
3. The samples were centrifuged at x 14,000 rpm for 15 min, supernatants were collected and pellets were resuspended with 100 µl PBS
4. Another 1 ml of the sample was taken for OD600 measurement, recorded in the following table:

- 1 - 25°C = 0.228 abs, 1 - 32°C = 0.279 abs, 1 - 37°C = 0.213 abs
- 8 - 25°C = 0.256 abs, 8 - 32°C = 0.274 abs, 8 - 37°C = 0.215 abs
- 10 - 25°C = 0.272 abs, 10 - 32°C = 0.327 abs, 10 - 37°C = 0.290 abs

- 12 - 25°C = to be measured tomorrow, 12 - 32°C = 0.362 abs, 12 - 37°C = 0.310
- S - 25°C = 0.308 abs, S - 32°C = 0.201 abs, S - 37°C = 0.105 abs
- S-G - 25°C = 0.338 abs, S-G - 32°C = 0.212 abs, S-G - 37°C = 0.171 abs
- C - 25°C = 0.205 abs, C - 32°C = 0.205 abs, C - 37°C = 0.222 abs
- C-G - 25°C = 0.304 abs, C-G - 32°C = 0.210 abs, C-G - 37°C = 0.206 abs

5. All absorbance measurements are 1 : 10 diluted with TB

x10 MES running buffer

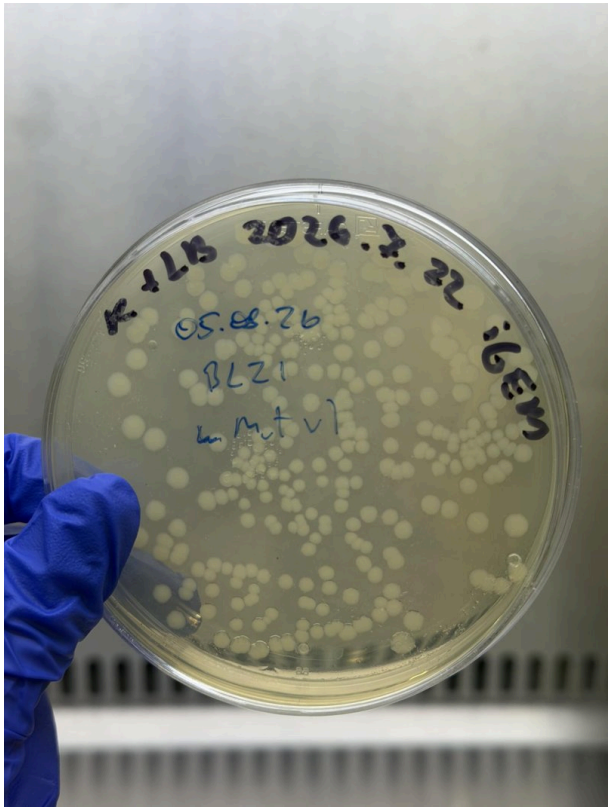
1. MES running buffer was prepared with:
    - 39.02 g MES
    - 24.24 g Tris base
    - 4 g SDS
    - 1.2 g EDTA
    - 400 ml ddH2O

2. x 10 MES running buffer was sterile filtered with 0.22 µm filter

Plate results of mutant transformants

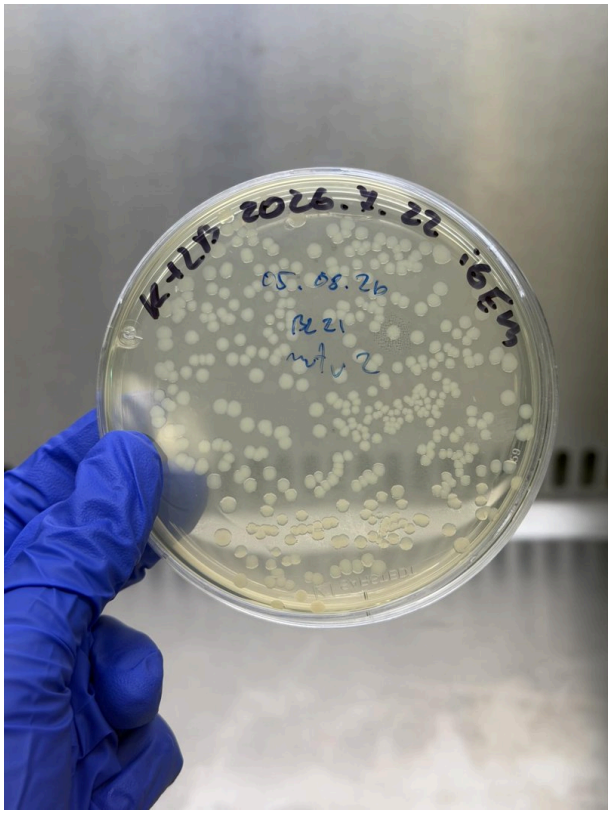
1. Mutant V1

IMG_1040.jpeg



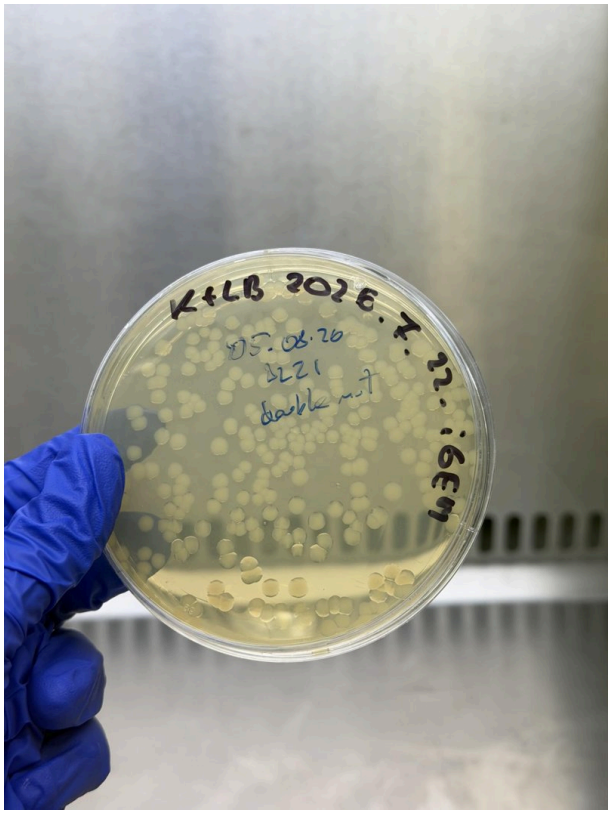
2. Mutant V2

IMG_1042.jpeg



3. Double cys mutant

IMG_1041.jpeg



20x MES-SDS Running Buffer - 300 mL

Introduction

Purpose: SDS-PAGE running buffer (MES system — good resolution for low-MW proteins).

Materials

- MES (48.8 g)
- Tris base (30.3 g)
- SDS (5 g)
- EDTA (1.5 g)
-

Procedure

1. Dissolve all components in ~200 mL ddH_2O.
2. Make up to 250 mL with ddH_2O.
3. Filter sterilize (0.22 µm) if storing long-term.
4. Dilute 1:20 in ddH_2O to make 1x working buffer before each run.

20x MES-SDS Running Buffer - 1L (make smaller stocks)

Introduction

Purpose: SDS-PAGE running buffer (MES system — good resolution for low-MW proteins). 1M MES 1M Tris-HCl 5g/L SDS

Materials

- 195.2g MES
- 121.2g Tris base
- 20g SDS
- 6g EDTA
- 1L ddH2O

Procedure

- [ ] 1. Dissolve all components in ~200 mL ddH_2O.
- [ ] 2. Make up to 250 mL with ddH_2O.
- [ ] 3. Filter sterilize (0.22 µm) if storing long-term.
- [ ] 4. Dilute 1:20 in ddH_2O to make 1x working buffer before each run.

07/08/26 - Friday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 06 Aug 2026 18:46:42 UTC
Entry Last Modified: 09 Aug 2026 17:15:49 UTC
Export Generated On: 11 Oct 2026 18:38:26 UTC

THURSDAY, 06/08/2026

Members

Vasily Vanifatov, Katie Wei, Hector Cross

Objectives

- ~~**Sterile filter 10x MES**~~
- ~~**Sonicate and spin down 12 samples (BLR 24hr timepoint)**~~
- Resuspension of all pellets
- ~~Make 12L TB send to autoclave by 3pm~~
- ~~Make destain~~
- ~~SDS-PAGE of expression tests with negative control~~

Sonication

1. Sonication follows the same protocol as yestarday (see 06/08/26 - Thursday)
2. Each sample was sonicated for 40 s (5 s pulse ON and 5 s pulse OFF) at amplitude 40%

SDS-PAGE

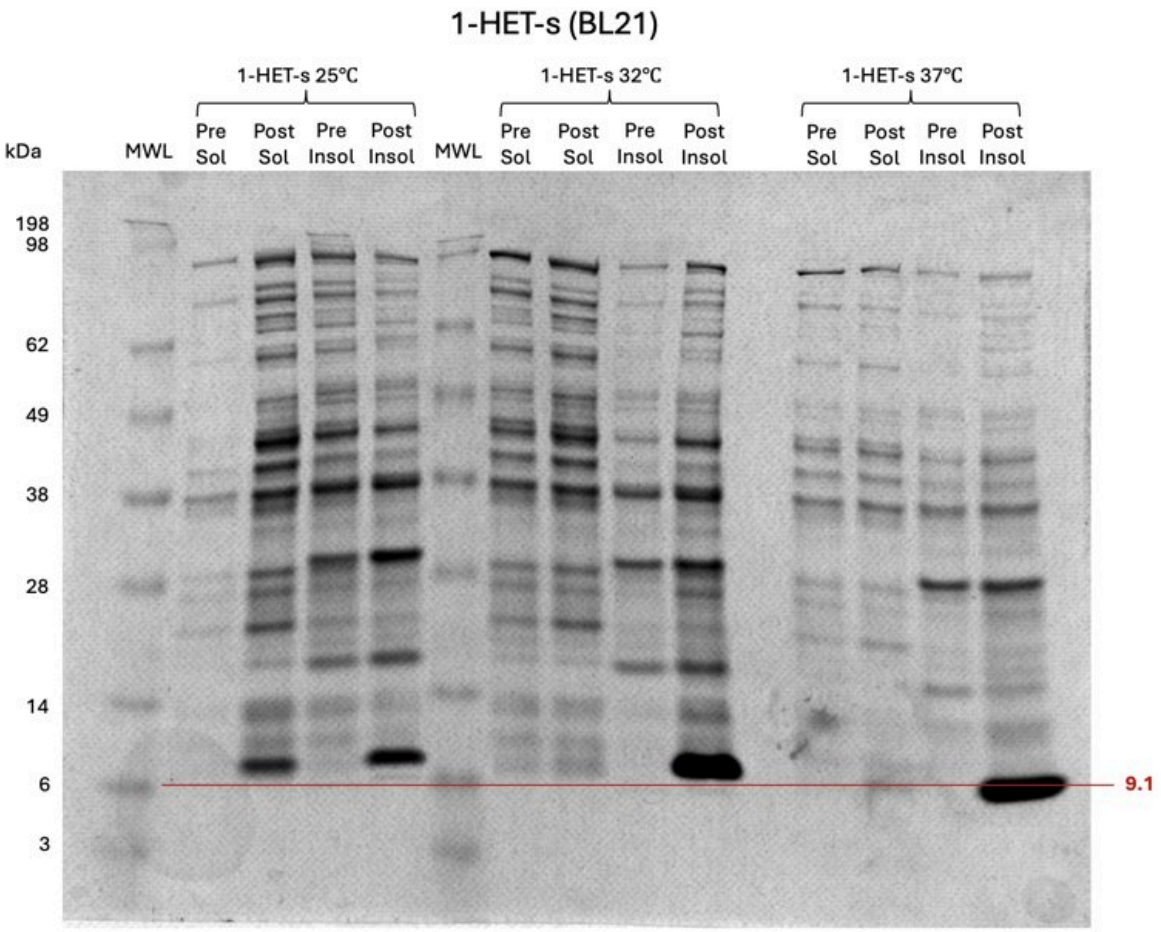
1. SDS-PAGE samples were prepared by centrifuging the sonicated samples for 10 min at 14,000 rpm
2. Supernatants were collected as the soluble sample and pellets were resuspended in 100 µl PBS as the insoluble sample
3. 15 µl of each sample was mixed with 15 µl of SDS loading buffer and were heated for 3 min at 100 °C heat block
4. Gel 1 and 2 used SurePAGE Bis-Tris gels and MES running buffer, the other four gels used ProteinEle Precast Tris-Glycine gel (4%-20%) and Tris-glycine running buffer
5. For all gels, 10 µl of sample and 5 µl of ladder was loaded

- Gel run 1 (MES with Genscript gels):
  Gel 1: 1-HET-s (BL21)
  Gel 2: 10-mCardinal-HET-s (BL21)

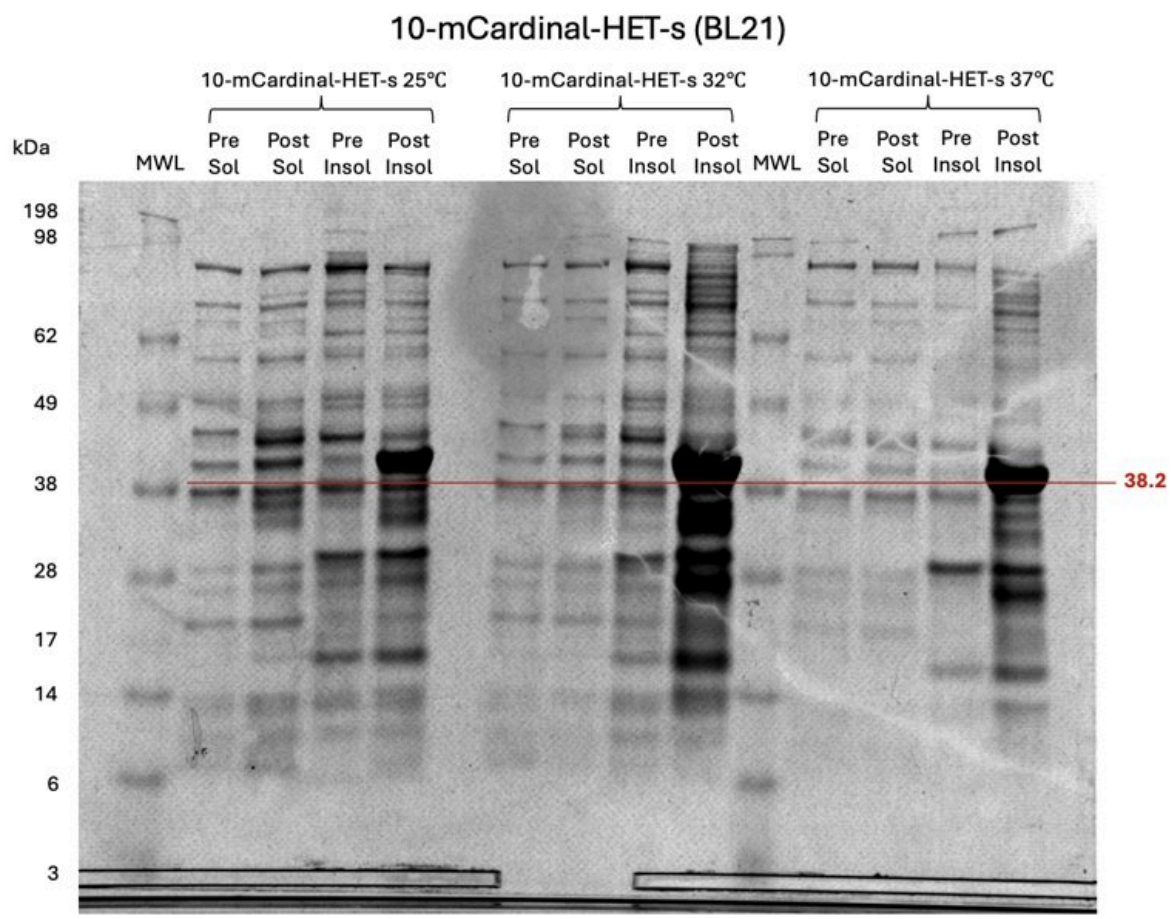
- Gel run 2 (Tris-glycine with Cliniscience gels):
  Gel 3: 7-seed (BLR)
  Gel 4: 11-seed-eGFP (BLR)
  Gel 5: 12-cap-eGFP (BL21)
  Gel 6: 12-cap-eGFP (BLR)

Gel image results

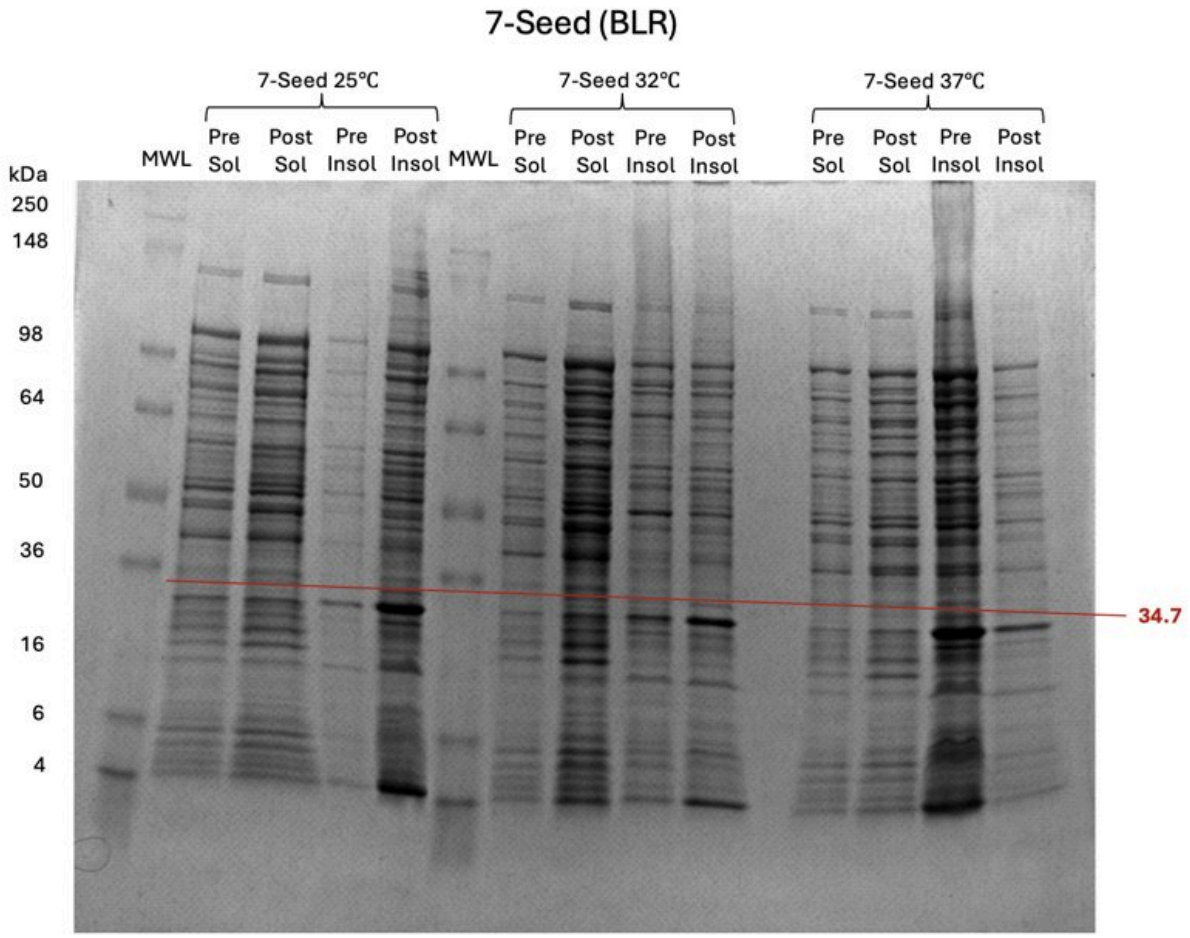
2026-08-07_1-HET-s.png



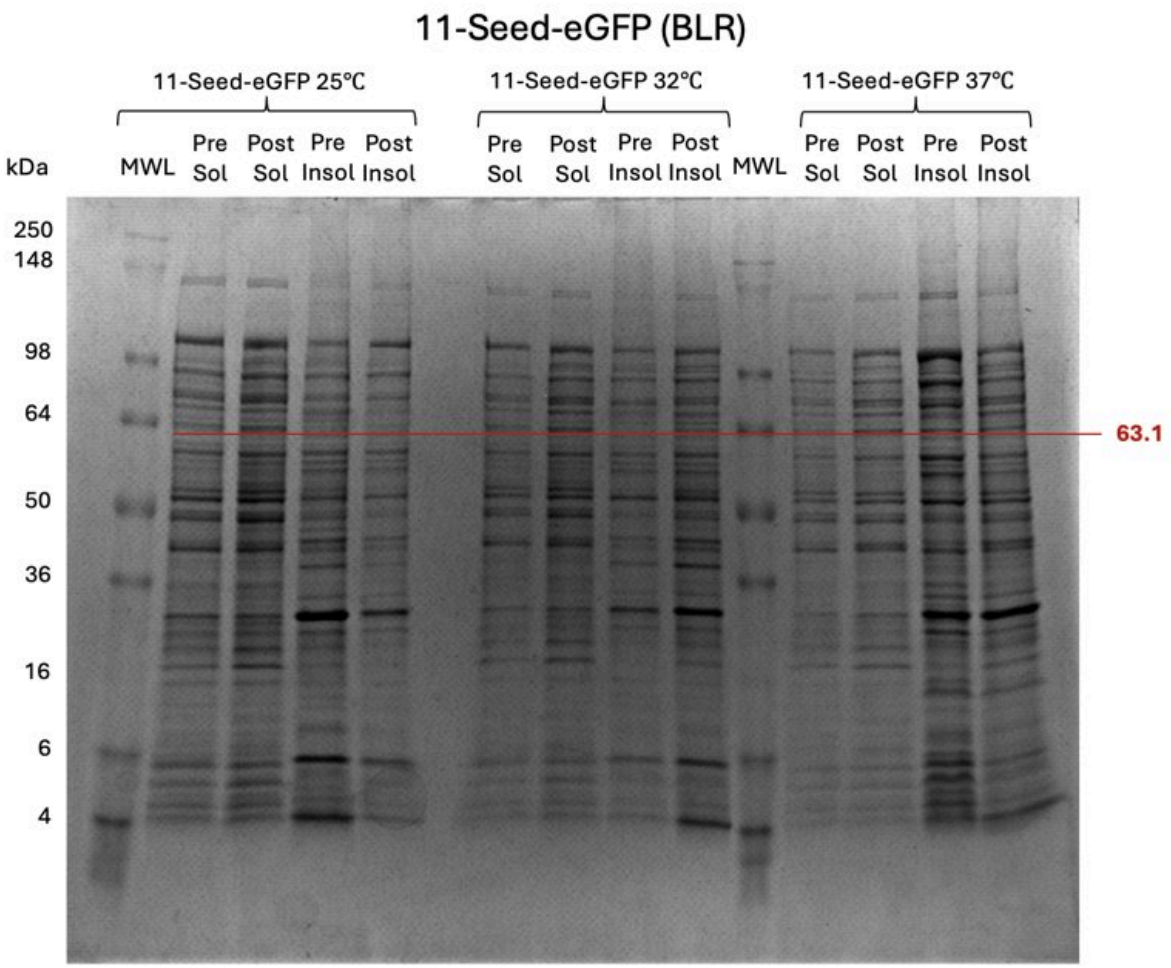
2026-08-07_10-mCardinal-HET-s.png



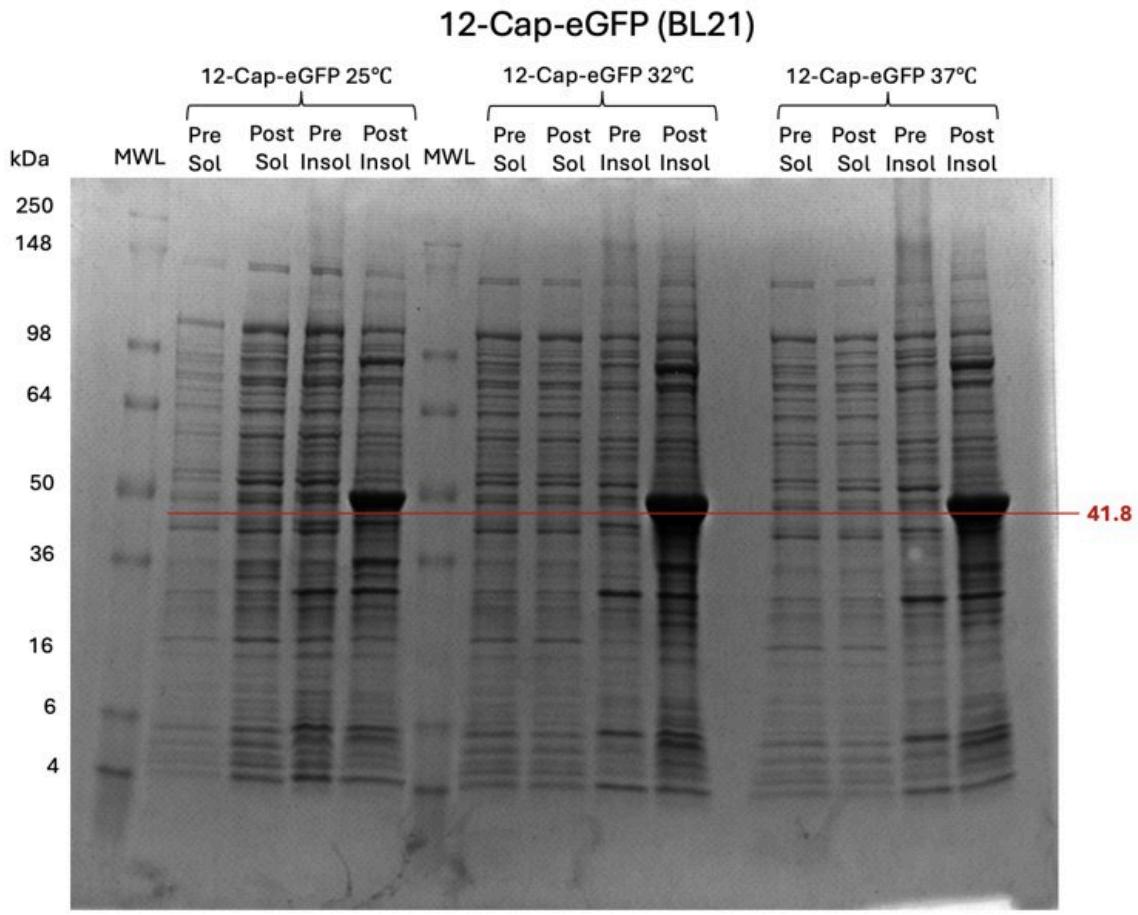
2026-08-07_7-Seed_BLR.png



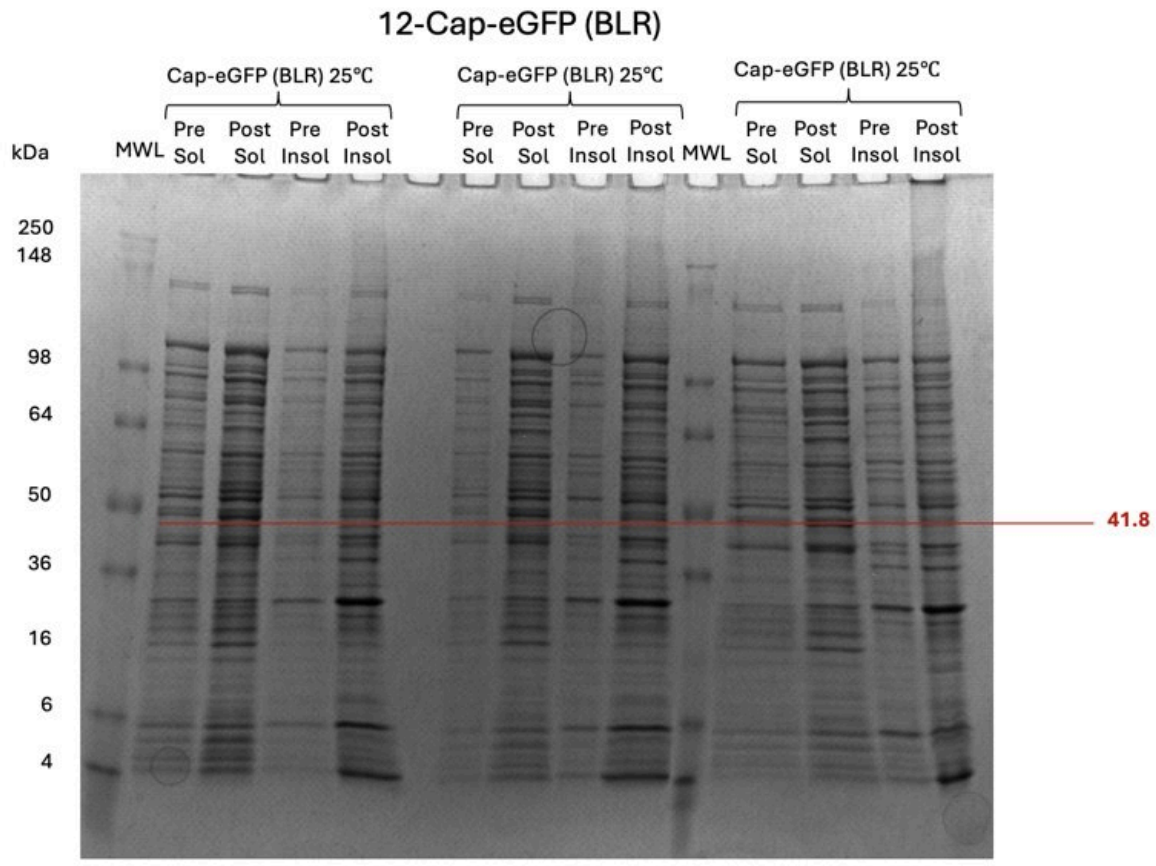
2026-08-07_11-Seed-eGFP_BLR.png



2026-08-07_12-Cap-eGFP_BL21.png



2026-08-07_12-Cap-eGFP_BLR.png



09/08/26 - Sunday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 07 Aug 2026 18:30:59 UTC
Entry Last Modified: 07 Aug 2026 18:37:02 UTC
Export Generated On: 11 Oct 2026 18:38:25 UTC

FRIDAY, 07/08/2026

Members

Hector Cross (Supervised by Ben Shin)

Objectives

20mL preculture of WT, double mut, mut v1 & mut v2

10/08/26 - Monday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 07 Aug 2026 18:44:01 UTC
Entry Last Modified: 10 Aug 2026 18:19:43 UTC
Export Generated On: 11 Oct 2026 18:40:13 UTC

FRIDAY, 07/08/2026

Members

Katie Wei, Hector Cross

Objectives

- ~~**Make 12L TB first thing in the morning**~~
- **Autoclave tips**
- ~~**Run Cap BL gel**~~
- **Send 1L TB+10x bottle for autoclave**
- ~~Make more destain~~
- ~~Put 4 monomer constructs shaking @37 ON~~
- ~~Transform Seed & Seed-GFP into BL21 for expression test~~
- Hector shows that he has bought a USB stick
- 50% glycerol stock sterile filtered or autoclaved
- Make Kan plates and make more Kan stock
- Sort out freezer storage

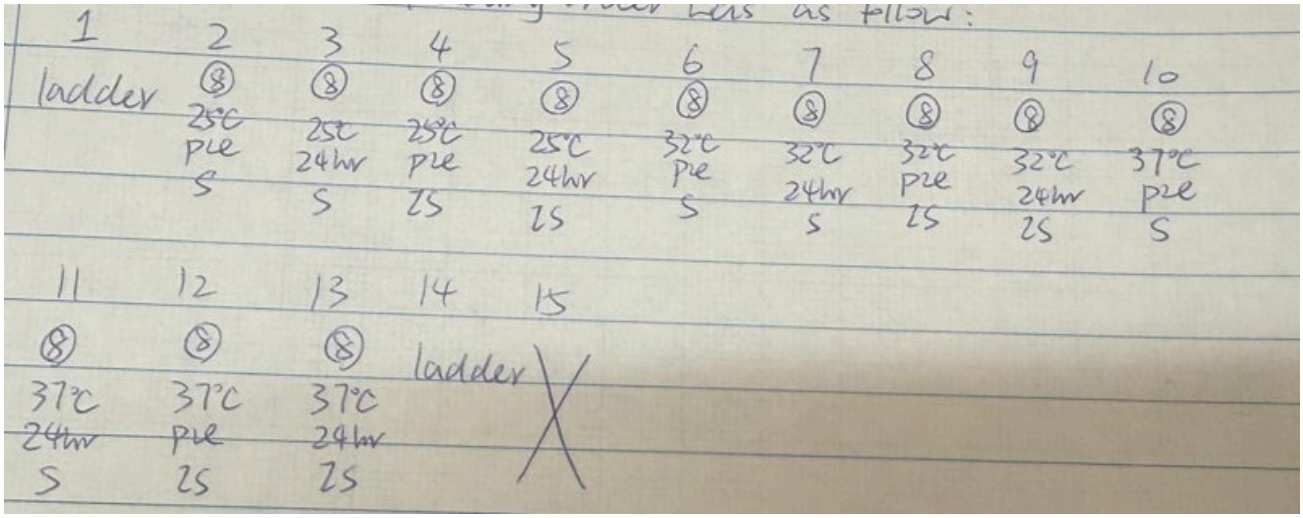
12 L TB culture

1. Weighed out 47.6 g of TB salt and added it to a 2 L conical flask
2. Added 920 ml ddH2O and seal the flask with double layerd aluminum foil
3. 3 L of culture was made for each construct

SDS-PAGE

1. 15 µl of samplw was mixed with 15 µl of loading buffer and were boiled at 100 °C heat block for 3 min
2. SurePAGE Bis-Tris gel and MES running buffer was used
3. 10 µl of the sample and 5 µl of the ladder was loaded, the loading order was as follow

IMG_1074.jpeg



4. Gel was run at 80 V until leaving the well, and then at 150 V until reaching the bottom
5. Gel was stained with Coomassie blue and destained with destaining solution

Transformation of seed and seed-eGFP into BL21 strain

1. The seed and seed-eGFP DNA were transformed into 50 µl BL21 competent cells
2. Transformation followed the same protocol (see 05/08/26 - Wednesday)

Inoculation

1. The OD600 of preculture were measured and results were recorded below:

Table1

	Construct	Absorbance	Adjusted Absorbance
1	WT1 (1:10)	0.302	3.02
2	WT2 (1:20)	0.168	3.36
3	WT3 (1:20)	0.165	3.30
4	V1-1 (1:20)	0.161	3.22
5	V1-2 (1:20)	0.158	3.16
6	V1-3 (1:20)	0.156	3.12
7	V2-1 (1:20)	0.177	3.54
8	V2-2 (1:20)	0.179	3.58
9	V2-3 (1:20)	0.164	3.28
10	Double cys-1 (1:20)	0.166	3.32
11	Double cys-2 (1:20)	0.157	3.14
12	Double cys-3 (1:10)	0.296	2.96

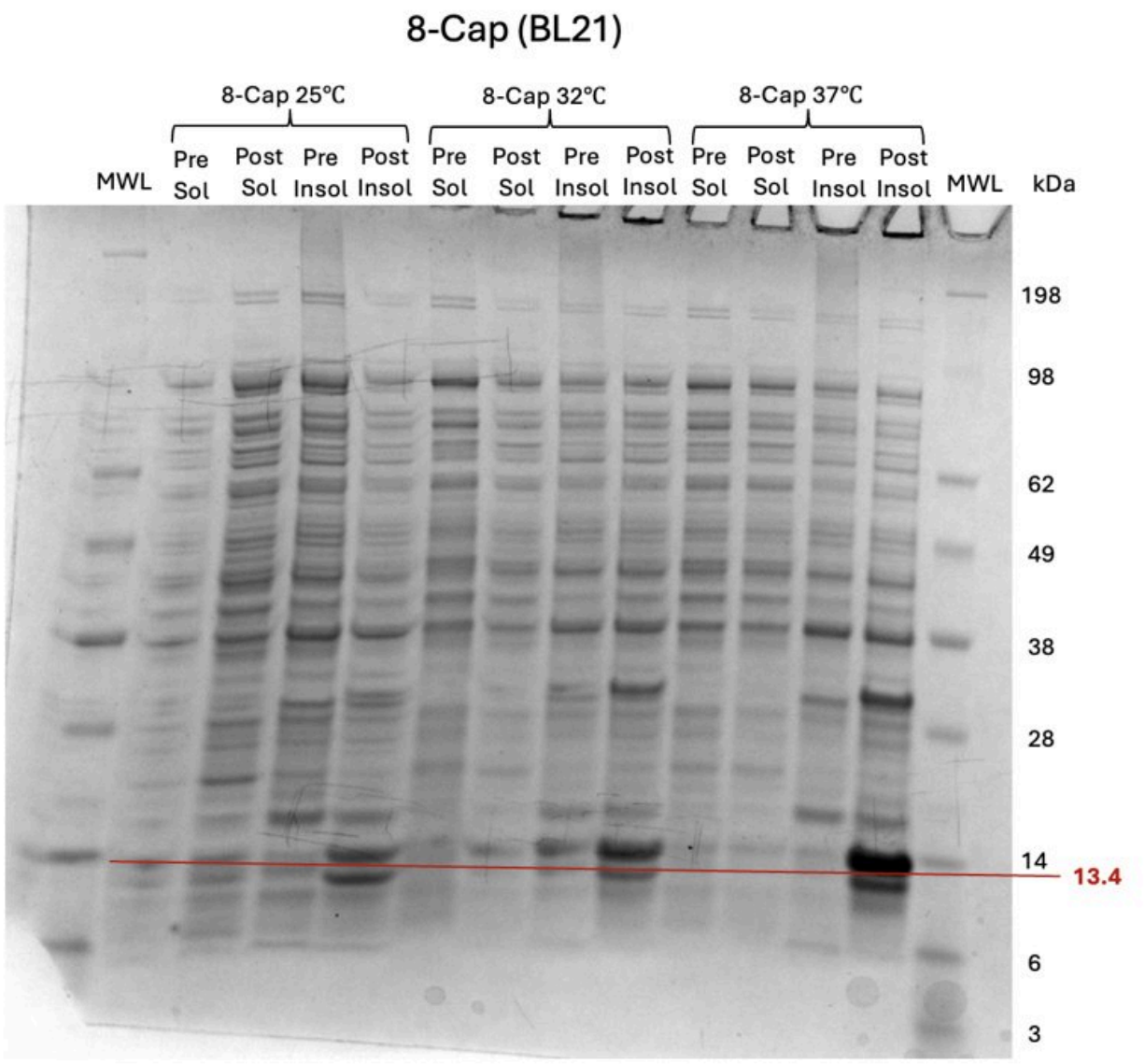
2. For each 1 L TB culture, 120 ml of auto-induction solution (100 ml x10 TB salt and 20 ml of x50 auto-induction solution) was added
3. 8 ml of each pre-culture was inoculated
4. The flasks were put in shaking incubator at 37°C for 24 hr

Glycerol stock

1. For glycerol stock, 1 ml of 50% glycerol was mixed with 1 ml of the pre-culture solution
2. The stock was then frozen at -80°C fridge

Gel image result

2026-08-10_8-Cap_BL21.png



11/08/26 - Tuesday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 10 Aug 2026 18:20:07 UTC
Entry Last Modified: 11 Aug 2026 18:34:54 UTC
Export Generated On: 11 Oct 2026 18:40:12 UTC

MONDAY, 10/08/2026

Members

Katie Wei, Hector Cross

Objectives

- ~~**Autoclave tips**~~
- ~~**Send 500mL TB+10x preculture stockbottle for autoclave (250mL bottles so 3g tryptone 6g yeast extract + 25mL 10x TB salt)**~~
- Hector shows that he has bought a USB stick
- Make Kan plates
- ~~Make Kan stocks~~
- **Sort out freezer storage**
- ~~**5mL cultures for glycerol stock of Cap & Cap-GFP**~~
- ~~**5mL precultures for seed & seed-GFP expression test**~~
- ~~Centrifuge tutorial with Liz McCormack on 4 monomer constructs from ON expression @ 37~~

TB culture

1. Weighed out 3 g of tryptone and 6 g of yeast extract and added it into a clean 250 ml bottle
2. Added 225 ml of MiliQ water to dissolve the powder
3. Added 25 ml of x10 TB salt
4. Labelled the bottles and sent them for autoclave

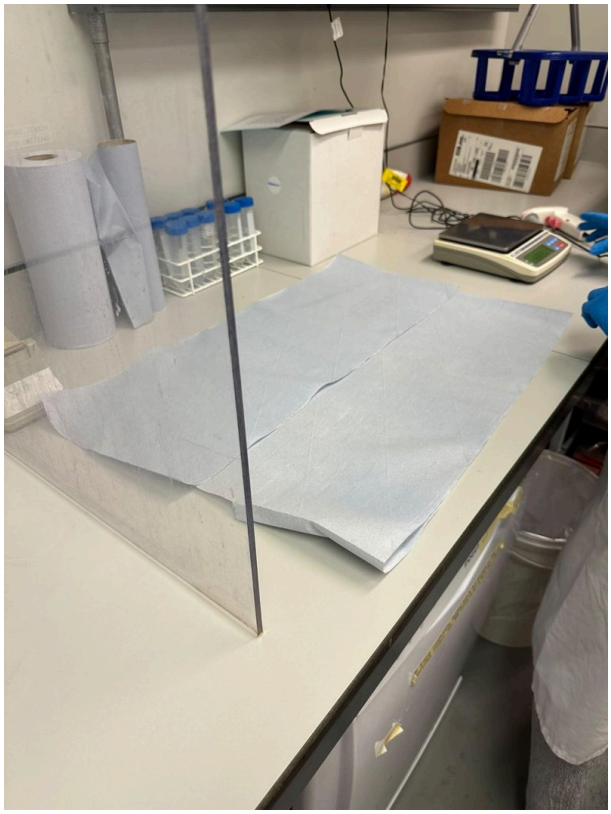
Kanamycin

1. Weighed out 1 g of kanamycin inside the fumehood
2. Added 10 ml of MiliQ water to dissolve kanamycin, making the stock concentration 100 mg/ml (x2000 kanamycin stock)
3. Sterile filter the kanamycin solution using syringe and 0.22 µm filter inside the clean bench or near bunsen burner

Centrifuge tutorial

1. Place blue towls on the bench to prevent culture contamination

589ac138-a4c6-40e5-ad9a-dff270febc48.JPG



2. Pour cultures from conical flask into centrifuge bottles (this step should be done on bench not on balance) until it fills up the entire bottle
3. Close the bottle with lid and weigh it on the electronic balance, then zero it

bac8aefc-975a-4b51-850b-035ecd010dec.JPG



4. Pour another culture into a separate centrifuge bottle and weigh it on the balance (with the lid) until the reading is zero again, use the dropper to add/remove liquid from the bottle

7e9579a9-618a-4c9b-ab6c-db02b9dfab91.JPG



5. Put the balanced bottles into the J6 centrifuge

d81a1ce1-cb76-4351-aab1-c5b7c9402284.JPG



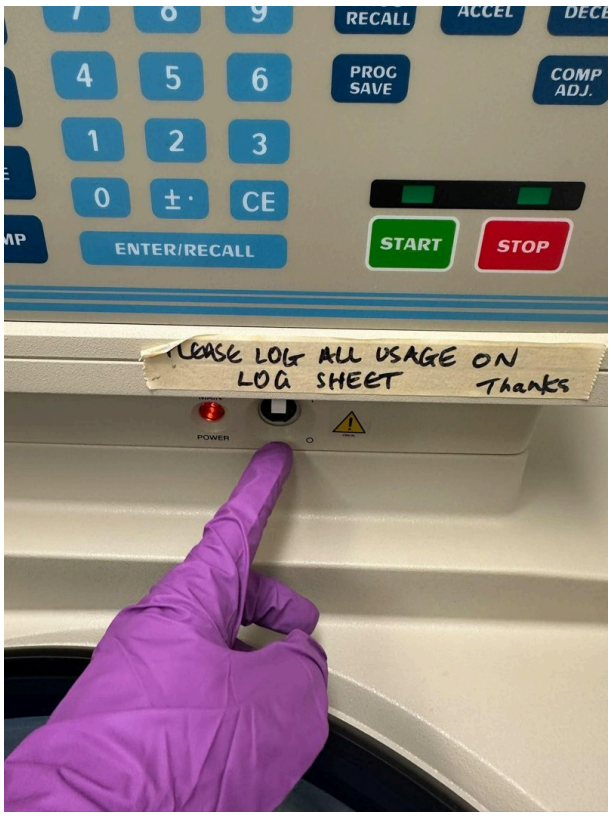
6. Put the blue lid on top of the rotor and close the centrifuge

60d43e4e-34d4-4844-b8e3-de04205dee5b.JPG



7. Switch the centrifuge on and set the program, the rotor is 4.2, the speed is 4,200 rpm, and the time is 20 min, then press start

ff83ca0e-858e-4a48-b172-0781cccde09d.JPG



8. During the centrifugation, prepare Tomson flasks with chemgene and make up the virkon solution

12993752-9502-4f5a-9350-9d4461e56585.JPG



9. After centrifugation is finished, pour off the supernatant into Tomson flasks
10. When the flasks are full, leave them in the box underneath the sink
11. For the conical flasks and the bottle, add virkon solutions and leave them for 20 min, then pour off the liquid down the sink and return the flasks downstairs, the bottles can be left on the bench

Resuspension

1. Added 25 ml of Lysis buffer and scrape the bacteria pellets at the bottom of the bottle with the stripette tips
2. Added some more Lysis buffer to clean the edge and the tip head, then transferred all of the bacterial resuspension into the pots
3. Placed the pots at -80°C freezer and left them on rollers overnight to thaw the cells

Pre-culture

- Pre-culture were prepared for seed, seed-eGFP, cap, cap-eGFP, all of them are BL21 transformants
- See 28/07/2026 - Tuesday for the protocol of pre-culture

12/08/26 - Wednesday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 11 Aug 2026 18:31:52 UTC
Entry Last Modified: 12 Aug 2026 19:03:22 UTC
Export Generated On: 11 Oct 2026 18:40:12 UTC

TUESDAY, 11/08/2026

Members

Katie Wei, Hector Cross

Objectives

- ~~**Making glycerol stocks for seed, seed-eGFP, cap, cap-eGFP**~~
- **Making 6 L TB and send them for autoclave**
- ~~**20 ml culture of seed, seed-eGFP and induction for expression test**~~
- ~~Protein purification of monomers and mutants~~
- Sort out freezer storage
- USB stick
- Make Kan plates

Glycerol stock

1. 1 ml of 50% glycerol was mixed with 1 ml of the precultures of seed, seed-eGFP, cap, cap-eGFP respectively
2. Sampleswere stored at -80°C fridge

Expression test of seed

1. Autoinduction culture was prepared with 147 ml of TB + x10 TB salt, 3 ml of x50 autoinduction solution, and 75 µl of kanamycin
2. 20 ml of the culture was aliquoted and respective amount of bacterial preculture was added to ensure that the starting OD600 was 0.05
3. The OD600 of precultures were as follow:

Table1

	Construct	Absorbance (1:10)	Adjusted Absorbance	Volume added (µl)
1	Seed	0.531	5.31	200
2	Seed-eGFP	0.790	7.90	130

4. OD600 was then measured again after the bacteria reaches their exponential phase, and the recordings were as follow (7 hours):
    - Seed = 0.542 abs
    - Seed-eGFP = 0.592 abs