Imperial College London

LABORATORY BOOK



DEPARTMENT OF LIFE SCIENCES

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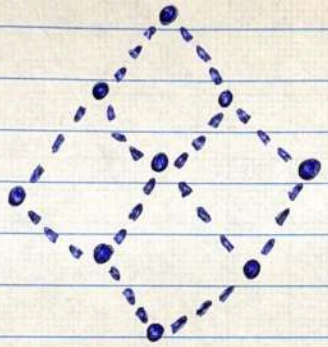
Please return to SAF 203



reGELerate

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In lab: Katie Wei, Mateo-Martin-Nieto, Vaira Knabikade

Tuesday 21/07/2026

Objective

Make the antibiotic stock concentrations.

Took the reagents from Paul & Freemond's lab measured out:

- Kanamycin: 202.3 mg
- chloramphenicol: 142.7 mg

We disolved kanamycin in ddH_2O to a final concentration of 100 mg/ml

Disolved chloramphenicol in ~~ddH_2O~~ ethanol to a final concentration of 50 mg/ml

Both were vortexed for ~5s and kept in the cold room (4°C).

DEBABRATA DUTTA

In lab: Katie Wei, Mateo-Martin-Nieto, Vaiva Kenabikaite

~~Monday~~ Wednesday 22/07/2026

Objective

Make LB-Kanamycin and LB-Kanamycin + Chloramphenicol agar plates.

Took already prepared LB broth: 350 mL for Kanamycin plates and 150 mL for Kanamycin + Chloramphenicol plates. and heat it up in a microwave

Added: (After we can hold the bottle in hands comfortably)

- LB-Kanamycin plate: 175 mL of Kanamycin stock (see p.3)
- LB-Kanamycin + Chloramphenicol: 75 µL of Kanamycin and 75 µL of Chloramphenicol stock (see p. 3)

Then we labeled the petri dishes and poured ~25 mL of LB + antibiotic into each plate. 15 plates were made for LB-Kanamycin and 6 plates for the LB-Kanamycin + Chloramphenicol.

DEBABRATA DUTTA

In lab: Hector Cross, Mateo Martin Nieto, Katie Wei, Vaiva Knabikaite

Objectives

Friday 24/07/2026

→ ~~Final~~ Autoclave induction
→ 10x TB salt 2L 90g Na_2HPO_4, 22g KH_2PO_4, 26g NH_4Cl, 7g Na_2SO_4
→ 50x TB auto induction 2L 250g Glycerol, 25g glucose, 100g lactose

Both added twice
(The masses written are for 1L)

Sent to autoclave at 16:00

DEBABRATA DUTTA
Dutt

In lab : Mateo Martin Nieto, Katie Wei

Monday 27/07/2026

Objectives:
-Prepare guanidine hydrochloride (GuHCl) buffers, TB ~~buffer~~ base
-Perform transformation of the four constructs for the expression test (01. 08. 10. 12)

To make the TB base (1L), we added:
12 g Tryptone
24 g Yeast extract
Weighed out tryptone and yeast extract on an electronic balance, dissolved it in 900ml MiliQ and mixed it, then make up the volume to 1L. TB base is then autoclaved

To make the ~~elution~~ GuHCl buffer, we used:
GuHCl 6M. ~~MAT~~
Tris base 50 mM
For ~~3~~ 4.5 L of ~~wash buffer~~ ~~GuHCl~~ Lysis buffer, we added:
2578.5 g GuHCl
27.27 g Tris base
Separately, we made an additional 2L of Lysis buffer by adding:
1146 g GuHCl
12.12 g Tris base

Took 1L of the lysis buffer and added 20.42 g imidazole to make 1L of the elution buffer with 300 mM imidazole

Took 3.5L of the Lysis buffer and added 7.15 g imidazole to make 3.5 L of the wash buffer with 30 mM imidazole

All buffers ~~are~~ were adjusted to pH 8.0 by adding 37% HCl and measuring the pH using a pH meter

All buffers ~~were~~ were sterilised using a vaccum filter with 0.2μm

Resuspended Twist DNA constructs with autoclaved MilliQ (Constructs 01, 08, 10, 12) to make a stock concentration of 10ng/μl
Added 3μl of all four constructs to eppendorf tubes and added ~~≅~~ 40μl of BL21(DE3) competence cell.
For control, added 5μl of pET-28a(+) and 40μl competence cell.
Incubated on ice for 30min
Heat shocked in 42°C water bath for 30s
Incubated on ice for 2min
Added 250μl SOC medium
Placed the eppendorf tubes in shaking incubator at 37°C ~~225~~ 220 rpm for 1h
Plated ~~5~~ ≅ 50μl and 200μl of bacteria on k+ LB plates for each construct and the control
Incubated the plates in 37°C overnight

~~Observation and~~

DEBABRATA DUTTA

In lab: Katie Wei, Mateo-Martin-Nieto, Vaiva Knabikaite

Tuesday 28/07/2026

Observations and notes from 27/07/2026

All petri dishes showed growth, including +ve control. However, all of them were overgrown, so next time 10 and 20 µL should be plated.

Objectives

- Prepare precultures

We put 20 mL TB, 2.2 mL 10× TB salt and 11 µL Kanamycin to the incubation tube. Then we selected a single colony from the petri dish and added it into the tube. This was done for all 4 constructs. The tubes were incubated at 37 °C, 220 rpm, overnight.

DEBASRATA DUTTA

DD

In lab: Hector Cross, Katie Vei, Mateo-Martin-Nieto, Vaiva Kuzbi-kaite

Wednesday 29/07/2026

Observations from 28/07/2026
All 4 tubes that were incubated overnight look cloudy.

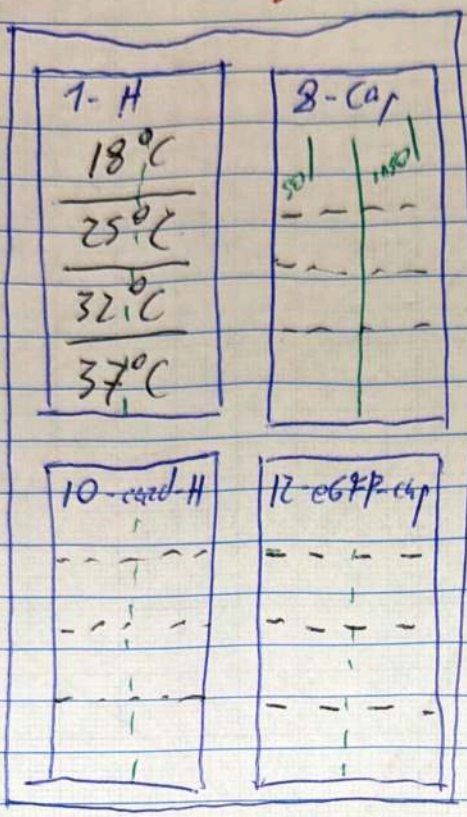
Objectives
- Prepare cultures for expression testing with auto-induction TB

Culture ODs - Taken at 11:30
The results from spectrophotometer 600nm
- Blanked with Auto-induction TB + Kan
01 - HET-s (1:10): 0.581 abs → 5.81 abs
08 - Ca, (1:10): 0.526 abs → 5.26 abs
~~09 - Poissoned cap (1:~~
10 - mCardinal - unit (1:10): 0.817 abs → 8.17 abs
12 - eGFP-cap (1:10): 1.077 abs → 10.77 abs

To make auto-induction TB solution (enough for all tubes), we added ~~BBOTB~~, 360 mL TB, 40 mL 10x TB salt, 8 mL auto-induction TB and 200 µL Kanamycin.

For each construct, we added 20 mL of the TB-autoinduction solution and ~~the rest~~ the respective volume of bacteria, such that the final concentration is 0.02 (01 ⇒ 70 µL, 08 ⇒ 80 µL, 10 ⇒ 50 µL, 12 ⇒ ⇒ 40 µL). For each construct 4 falcon tubes were prepared, ready to be incubated at 37°C.

Tube labeling - 18 hr / 24 hr



Nomenclature example

1-H
18°C, 18 hr
sol

Culture ODs - Taken at 16:15 (after being incubated at 37°C)
Measured with spectrophotometer at 600nm; blanked with auto-induction TB + Kan.
01 - Het-s: 0.085 abs
08 - Cap: 0.162 abs
10 - mCardinal-cmt: 0.073 abs
12 - eGFP-cap: 0.063 abs

Culture ODs - Taken at 18:30 (after being incubated at 37°C)
Measured at 600nm, blanked (see above)
01 - Het-s: ~~0.590~~ 0.592
08 - Cap: 0.855
10 - mCardinal-cmt: ~~0.507~~ 0.510
12 - eGFP-cap: 0.297

Culture ODs - Taken at 7:30 pm

01 - Host:
08 - Cap:
10 - mCardinal-cap:
12 - eGFP-cap:

18°C, 12-eGFP-cap: 0.526 abs
25°C, 12-eGFP-cap: 0.588 abs
32°C, " : 0.421 abs
37°C, " : 0.495 abs

We start induction at 7:30 pm

DEBABRATA DUTTA

In lab: Mateo-Martin-Nieto, Katie Wei, Vaiva Knabikaite, Vasily Vanifator

Thursday 30/07/2026

Observations from 29/07/2026
All tubes looked cloudy

Objectives
- Lyse the cells ~~with~~ via sonication
~~- Run an SDS get~~

Time: 13:30

OD measurements of 18hr

01 - H 18°C: 1.383 abs
01 - H 25°C: 1.656 abs
01 - H 32°C: 1.530 abs
01 - H 37°C: 1.336 abs
08 - Cap 18°C: 1.567 abs
08 - Cap 25°C: 1.480 abs
08 - Cap 32°C: 1.430 abs
08 - Cap 37°C: 1.289 abs
~~10 - card - H~~
10 - card - H 18°C: 1.611 abs
10 - card - H 25°C: 1.535 abs
10 - card - H 32°C: 1.620 abs
10 - card - H 37°C: 1.712 abs
12 - eGFP - cap 18°C: 1.727 abs
12 - eGFP - cap 25°C: 1.761 abs
12 - eGFP - cap 32°C: 1.782 abs
12 - eGFP - cap 37°C: 1.781 abs

Sonication
Centrifuge 1 mL sample ~~and~~ at 1200 rpm for 3 min and remove the supernatant. Resuspend the pallet with 400 µL PBS solution. Then sonicate for 5 mins: pulse ~~5~~ on for 5 sec and pulse off 10 sec. Keep the epp tubes in ice.

amplitude 30%

12

(1:5)

OD measurements of 24h (600nm) Time 19:20

01-H, 18°C: 0.444 abs ⇒ 2.22
01-H, 25°C: 0.528 abs ⇒ 2.64
01-H 32°C: ~~0.631 abs~~ ⇒ 0.435 abs ⇒ 2.18
01-H, 37°C: 0.405 abs ⇒ 2.03
08-Cap, 18°C: 0.536 abs ⇒ 2.68
08-Cap, 25°C: 0.437 abs ⇒ 2.19
08-Cap, 32°C: 0.401 abs ⇒ 2.01
08-Cap, 37°C: 0.390 abs ⇒ 1.95
10-Card H, 18°C: 0.564 abs ⇒ 2.82
10-Card H 25°C: 0.477 abs ⇒ 2.39
10-Card H 32°C: ~~0.52~~ 0.513 abs ⇒ 2.57
10-Card H 37°C: 0.567 abs ⇒ 2.84
12-eGFP-cap, 18°C: 0.638 abs ⇒ 3.19
12-eGFP-cap, 25°C: 0.690 abs ⇒ 3.45
12-eGFP-cap, 32°C: 0.631 abs ⇒ 3.16
12-eGFP-cap, 37°C: 0.680 abs ⇒ 3.40

24h samples were put in a -20°C freezer for another day measurements

DEBABRATA DUTTA
@dutta

In lab: Mateo-Martin-Nieto, Katie Wei, Vaiva Knabikaite, Vasily Vanifatov

Friday 31/07/2026

Objectives
- Sonicate the 24h samples
- Learn how to stain SDS gel for prions (silver stain)
- Run SDS gels

The same sonication procedure was performed as for 18h samples (see 30/07/2026, p. 12).

Spillage in 10-Card 37°C 24h insol tube (volume left in the epp tube is 230 µL). ~~We will~~ ~~This sample was redone~~.

The proteins samples after sonication were centrifuged at 1400 rpm for 15 mins to seperate the soluble and insoluble protein. The ~~pellets~~ supernatants are then seperated and the pellets are resuspended in 100 µL PBS.

SDS page

900 mL of ~~mq~~ Mili-Q and 100 mL of Tris-glycine SDS 10x were mixed for the running buffer. For the silver stain: 3 µL of sample, 3 µL of PBS and 5 µL of blue loading buffer. For the Commasie stain: 15 µL of sample and 15 µL of blue buffer. The samples are then incubated at 100°C for 10 mins on the heat block. Spin down briefly after.

Samples are briefly centrifuged to spin them down

Should be 2 µL next time (for the total volume to be 10 µL)

The gels used: Mini-PROTEAN TGX precast gel (BioRad), 12%, 10-well comb, 50 µL/well (Cat. #456-1044)

For loading the gel: take off the tape at the bottom, remove the comb, adjust the wells with a ~~pipe~~ pipette tip. When putting in the gel, the smaller gel must be towards the inside. After pouring in the running buffer, clean the wells by pipetting up and down.

When loading the ladder, it should be of the same volume. If there are any empty wells left, they should be loaded with the same volume of the ~~loading buffer~~ PBS or water, so that when the samples are running, ~~the~~ the weight across the gel is the same.

1 → 10

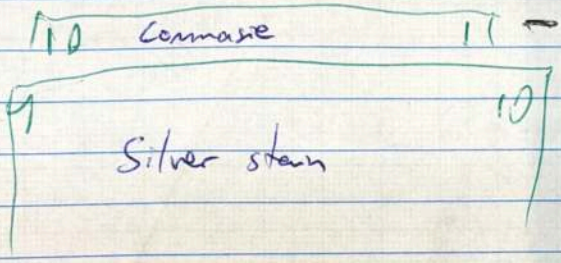
Gel loading seq. (left → right)

| MWL | 18°C, sol | 25°C, sol | ... | 18°C ins. | 25°C ins. ... |

| PBS or water

First put a small voltage (80V), so samples settle into the gel and there are bubbles in the tub. Then run the gel at 200 V for ~40 mins.

~~The one~~ The gel closer to the tub edge is the silver-stain



Stop the gel when you see the blue ~~of~~ stain just running off the gel. Then take the gel and break the plastic and cut away the wells. ~~Put the gel into Milli-Q water (for the silver-stained gel) and for the Commasie-stained gel into Commasie stain. Keep them there for ~30 min~~

~~For silver-stain two rounds of washing for 5 min in Milli-Q and then 1 min of Sensitizer sol., then x2 Milli-Q for 1 min, then 30 min in Stain Working solution. (see protocol for specific sol. ratios)~~ ~~Sol. prep.~~

For Commasie - ~~30~~ mins of staining, while shaking. If protein concentration is low, ~~20~~ the staining can be left ~~to~~ overnight

For silver stain: ×2 Mili-Q for 5 min, ×2 6:3:1 water: ethanol: acetic acid for 15 min, ×2 10% ethanol for 5 min, ×2 Mili-Q for 5 min, Sensitizer Working Sol. for 1 min, ×2 Mili-Q for 1 min, Stain Working Sol. for 30 min, ×2 Mili-Q for 20 sec, Developer Working Sol. for 2-3 min.

Sensitizer Working Sol.: 1 part Silver Stain Sensitizer with 500 parts Mili-Q (e.g., 50 µL Sensitizer + 25 µL water)

Stain Working Sol.: 1 part Silver Stain Enhancer with 50 parts Silver Stain (e.g. 0.5 mL of Enhancer + 25 mL Stain)

Developer Working Sol.: 1 part Silver Stain Enhancer with 50 parts Silver Stain Developer (e.g., 0.5 mL Enhancer + 25 mL Developer).

When desired band intensity is reached, replace with Stop Sol. (5% acetic acid) for 10 min.

Note: G5 → MOPS ~~tris-Gly~~
4-12%, 15 wells, 10/pk
~~&~~ C15 4-20%, 15 wells, 10/pk

DEBABRATA DUTTA

In lab : Vasily Vanifatov, Katie Wei, Hector Cruz

Monday 03/08/2026

Objectives
- Sonicate the last two 24hr 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 Construct

Sonication

Sample 12-eGFP-cap 32°C 24hr and 12-eGFP-cap 37°C 24hr ~~were~~ were sonicated ~~for~~ for 5 min, pulse ON 5s, pulse OFF 10s, amplitude 30%. All of the 24 hr samples were centrifuged at 14,000 rpm for 15 min to separate soluble and insoluble proteins. Supernatants were separated and pellets were resuspended with 100 µl PBS.

Destaining Comassie blue gel

Destaining solutions were prepared with 30% methanol, 10% acetic acid, and 60% MiliQ, this was prepared inside a fume hood.

SDS-PAGE

SDS-PAGE was performed with 1-Het-s 18 hr; 10-mCardinal-Het-s 18hr, and 12-eGFP-cap 18 hr samples. The gel used was ~~Sure PAGE~~ ProteinEle Precast Tris-Glycine gel (4-20%), 15 µl of each sample and 3 µl ladder is loaded. SDS-PAGE was run at 180V until the bands reaches the bottom

Sample loading:

Gel 1

well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	ladder	① 18°C 18hr sol	① ~~18°C~~ 18hr insol	① 18°C 18hr insol	① 32°C 18hr sol	① 32°C 18hr insol	① 37°C 18hr sol	① 37°C 18hr insol	ladd er	⑰ 18°C 18hr sol	⑩ 18°C 18hr insol	⑫ 25°C 18hr sol	⑫ 25°C 18hr insol	⑫ 32°C 18hr sol	⑩ 32°C 18hr insol

Gel 2

well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	ladder	⑩ 37°C 18hr sol	⑩ 37°C 18hr insol	ladder	⑫ 18°C 18hr sol	⑫ 18°C 18hr insol	⑫ 25°C 18hr sol	⑫ 25°C 18hr insol	⑫ 32°C 18hr sol	⑫ 32°C 18hr insol	⑫ 37°C 18hr sol	⑫ 37°C 18hr insol			

Gels were stained with Comassie blue solutions overnight on shaking bed

Transformation of seed and cap to BLR(DE3)

seed DNA from Twist was resuspended in MiliQ to make the final concentration 10 ng/µl, 1.5 µl of the resuspended DNA for seed, seed-eGFP, cap, cap-eGFP were added 45 µl of the BLR(DE3) Physis competent cells. Cells were left on ice for 30 min, heat shocked in 42°C water bath for 30s. and then incubated on ice for another 2 min. 950 µl SOC medium was added to each sample and they were recovered for 40 min at 37°C 220 rpm shaking incubator. 50 µl of each sample was plated on LB+K+C plates and incubated at 37°C overnight

Pre-culture

Pre-culture medium was prepared with 20 ml TB base, 2.2 ml TB salt, and 11 µl kanamycin, 5µl of the medium were added to four tubes. A single, ~~dim~~ distinct colony was picked from the frozen plates of 1-HET-S, 8-cap, 10-mCardinal-HET-S, 12-eGFP-cap BL21(DE3) transformants and inoculated into each tube. The tubes were grown overnight in a shaking incubator at 37°C 220 rpm

DEBABRATA DUTTA

In lab: Katie Wei, Hector Cross

Tuesday 04/08/2026

Objectives:
- Destain two gels from yesterday (18 h samples of 1, 10, 12)
- Run SDS-PAGE for the remaining 24 h samples of 1, 8, 10, 12
- Pre-culture of seed and cap of BLR (DE3) transformants

Pre-culture OD normalisation

1-HET-S (1:10) = 0.812 abs → 8.12 abs

8-cap (1:10) = ~~0.571~~ 0.598 abs → 5.98 abs

10-mCardinal-HET-S (1:10) = 0.715 abs → 7.15 abs

12-eGFP-cap (1:10) = 0.828 abs → 8.28 abs

1-HET-S → 123 µl, 8-cap → 167 µl, 10-mCardinal-HET-S → 139 µl, 12-eGFP-cap → 120 µl

Prepared 100 ml of culture medium with 90 ml TB base and 10 ml 10x TB salt, added 50 µl kanamycin
Transferred 20 ml to each tube and added respective volume of bacterial culture to make the starting OD 0.05
Placed the tubes in 37°C rpm 220 shaking incubators overnight

Preparation of MES running buffer

For 1L, added:

MES 162.6g, Tris base 101g, SDS 16.6g, ~~&~~ EDTA 5g

SDS-PAGE of 24 hr samples

SDS-PAGE was performed with 24hr samples of 1-HET-S; 8-cap; 10-mCardinal-HET-S, and 12-eGFP-cap. ProteinEle Precast Tris-Glycine gel (4-20%) was used, 15µl of each sample ~~is~~ was added and 3µl of the ladder was added. SDS-PAGE was run at 80V at the beginning for samples to leave the well. Once the samples left the wells, they were run at 150 V until reaching the bottom. Gels were then stained with Coomassie blue and then destained.

Sample loading

Gel 1:

Well	1	2	3	4	5	6	7	8	9	10	11	12	13
	ladder	I-H 18°C 24hr sol	I-H 18°C 24hr insol	I-H 25°C 24hr sol	I-H 25°C 24hr insol	I-H 32°C 24hr sol	I-H 32°C 24hr insol	I-H 37°C 24hr sol	I-H 37°C 24hr insol	ladder	⑧ 18°C 24hr sol	⑧ 18°C 24hr insol	⑧ 25°C 24hr sol

14	15
⑧ 25°C 24hr insol	X

Gel 2:

Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	ladder	⑧ 32°C 24hr sol	⑧ 32°C 24hr insol	⑧ 37°C 24hr sol	⑧ 37°C 24hr insol	ladder	⑫ 18°C 24hr sol	⑫ 18°C 24hr insol	⑫ 25°C 24hr sol	⑫ 25°C 24hr insol	⑫ 32°C 24hr sol	⑫ 32°C 24hr insol	⑫ 37°C 24hr sol	⑫ 37°C 24hr insol	X

Gel 3:

Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	X	ladder	⑩ 18°C 24hr sol	⑩ 18°C 24hr insol	⑩ 25°C 24hr sol	⑩ 25°C 24hr insol	⑩ 32°C 24hr sol	⑩ 32°C 24hr insol	⑩ 37°C 24hr sol	⑩ 37°C 24hr insol					

DEBABRATA DUTTA

2n lab: Katie Wei, Hector Closs

Wednesday 05/08/2026

Objectives
- Destain the 24hr sample gel from yesterday
- Transform mutants into BL21 strain
- Expression test of 8 constructs

Expression test

OD measurement of the pre-culture
1-HET-S (1:10) = 0.253 abs → 2.53 abs
8-cap (1:10) = ~~0.252~~ 0.243 abs → 2.43 abs
10-mCardinal-HETS (1:10) = 0.248 abs → 2.48 abs
12-eGFP-cap (1:10) = 0.205 abs → 2.05 abs
seed (BLR) (1:10) = 0.444 abs → 4.44 abs
seed-eGFP (BLR) (1:10) = 0.422 abs → 4.22 abs
cap (BLR) (1:10) = 0.487 abs → 4.87 abs
cap-eGFP (BLR) (1:10) = 0.519 abs → 5.19 abs

Volume of each pre-culture added for OD600 = 0.05

1-HET-S 400 µl	seed (BLR) 225 µl
8-cap 415 µl	seed-eGFP (BLR) 240 µl
10-mCardinal-HET-S 405 µl	cap (BLR) 205 µl
12-eGFP-cap 490 µl	cap-eGFP (BLR) 195 µl

Prepared 250 ml cultures for BL21 strains and 250 ml cultures for BLR strain. For the BL21, added 25 ml 10X TB salt, 5ml 50X auto-induction, and 220 ml TB base, and 125 µl Kanamycin. For BLR, added 25 ml 10X TB salt, 5ml 50X auto-induction, 220 ml TB base, 125 µl kanamycin, and 125 µl chloramphenicol. Transfered 25 ml to respective tube and added different volume of bacterial precultre to make the starting OD600 = 0.05
Placed all tubes in 37°C 220 rpm shaking incubator and ~~measure~~ switch them to different expression temperature when the OD is 0.5-0.8, the three different temperatures are

↑ 25°C, 32°C, 37°C.
1 ml of the pre-induction culture were collected for negative control
1 ml of the culture were collected 24h after induction

Transformation of mutant V1, V2, double cys

Mutant DNA V1, V2, double cys from Genscript were resuspended in 400 µl MiliQ, 1.5 µl of each DNA was transfered to 50 µl of BL21 (DE3) competent cells. Cells were incubated on ice for 30 min, heat-shocked for 30s, incubated on ice for another 2 min and 950 µl of SOC medium was added. Cells were recovered for 1hr in 37°C 220 rpm shaking incubator and 50 µl was plated on LB+K plates and grown overnight in 37°C incubator

OD_{600} measurement of culture

After pre-induction samples were collected, their OD_{600} was measured to ensure that it is within the range of 0.5 - 0.8. The following is the OD_{600} measurement

1 - HET-S (BL21) = 0.876 abs
8 - cap (BL21) = 1.031 abs
10 - mCardinal - HET-S (BL21) = 0.917 abs
12 - eGFP - cap (BL21) = 1.054 abs
Seed (BLR) = 0.754 abs
Seed - eGFP (BLR) = 0.756 abs
Cap (BLR) = 0.743 abs
Cap - eGFP (BLR) = 0.758 abs

1 ml of the sample was collected and centrifuged for 3min at X 12,000 rpm, supernatants were discarded and 400 µl PBS was added to resuspend the pellet.

DEBAPRATA DUTTA

In lab: Katie Wei, Hector Cross

Thursday 06/08/2026

Objectives
- BMG Labtech plate reader training
- MES running buffer preparation
- 24 hr ~~sele~~ sample collection and sonication, SDS-PAGE

BMG Labtech plate reader
Long press button on the front to switch the machine on and off
- Three different filters (excitation, dichroic, emission)
- Number 1 to open up the filter and cycle different filters
- Number 2 closes all of the filter
- Short press the button on the front to put plates in

Software Voyager
- Setting → filters → select the filter you put in
- New protocol (name it) → select the plate (greiner 96) → by defaut read all 96 wells (can also select the wells individually)
- Data would be normalised with blank value
- Fill in standard concentrations and dilutions
- Click Apply to save the plate, + → measure → detection mode (e.g. absorbance) → Precision and speed (number of flashes and setting time)
- Fluorescence ~~330 — 470 nm~~ select to excitation and emission wavelength
- Advanced settings (detector position and measurement pattern)
- For kinetic measurement, select "Loop" and input total duration and loop duration, shake between readings (different shake modes, most people use double orbital 3, freqency 200 rpm)
- Fluorescence polarisation (needs a polarisation filter)
- Add temperature (can be built-in or protocol-specific)
  25°C room temperature - 45°C
- Enhanced dynamic range can adjust the signal range

- For fluorescence polarisation, gain needs to be set with no-binding
- Data are available in result section which opens up the analysis software
- Turn colour on for viewing data
- Wizard can be used to plot standard curves
- F1 key opens up the help tab
- Edit Layout to change the labelling of each well

~~Sonnt~~ Sonication of 24 hr samples

1 ml of the sample was collected and centrifuged for 3min at X ~~12,000~~ 12,000 rpm, supernatant was discarded and 400 µl PBS was added to resuspend the pellet.

~~The~~ The ~~pellet~~ resuspension was then Sonicated for 40s (5s pulse ON and 5s pulse OFF) at amplitude 40%.

The samples were centrifuged at 14,000 rpm for 15min. Supernatants were collected and pellets were resuspended with 100 µl PBS.

Another 1 ml of the sample was taken for OD600 measurement

① -25°C = 0.228 abs	S -25°C = 0.308 abs
⑧ -25°C = 0.256 abs	S-G -25°C = 0.338 abs
⑩ -25°C = 0.272 abs	C -25°C = 0.205 abs
⑫ -25°C =	C-G -25°C = 0.304 abs

① -32°C = 0.279 abs	S -32°C = 0.201 abs
⑧ -32°C = 0.274 abs	S-G -32°C = 0.212 abs
⑩ -32°C = 0.327 abs	C -32°C = 0.205 abs
⑫ -32°C = 0.362 abs	C-G -32°C = 0.210 abs

① -37°C = 0.213 abs	S -37°C = 0.105 abs
⑧ -37°C = 0.215 abs	S-G -37°C = 0.171 abs
⑩ -37°C = 0.290 abs	C -37°C = 0.222 abs
⑫ -37°C = 0.310 abs	C-G -37°C = 0.206 abs

All absorbance measurements are 1:10 dilution with TB

x10MES running buffer

MES running buffered was prepared with 39.02g MES, 24.24g Tris base, 4g SDS, 1.2g EDTA, 400 ml ddH_2O.

~~Reagents were dissolved~~ x10 MES running buffer ~~was~~ were sterile filtered with 0.22 µm filter

DEBABRATA DUTTA

Inlab: Vasily Vanifatov, Katie Wei, Hector Cross

Friday 07/08/2026

Objectives

- Finish sonication of the remaining samples
- Run SDS-PAGE with all constructs
- Stain/destain gel and take images

Sonication

Sonication follows the same protocol as yesterday, each sample were sonicated for 40s (5S pulse ON and 5S pulse OFF) at amplitude 40%

SDS-PAGE

SDS-PAGE samples were prepared by centrifuging the sonicated sample for 10 min at 14,000 rpm, supernatants were collected as the ~~TES~~ soluble sample and pellets were resuspended in 100 µl PBS as the insoluble sample. ~~Samples were then~~ 15µl of each sample was mixed with 15µl of SDS loading buffer, and ~~were~~ heated at 100°C on a heat block for 3min.

Gel 1 and 2 were SurePAGE Bis-Tris gels that uses MES running buffer, these were used for small molecular weight proteins such as HET-s, the loading order were as follow, 5µl of the ladder and ~~15~~ 10µl of the sample was loaded:

Gel 1

well	1	2	3	4	5	6	7	8	9	10
	ladder	① 25°C pie S	① ~~25°C~~ 24hr S	① ~~25°C~~ pie ~~2S~~	① ~~25°C~~ 24hr ~~2S~~	ladder	① 32°C pie S	① ~~32°C~~ 24hr S	① ~~32°C~~ pie ~~2S~~	① ~~32°C~~ 24hr ~~2S~~

11	12	13	14	15
X	① ~~37°C~~ pie S	① ~~37°C~~ 24hr S	① ~~37°C~~ pie ~~2S~~	① ~~37°C~~ 24hr ~~2S~~

Gel 2 :

well	1	2	3	4	5	6	7	8	9	10
	ladder	⑩ 25°C pre S	⑩ 25°C 24hr S	⑩ 25°C pre 2S	⑩ 25°C 24hr 2S	X	⑩ 32°C pre S	⑩ 32°C 24hr S	⑩ 32°C pre 2S	⑩ 32°C 24hr 2S

11	12	13	14	15
ladder	⑩ 37°C pre S	⑩ 37°C 24hr S	⑩ 37°C pre 2S	⑩ 37°C 24hr 2S

Gel 3 :

well	1	2	3	4	5	6	7	8	9	10
	ladder	S 25°C pre S	S 25°C 24hr S	S 25°C pre 2S	S 25°C 24hr 2S	ladder	S 32°C pre S	S 32°C 24hr S	S 32°C pre 2S	S 32°C 24hr 2S

11	12	13	14	15
X	S 37°C pre S	S 37°C 24hr S	S 37°C pre 2S	S 37°C 24hr 2S

Gel 4 :

well	1	2	3	4	5	6	7	8	9	10
	ladder	S-G 25°C pre S	S-G 25°C 24hr S	S-G 25°C pre 2S	S-G 25°C 24hr 2S	X	S-G 32°C pre S	S-G 32°C 24hr S	S-G 32°C pre 2S	S-G 32°C 24hr 2S

11	12	13	14	15
ladder	S-G 37°C pre S	S-G 37°C 24hr S	S-G 37°C pre 2S	S-G 37°C 24hr 2S

Gel 5 :

well	1	2	3	4	5	6	7	8	9	10
	ladder	⑫ 25°C pre S	⑫ 25°C 24hr S	⑫ 25°C pre 2S	⑫ 25°C 24hr 2S	ladder	⑫ 32°C pre S	⑫ 32°C 24hr S	⑫ 32°C pre 2S	⑫ 32°C 24hr 2S

11	12	13	14	15
X	(12)	(12)	(12)	(12)
	37°C	37°C	37°C	37°C
	pre	24hr	pre	24hr
	S	S	2S	2S

Gel 6:

well	1	2	3	4	5	6	7	8	9	10
	ladder	C-G	C-G	C-G	C-G	X	C-G	C-G	C-G	C-G
		25°C	25°C	25°C	25°C		32°C	32°C	32°C	32°C
		pre	24hr	pre	24hr		pre	24hr	pre	24hr
		S	S	2S	2S		S	S	2S	2S

11	12	13	14	15
	C-G	C-G	C-G	C-G
	37°C	37°C	37°C	37°C
	pre	24hr	pre	24hr
	S	S	2S	2S
ladder				

DUTTA

DEBABRATA

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In Lab: Hector Cross (Ben Shin supervising)

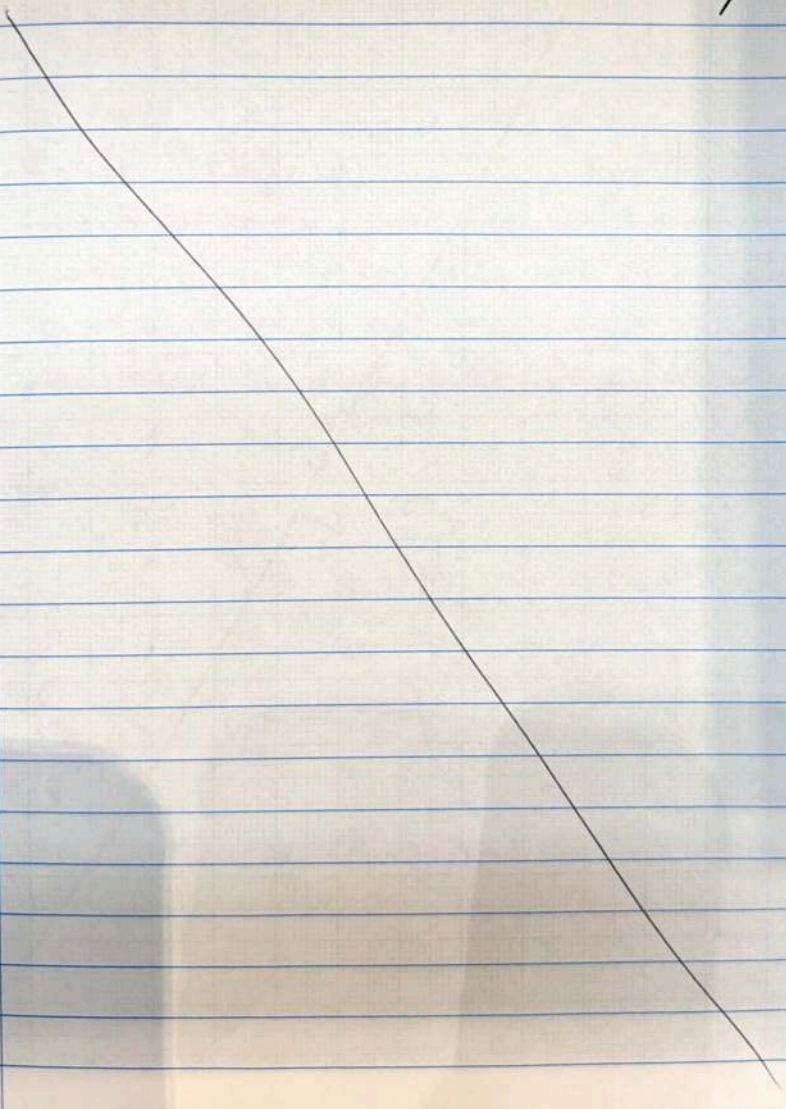
Sunday 11/08/26

Objectives

-> Prepare precultures of Het-s WT, mutant v1, v2 & double sys mutant

Inoculation

- Prepared 12x 15mL samples of TB broth + 10x salt for 45mL of preculture per construct (3 falcon tubes). Picked a healthy transformed colony from kan selection plates (parafilm wrapped). All work done with aseptic technique



BEN SHIN

In lab: Katie Wei · Hector Cross

Monday 10/08/26

Objectives:
- make 12 L TB and send them for autoclave
- Run SDS-PAGE of cap BL21 with genscript gels and MES
- Prepare more destaining solution
- inoculate 4 constructs into the 3 L TB culture
- transformation of seed and seed-eGFP into BL21 strain

12L TB culture
Weighed out 47.6g of TB salt and added it to a 2 L conical flask, added 920 ml ddH2O and seal the ~~bottle~~ flask with double layers of aluminum foil. 3 L of culture was made for each construct

SDS-PAGE
~~E~~ 15 µl of sample was mixed with 15 µl of loading buffer and were boiled at 100°C heat block for 3 min.
SurePAGE Bis-Tris gel was used and MES running buffer was added to the tank, 10 µl of the sample and 5 µl of the ladder was loaded. The loading order was as follow:

well	1	2	3	4	5	6	7	8	9	10
	ladder	⑧ 25C pre S	⑧ 25C 24hr S	⑧ 25C pre 2S	⑧ 25C 24hr 2S	⑧ 32C pre S	⑧ 32C 24hr S	⑧ 32C pre 2S	⑧ 32C 24hr 2S	⑧ 37C pre S

11	12	13	14	15
⑧ 37C 24hr S	⑧ 37C pre 2S	⑧ 37C 24hr 2S	ladder	X

Gel was then ~~dye~~ stained with Coomassie blue and destained with destaining solution

Transformation of seed and seed-eGFP into BL21 strain

The seed DNA and the seed-eGFP DNA were transformed into 50 µl BL21 Competence Cell. Transformation followed the same protocol as in 05/08/2006 (p.22)

Inoculation

The OD600 was measured and the results were recorded as follow:

WT1 (1:10) = 0.302 abs → 3.02 abs
WT2 (1:20) = 0.168 abs → 3.36 abs
WT3 (1:20) = 0.165 abs → 3.30 abs
V11 (1:20) = 0.161 abs → 3.22 abs
V12 (1:20) = 0.158 abs → 3.16 abs
V13 (1:20) = 0.156 abs → 3.12 abs
V21 (1:20) = 0.177 abs → 3.54 abs
V22 (1:20) = 0.179 abs → 3.58 abs
V23 (1:20) = 0.164 abs → 3.28 abs
double cys1 (1:20) = 0.166 abs → 3.32 abs
double cys2 (1:20) = 0.157 abs → 3.14 abs
double cys3 (1:10) = 0.296 abs → 2.96 abs

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

Glycerol stock

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

In lab: Katie Wei, Hector Cross

Tuesday 11/08/26

Objectives:
- Spin down cell pellets for 12 L cultures
- make kanamycin and TB and send them for autoclave
- Prepare pre-cultures for glycerol stock of cap / cap-eGFP
- Prepare pre-culture for seed / seed-eGFP expression test

TB culture preparation
Weighed out 3g tryptone and 6 g yeast extract and added them into a clean 250 ml bottle. Added 225 ml MiliQ water to dissolve the powder. Added 25 ml of x10 TB salt and autoclaved.

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

Centrifugation tutorial
- Place blue towl on bench to prevent spillage
- Pour cultures from conical flask into centrifuge bottles (this step should be done on bench not balance) until filling up the entire bottle
- Close the bottle with its lid and put it on electronic balance, zero it
- Pour another culture into a separate bottle and weigh it on the balance (with the lid) until the reading is zero again, use the dropper to add / remove solution from the bottle (it is better to pour more solutions into the bottle then remove it)
- Put the balanced bottle into J6 centrifuge, put the blue lid on the top of the rotor and close the centrifuge

- switch the centrifuge on and set the program, press start Rotor 4.2, speed 4,200 rpm, time 20 min
- During centrifugation, prepare Tomson flasks with Clemgene and Virkon solution
- After centrifugation is finished, pour off supernatant into Tomson flask, when the flasks are full, leave them in the box underneath the sink
- For the conical flask and the used bottles, add virkon solution and leave them for 20min, then pour off the liquid down the sink and return the flasks downstairs, bottles can stay on the bench

Resuspension

- Added 25 ml lysis buffer and scrape the pellets on the bottom with stripette tips, added some more lysis buffer to clean the edge and tip head. transfered all bacterial resuspension into pots placed the pots at -80°C freezer then leave them on rollers overnight to thaw the cells

Pre-culture

Pre-cultures for seed, seed-eGFP, cap, cap-eGFP were prepared (all BL21 transformant), see 03/08/2026 for protocol (p. 17)

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In lab: Katie Wei, Hector Cross

Wednesday 12/08/26

Objectives:
- making glycerol stocks for seed, seed-eGFP, cap, cap-eGFP
- making 6L TB cultures and send them for autoclave
- 20 ml culture of seed, seed-eGFP and induction for expression test
- Protein purification of monomers and mutants
- Pre-culture for seed and cap expression

Glycerol stock

1 ml of 50% glycerol is mixed with 1 ml of preculture of seed, seed-eGFP, cap, and cap-eGFP, samples were freezed at -80°C

Expression test of seed

Autoinduction culture was prepared with 147 ml TB + x10 TB salt, 3 ml x50 autoinduction solution, and 75 µl of kanamycin 20 ml of the culture was aliquoted and respective amount of bacterial pre-culture was added to ensure that the starting OD_{600} was 0.05, the OD_{600} of precultures were as follow

seed (1:10) = 0.531 abs → 5.31 abs → 200 µl

seed-eGFP (1:10) = 0.790 abs → 7.90 abs → 130 µl

OD_{600} was then measured again after reaching the exponential phase, and the recordings were as follow, 1 ml of pre-expression sample was collected

seed = 0.542 abs

seed-eGFP = 0.592 abs

Bacterial cultures were then induced at different expression temperatures, 25°C, 32°C, and 37°C

Pre-induction samples were spin down and the supernatant were discarded, pellets were resuspended with 400 µl PBS and freezed at -20°C for sonication tomorrow

Protein purification

- Equilibrate nickle resin with 2x ddH2O and 2x Lysis buffer by ~~spin~~ centrifuging it for 12 min at 4,200 rpm
- Centrifuge the cell lysate for 30 min at 18.000 rpm and mix the supernatant with the resin
- Leave the lysate and resin to bind on a roller at 4°C for 2 hours
- Centrifuge the lysate-resin mixture for 25 min at 4.200 rpm and collect the supernatant as the wash in the wash pot
- Resuspend the resin with ~45 ml of wash buffer and centrifuge for 25 min at 4.200 rpm. Collect the supernatant as the FT in the FT pot
- Repeat the wash process again with the centrifuge (don't pour off the supernat ant)
- Set up the clamp and the purification column and rinse the column with ddH2O and wash buffer.
- Pour the second wash-resin mixture into the column and collect the liquid flowing through the column in the FT pot (always ensure that the resin is moist and not dried out)
- Add ~ 45 ml of the elution buffer into the column and collect the liquid flowing through the column in the elute tubes
- Clean the column with MiliQ water and let it run through, then add 1 ml of 10% sodium azide and 99 ml of MiliQ
- Store the elution at -20°C fridge, the wash and FT pot in cold room, and the columns in the cold room

Pre-culture of seed and cap

- Added 15 ml of TB + x10 TB salt and 7.5 µl of kanamycin
- Added 65 µl of the seed and cap pre-culture respectively
- Overnight incubation at 37°C, 220 rpm.

Sodium azide solution

- Dissolved 5 g of sodium azide in 50 ml water in fumehood and sterile filter it with 0.22 µm filter.

In lab: Katie Wei, Heeba Cross

Thursday 13/08/26

Objectives:
- Concentrate HET-S proteins
- Bulk expression of seed and cap
- SDS-PAGE of purified HET-s proteins and expression test samples

OD measurement of expression test
Time: 9:00 A.M.
seed-25°C (1:10) = 0.285 abs
seed-G-25°C (1:10) = 0.309 abs
seed-32°C (1:10) = 0.300 abs
seed-G-32°C (1:10) = 0.285 abs
seed-37°C (1:10) = 0.270 abs
seed-G-37°C (1:10) = 0.302 abs
Time: 12:30 A.M.
seed-25°C (1:10) = 0.288 abs
seed-G-25°C (1:10) = 0.309 abs
seed-32°C (1:10) = 0.298 abs
seed-G-32°C (1:10) = 0.293 abs
seed-37°C (1:10) = 0.246 abs
seed-G-37°C (1:10) = 0.267 abs

1 ml of 24hr sample was collected and centrifuged for 1 3/5 min at 12,000 rpm, discard the supernatant, and resuspend it with 400 µl PBS. Samples were sonicated for 40s (5s pulse ON, 5s pulse OFF) at amplitude 40%. Sonicated samples were then centrifuged for 15 min at 14,000 rpm. Supernatant was collected into a separate tube and pellet was resuspended with 4µl ~~15µl of~~ PBS. 15µl of sample was mixed with 15µl loading buffer, samples were boiled for 3 min on 100°C heat block.

Concentrate purified proteins

- ~~5kDA~~ 5kDa Concentrators were washed with ddH2O ~~for~~ three times and with Lysis buffer twice
- Pour elution solution (thawed ~~in~~ in warm water beforehand) and spin it at 4200 rpm, continue adding elution solution (the Concentrator only takes 15 ml) until all of them were spinned down, collect the flow through in a separate ~~tube~~ tube as Concentrate FT
- Leave 5 ml ~~in the~~ of the solution in the Concentrator column and take 25 µl of it ~~to~~ for sample preparation
- Take 25 µl of the Concentrate FT for sample preparation
- Take the 5 ml solution out of the Concentrator into a tube, freeze it at -20°C, freeze the Concentrate FT as well
- Clean the Concentrator with 3 rounds of water and 3 rounds of glycerol + azide wash, store it in cold room with the final wash

SDS-PAGE

For Wash, FT, elution, Concentrated elution, and Concentrator FT samples, 25µl of each sample was mixed with 950 µl of absolute ethanol. Samples were freezed at -80°C for 30 min They were then thawed and centrifuged to remove the supernatant. Pellets were resuspended in 30 µl loading buffer and boiled at 100°C for 3 min.

SDSPAGE was run with 80 V at the start until samples leave the well, then at 150V for Bis-Tris gel and at 200V for Tris-glycine gel, loading order were as follow:

Gel 1 (Bis-tris gel)

well	1	2	3	4	5	6	7	8	9	10
	ladder	WT W	WT FT	WT E	~~#~~ V1 W	~~##~~ V1 ~~##~~ FT	~~##~~ V1 E	V2 W	V2 FT	V2 E

11	12	13	14	15
DC W	DC FT	DC E		

Gel 2 (Tris - glycine)

well	1	2	3	4	5	6	7	8	9	10
		ladder	WT Conc.	WT Conc. FT	VI Conc. [redacted]	VI Conc. [redacted]	VI Conc. FT		V2 Conc.	V2 Conc. FT

11	12	13	14	15
	DC Conc.	DC Conc. FT		

Gel 3 ([redacted] Tris-glycine)

well	1	2	3	4	5	6	7	8	9	10
	ladder	S pre 25°C	S 24 25°C	LS pre 25°C	LS 24 25°C	S pre 32°C	S 24 32°C	LS pre 32°C	LS 24 32°C	S pre 37°C

11	12	13	14	15
S 24 37°C	S LS pre 37°C	S LS 24 37°C		

Pre-culture of seed and cap

OD_{600} measurement

seed [redacted] (1:10) = 0.134 abs

cap (1:10) = 0.138 abs

3 L ~~culture~~ TB culture prepared with 47.6 g Tris salt, 880 ml ddH_2O and 120 ml of TB x10 salt + x50 antiinduction solution

5 ml of pre-culture was inoculated and cultures were grown at 37°C

In lab = Katie Wei, Hector Closs

Friday 14/08/26

Objectives:
- Make x10 TB salt and 50% glycerol for autoclave
- SDS-PAGE of seed-eGFP samples for expression test
- Spin down seed and cap bulk cultures
- Buffer exchange of Concentrated HET-s monomers

x10 TB salt

For 2L of x10 TB salt, added
90 g Na_2HPO_4, 22g KH_2PO_4, 26g NH_4Cl, 7g Na_2SO_4
Added MiliQ water to dissolve them with a magnetic spinner

50% glycerol

For 200ml of 50% glycerol, added 164g glycerol stock, then added MiliQ water to make up 200ml

SDS-PAGE

The protocol of SDS-PAGE is the same as Thursday 13/08/26 (p.37)
The loading order of the sample is as follow:
Gel 1

well	1	2	3	4	5	6	7	8	9	10
	ladder	S-G S pre 25°C	S-G S 24 25°C	S-G IS pre 25°C	S-G IS 24 25°C	S-G S pre 32°C	S-G S 24 32°C	S-G IS pre ~~25°C~~ 32°C	S-G IS 24 32°C	S-G S pre 37°C

11	12	13	14	15
S-G S 24 37°C	S-G IS pre 37°C	S-G IS 24 37°C	Conc. WT	Conc. WT

Spin down of bulk cultures

See Tuesday 11/08/26 for centrifuge tutorial (p.32)
3L of seed and 3L of cap were spinned down at 4,200 rpm for 23 min. The supernatant was discarded and pellets were

resuspended in Lysis buffer. They were freezed in -80°C fridge over the weekend

Buffer exchange

~~Concentr~~ Concentrate the protein to 2.5 ml using the 4,200 rpm centrifuge. Wash the ~~col~~ PD-10 Column with MilliQ water then equilibrate it with 25 ml of Lysis buffer. Add 2.5 ml of the concentrated protein into the column and collect the FT, then added 3.5 ml of Lysis buffer and collect the FT, the second FT should contain concentrated proteins in Lysis buffer.

~~Bio~~Biospectrophotometer measurement

~~Sa~~ Using two different blanks, the lysis buffer and the elution buffer, the concentration of the protein is measured
Samples were prepared with 2 µl of the concentrated protein and 38 µl of the blank to make a 1:20 dilution
2 µl of sample was added to ~~m~~cuvette and close it to see a column forming. Put the cuvette in the biospectrophotometer and close the lid. Press blank for calibrating biospectrophotometer and press sample to take the reading
Clean the cuvette with whatman filter paper

Blank (Lysis buffer)

WT	V1	V2	Double cys
0.214 abs	0.310 abs	0.160 abs	0.276 abs

Blank (Elution buffer)

WT	V1	V2	Double cys
-0.002 abs	-0.108 abs	-0.014 abs	-0.164 abs

Blank (Lysis buffer)

V1 (1:10)	V2 (1:10)	V1 FT	V1 undiluted	V2 FT	V2 undiluted
0.130	0.173	-0.014	0.868	0.100	0.663

In lab: Katie Wei, Hector Cross, Mateo-Martin-Nieto

Monday 17/08/26

Objectives:
- Buffer exchange of WT and double cys
- Make kan plates
- Transformation of sortase, eGFP-sortase, HET-s sortase, enzyme-sortase
- SDS-PAGE of concentrated monomers

Buffer exchange

Buffer exchange of V1 and V2, see 14/08/26 (p.40) for detail of the protocol, measure the concentration of the buffer exchange samples and buffer exchange FT. results are listed as follow

Blank (Lysis buffer)

WT undiluted	WT FT	DC undiluted	DC FT
0.591 abs	0.350 abs	1.239 abs	0.102 abs

Kan plates

15 kan + LB plates were made, refer to 22/07/26 for detail of the protocol, plates were stored in the cold room

Transformation

Transformed 2-HET-s-sortase; 15-eGFP-sortase; 17-CBHII-sortase; 18-CellA-sortase; 14-sortA-5M; 20-sortA-7M into BL21 competent cells.

DNA were resuspended with MiliQ to make a stock concentration of 30 ng/µl, and 0.5 µl of the DNA were added to 50 µl BL21 competent cells, then follows the protocol at 05/08/26 (p.22)

SDS-PAGE

25 µl of the buffer exchange and buffer exchange FT of WT, V1, V2, DC were mixed with 950 µl of absolute ethanol, and freezed at -80°C fridge for 30 min. Samples were then centrifuged for 20 min at 14.000 rpm and supernatants were discarded, pellets

were resuspended with 100 µl PBS. 15 µl of the resuspended sample was then mixed with 15 µl of the loading buffer, samples labelled 0 min were not boiled; 3 min were boiled on 100°C heat block for 3 min, and 10 min for 10 min. The gel used was Bis-Tris with MES running buffer. The loading order was as follow:

well	1	2	3	4	5	6	7	8	9	10
	ladder	WT 0min	WT 3min	WT 10min	WT FT	V1 0min	V1 3min	V1 10min	V1 FT	V2 0min

11	12	13	14	15
V2	V2	DC	DC	DC
10 3min	FT	0min	10min	FT

In lab: Katie, Mateo, Hector Tuesday 18/08/2016

Objectives
- Preculture transformed cultures from yesterday
- Purify seed & cap
- Sequence plasmids

Sequencing order - Full circle
Full circle - Bag code KTPQ8FC

#1 01-HET-s (TWIST)
#2 02-N-term-HET-s (Ansa)
#3 03-Cys mut V1 (GeneScript)
#4 04-Cys mut V2 (GeneScript)
#5 05-Cys mut V3 (Ansa)
#6 06-Double Cys mut (GeneScript)
#7 07-Seed (TWIST)
#8 08-Cap (TWIST)
#9 09-Poissoned Cap (Ansa)
#10 10-mCard HET-s (TWIST)
#11 11-eGFP seed (TWIST)
#12 12-eGFP cap (TWIST)
#13 13-eGFP Poissoned cap (Ansa)
#14 14-eSortA MS (Ansa)
#15 15-GGG-eGFP ~~eSortA~~ (GeneScript)
#16 16-GGG-CBHI (Ansa)
#17 17-GGG-CBHII (Ansa)
#18 18-GGG-C1A (Ansa)
#19 19-GGG-CoFA (Ansa)
#20 20-eSortA (7H) (Ansa)
#21 07-Seed (Ansa)
#22 22-eGFP-seed (Ansa)

Purification of seed and cap

Refer to 12/08/26 (p.35) for detail of protein purification protocol
seed and cap constructs were purified, and the FT, Wash, Elution samples were used to run SDS-PAGE

Pre-culture

For 17-GGGG-CBH2I and 18-GGGG-CelIA, 5 ml precultures were made with 5 ml LB and 2.5 μl kanamycin
For 2-HE, 14-SortA-SM, 20-SortA-7M, 15-GGG-eGFP, ~~25 ml~~ 20 ml precultures were made with ~~25~~ 20 ml LB and 10 μl kanamycin
Individual colony was inoculated and tubes were incubated at 37°C, 220 rpm overnight

12 L TB culture

Refer to 10/08/26 (p.30) for the protocol, add 880 ml of ddH₂O instead of 920 ml.

SDS-PAGE

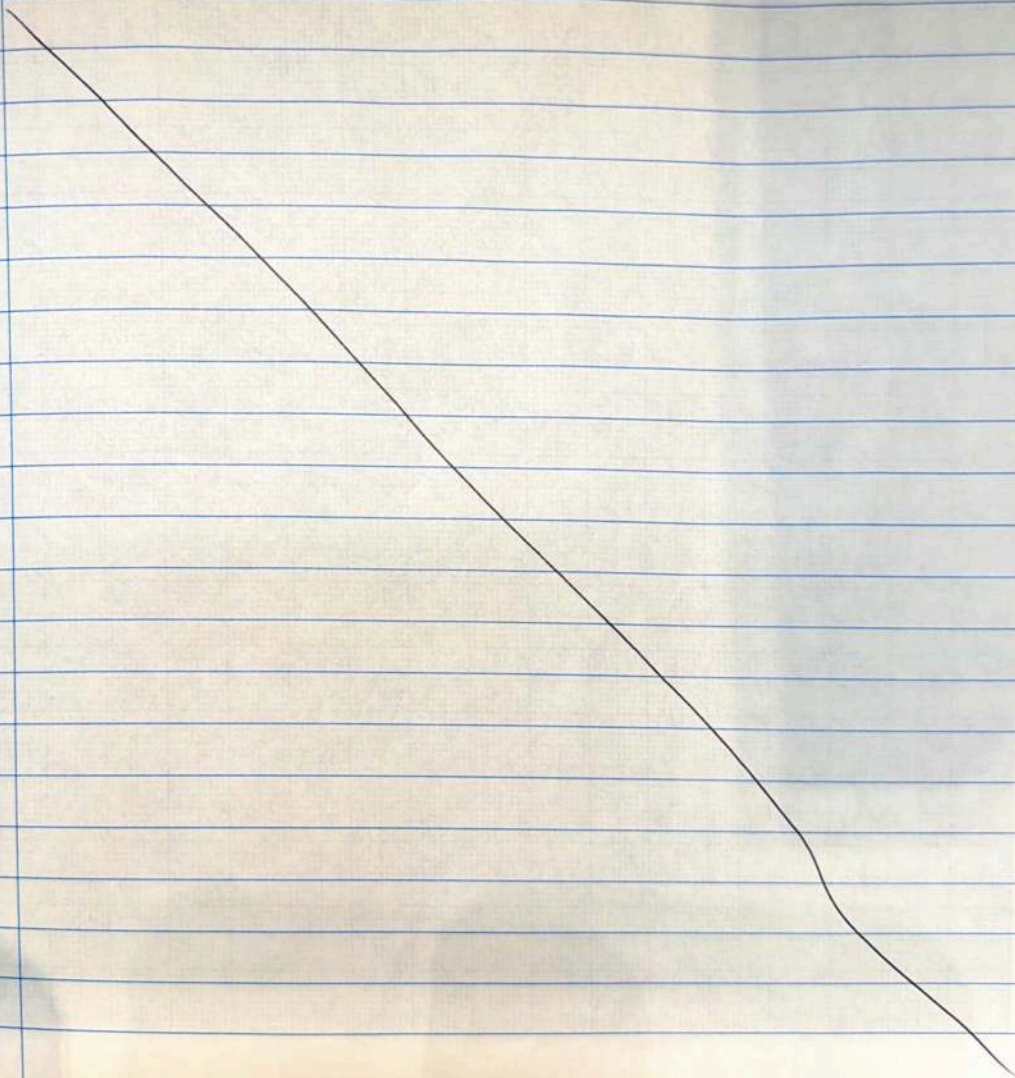
FT, wash, elution samples were prepared for purified cap and seed, following the protocol in 17/08/26 (p.41). FT was resuspended in 200 μl PBS, wash in 100 μl PBS, and Elution in 15 μl PBS
Concentrated samples were also prepared for WT, V1, V2, DC, all resuspended in 100 μl PBS
For cap and seed samples, 15 μl of resuspension was mixed with 10 μl of newly made SDS-PAGE loading buffer (with BME); for old WT, V1, V2, DC samples, 15 μl resuspension was mixed with 15 μl of old SDS-PAGE loading buffer; and for new WT, V1, V2, DC samples, 15 μl resuspension was mixed with 10 μl of new loading buffer.
Tris-glycine gel was used and the ~~loading~~ loading order is as follow:

well	1	2	3	4	5	6	7	8	9	10
	ladder	cap FT	cap W	cap E	seed FT	seed W	seed E	WT old	WT new	V1 old

11	12	13	14	15
V1 new	V2 old	V2 new	DC old	DC new

SDS-PAGE loading buffer

For 1ml of SDS-PAGE, added 200μl BME (20% w/v), which is a x2 loading buffer, BME can be found in the fumehood in the weighing area of Lab 245, and needs to be added freshly before making the samples



In lab: Kaitle, Hector, Mateo

19/08/26 Wednesday

Objectives
- inoculation of four constructs in 2L TB cultures
- Buffer exchange and lyophilisation with Ben
- Making buffers for non-denaturing purification

OD600 of preculture (1:10)
02 = 0.275 abs
14 = 0.256 abs
15 = 0.263 abs
20 = 0.277 abs
17 = 0.431 abs
18 = 0.482 abs

Prepared mixture of X10 TB salt and X50 autoinduction solution. added 120 ml of the mixture to each 1L flask, added 9.5 ml of the precultures for 02, 14, 15, 20 constructs, Put the flasks in 37°C, 220 rpm incubator overnight

Buffer exchange with ~~Ben~~
Set up the PD10 columns with the clamps and stands. equilibrate them with five rounds of MilliQ water. Add 2.5ml of the concentrated proteins (WT, V1, V2, DC, seed, cap) and ~~Collect~~ discard the flow through. Add 3.5 ml of water and Collect the flow through in eppendorf tubes. Centrifuge the E tubes at 14,000 rpm for 5 min. Collect the supernatant (there may not be any pellets) and combine them into a glass bottle. Use nanodrop to estimate the concentration of the protein ~~as~~ with 1:10 dilution, and aliquot the protein in eppendorf tubes to ~~make the concentration~~ ensure 2 mg protein per tube (the tubes should have a hole on the cap for lyophilisation). Fast-freeze the tubes in liquid nitrogen. Lyophilise tubes (-100°C and 100 mTorr) overnight.

Protein Concentration 1	Protein Concentration 2
WT (1:10) A_{280} = 2.54 abs	WT (1:10) 4 A_{280} = 2.64 abs
V1 (1:b) A_{280} = 3.69 abs	V1 (1:10) A_{280} = 3.68 abs
V2 (1:10) A_{280} = 3.73 abs	V2 (1:10) A_{280} = 4.25 abs
DC (1:10) A_{280} = 3.31 abs	DC (1:10) A_{280} = 3.29 abs
~~seed~~ cap (1:10) A_{280} = 2.37 abs	cap (1:10) A_{280} = 2.91 abs
~~cap (1:10)~~ seed A_{280} = 0.485 abs	~~seed A_{280}~~

Buffer

① Lysis buffer (3mM imidazole, 50 mM Tris, 300 mM NaCl, pH 8.0)

2.5 L of the lysis buffer was made with

0.51 g imidazole, 15.1 g Tris base, ~~43~~ 43.83 g NaCl

② Wash buffer (30 mM imidazole, 50mM Tris, 300 mM NaCl, pH 8.0)

Use 1L of lysis and added

1.84g imidazole

③ Elution buffer (300 mM imidazole, 50 mM Tris, 300 mM NaCl, pH 8.0)

Use 0.5 L of lysis and added

10.1 g imidazole

④ Stripping buffer (500 mM ~~50mM, 10m~~ NaCl, 50 mM EDTA·$2H_2O$, 10 mM NaH_2PO_4, 10 mM Na_2HPO_4)

0.5 L of stripping buffer was made with

14.61g NaCl, 9.31g EDTA·$2H_2O$, 0.6g NaH_2PO_4, 0.71g Na_2HPO_4

⑤ Nickle recharging solution

0.5 L of nickle recharging solution was made with

13 g $NiSO_4 \cdot 6H_2O$