21/07/2026 - Tuesday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 14 Jul 2026 11:13:02 UTC
Entry Last Modified: 28 Jul 2026 11:27:17 UTC
Export Generated On: 11 Oct 2026 18:39:39 UTC

TUESDAY, 14/07/2026

Members

Mateo, Vaiva, Katie

Objective

1. Prepare antibiotics (kanamycin and chloramphenicol) stock concentrations

Procedure

1. Reagents were taken from Paul Freemond's lab and measure out the mass of the antibiotics
    - Kanamycin: 202.3 mg
    - Chloramphenicol:
2. Dissolved kanamycin in ddH2O to reach a final concentration of 100 mg/ml
3. Dissolved chloraphenicol in absolute ethanol to reach a final concentration of 50 mg/ml
4. Both were vortexed for around 5 s and kept in the cold room (4 °C)

Supervisor

Debabrata Dutta

7.1B - Expression testing w. IPTG TB

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Terrific broth (TB): 18mL
  - 12 g/L tryptone, 24 g/L yeast extract
- 10x TB salt: 2mL
  - 90 g/L Na_2HPO_4, 22 g/L KH_2PO_4, 26 g/L NH_4Cl, 7 g/L Na_2SO_4
- Glycerol: 80µL glycerol
- IPTG stock
- 25 mL culture tube

Procedure

Day 1

- [x] 1. The day before protein expression pick a colony from the Kan plate (BL21(DE3) or BLR(DE3)) and inoculate 50µL of TB with 50µg/mL kanamycin added
- [x] 2. Grow preculture overnight at 37°C shaking at 200RPM

Day 2

- [x] 3. The next day add 2mL of 10x TB salt and 400µL of 50x Auto-induction solution to 18mL TB base.
- [ ] 4. Add 80µL of glycerol (0.4% v/v).
- [ ] 5. Add the dense preculture mixture and grow at 37°C shaking at 200RPM for 3-6 hours until OD~0.6-0.8.
- [ ] 6. Add IPTG to the final desired concentration.
- [ ] 7. Reduce the temperature to 25°C and leave to induce overnight shaking at 200RPM.

22/07/2026 - Wednesday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 22 Jul 2026 10:28:55 UTC
Entry Last Modified: 24 Jul 2026 14:34:24 UTC
Export Generated On: 11 Oct 2026 18:39:39 UTC

WEDNESDAY, 22/07/2026

Members

Mateo, Vaiva, Katie

Objective

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

Procedure

1. Took already prepared LB broth and heat it up in a microwave:
    - 350 ml for kanamycin plates
    - 150 ml for kanamycin + chloramphenicol plates
2. Waited for LB to cool down until we can hold the bottles in hands comfortably
3. Added the antibiotics to the LB in the clean bench:
    - 175 µl of kanamycin stock (21/07/2026 - Tuesday) for kanamycin plates
    - 75 µl of kanamycin stock (21/07/2026 - Tuesday) and 75 µl of chloramphenicol stock (21/07/2026 - Tuesday) for the kanamycin + chloramphenicol plates
4. Labelled the petri dishes and poured around 25 ml of LB + antibiotics into each plate:
    - 15 plates were made for LB + kanamycin plates
    - 6 plates were made for LB + kanamycin + chloramphenicol plates

Supervisor

Debabrata Dutta

24/07/2026 - Friday

Project: iGEM 2026 Shared Project

Author: Vaiva Knabikaite

Status: In progress

Entry Created On: 24 Jul 2026 13:21:21 UTC

Entry Last Modified: 24 Jul 2026 14:31:18 UTC

Export Generated On: 11 Oct 2026 18:39:39 UTC

FRIDAY, 24/07/2026

Members

Heck-tor, Mateo, Vaiva, Katie

Objectives

- Have an autoclave induction
- Make 10X TB 2 L
- Make 50X TB auto-induction 2 L

Procedure

1. 10X TB 2 L
    - 90 g Na_2HPO_4, 22 g KH_2PO_4, 26 g NH_4Cl, 7 g Na_2SO_4 weighted twice (The masses written down are for 1 L)
2. 50X TB auto-induction 2 L
    - 250 g Glycerol, 25 g glucose, 100 g lactose weighted twice (The masses written down are for 1 L)
3. Sent to autoclave at 16:00

Supervisor

Debabrata Dutta

27/07/2026 - Monday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 28 Jul 2026 11:27:31 UTC
Entry Last Modified: 03 Aug 2026 17:19:24 UTC
Export Generated On: 11 Oct 2026 18:42:25 UTC

TUESDAY, 28/07/2026

Members

Mateo, Katie

Objective

- Prepare guanadine hydrochloride buffers, TB base
- Perform transformation of the four constructs for the expression test (01, 08, 10, 12)

Procedure

TB base preparation

1. To make 1 L of the TB base, we added:
    - 12 g of tryptone
    - 24 g of yeast extract
2. Weighted out tryptone and yeast extract on an electronic balance, dissolved it in 900 ml MiliQ and mixed it
3. Made up the volume to 1L
4. TB base is then autoclaved

GuHCl buffer preparation

1. To make the GuHCl buffer, we used:
    - GuHCl 6 M
    - Tris base 50 mM
2. For 4.5 L of lysis buffer, we added:
    - 2578.5 g GuHCl
    - 27.27 g Tris base
3. Separately, we made an additional 2 L of lysis buffer by adding:
    - 1146 g GuHCl
    - 12.12 g Tris base
4. Took 1 L of the lysis buffer and added 20.42 g imidazole to make 1 L of the elution buffer with 300 mM imidazole
5. Took 3.5 L of the lysis buffer and added 7.15 g imidazole to make 3.5 L of the wash buffer with 30 mM imidazole
6. All buffers were adjusted to pH 8.0 by adding 37% HCl and measuring the pH using a pH meter
7. All buffers were sterilised using a vaccuum filter with 0.2 µm

Transformation

1. Resuspended Twist DNA constructs with autoclaved MiliQ (constructs 01, 08, 10, 12) to make a stock concentration of 10 ng/µl
2. Added 3 µl of all four constructs to eppendorf tubes and added 40 µl of BL21(DE3) competence cell

3. For control, added 5 µl of pET-28a(+) and 40 µl competence cell
4. Incubated on ice for 30 min
5. Heat shocked in 42 °C water bath for 30 s
6. Incubated on ice for 2 min
7. Added 250 µl SOC medium
8. Placed the eppendorf tubes in shaking incubator at 37 °C and 220 rpm for 1 h
9. Plated 50 µl and 200 µl of bacteria on K + LB plates for each construct and the control
10. Incubated the plates in 37 °C overnight

Wash buffer - 1 L

Introduction

Purpose: Removes weakly/non-specifically bound protein from Ni-NTA resin prior to elution.

Materials

- GuHCl (6 M, 573.2 g)
- Tris base (50 mM, 6.06 g)
- Imidazole (30 mM, 2.04 g)

Procedure

1. Dissolve GuHCl in ~700 mL ddH_2O.
2. Add Tris base and imidazole.
3. Adjust pH to 8.0 with HCl.
4. Make up to 1 L with ddH_2O.
5. Filter (0.22 µm).
6. **Storage:** RT, indefinite.

28/07/2026 - Tuesday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 28 Jul 2026 11:28:20 UTC
Entry Last Modified: 28 Jul 2026 12:15:25 UTC
Export Generated On: 11 Oct 2026 18:42:25 UTC

TUESDAY, 28/07/2026

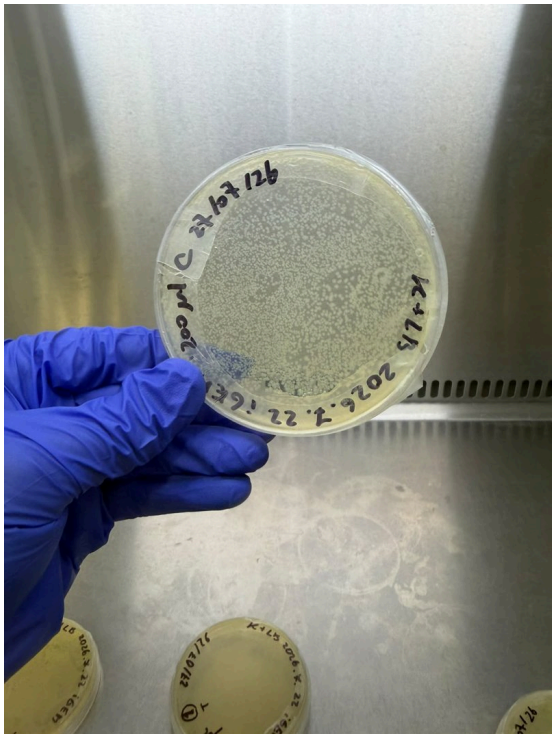
Members

Mateo, Vaiva, Katie

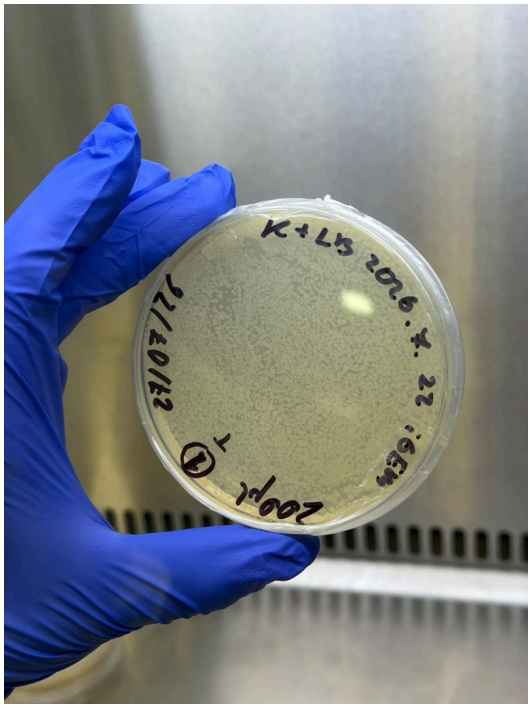
Observations and notes from 27/07/2026 - Monday

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

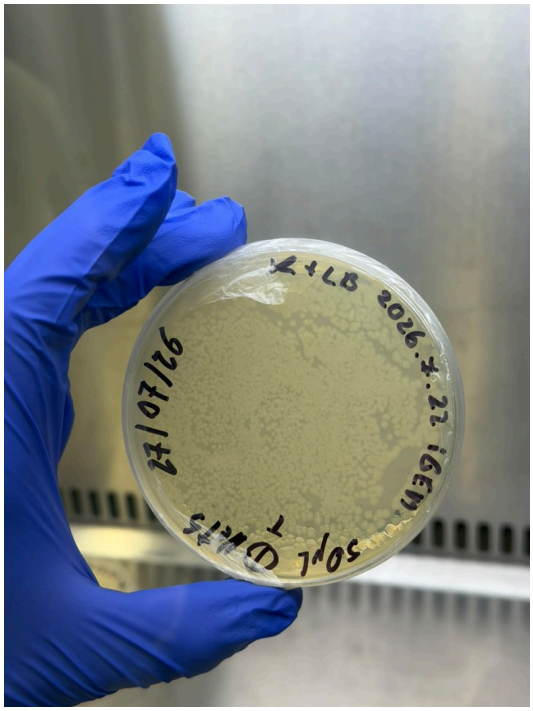
Positive Control (200µL)



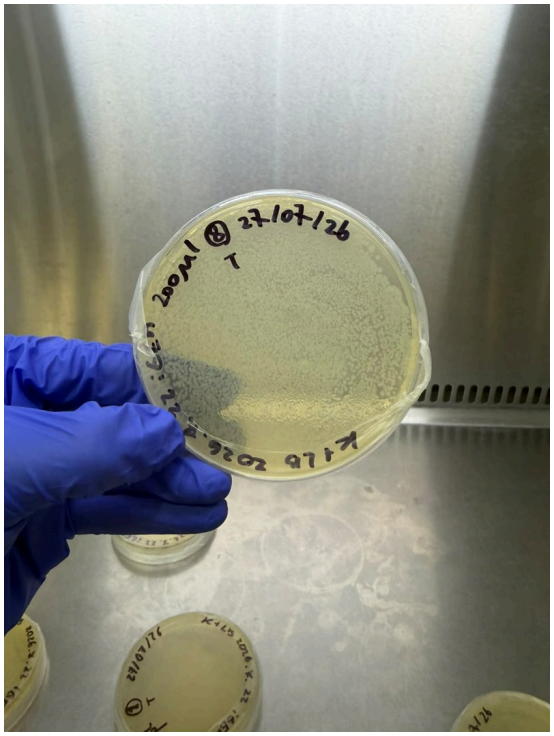
Construct ID.1 (200µL)



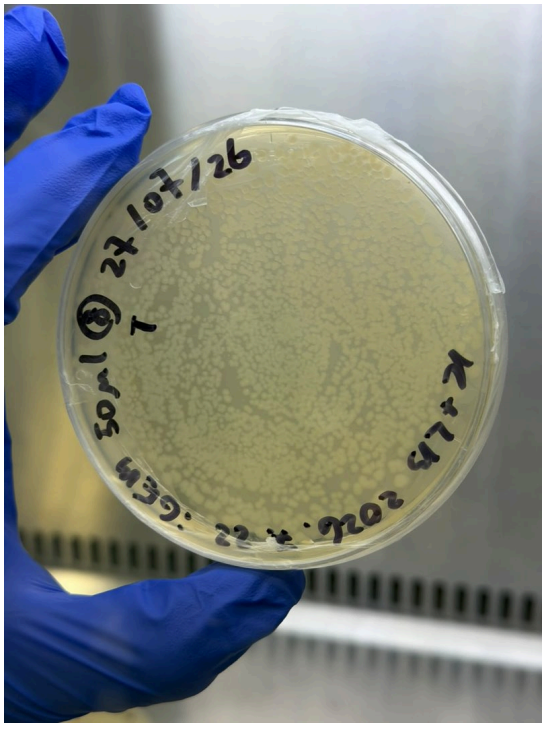
Construct ID.1 (50µL)



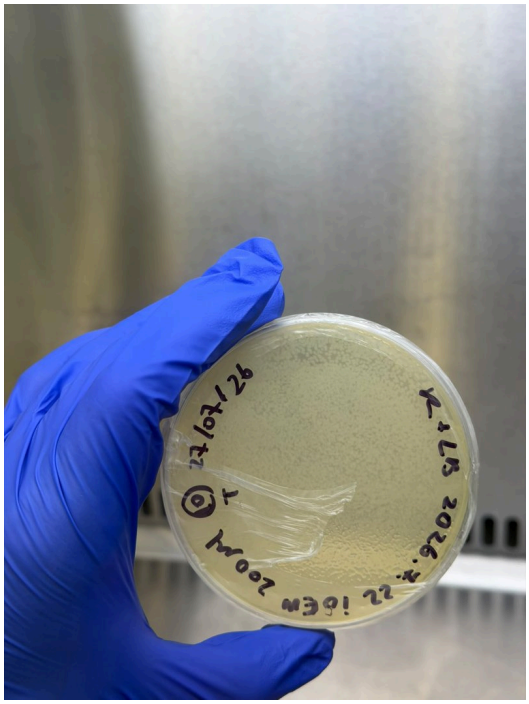
Construct ID.8 (200µL)



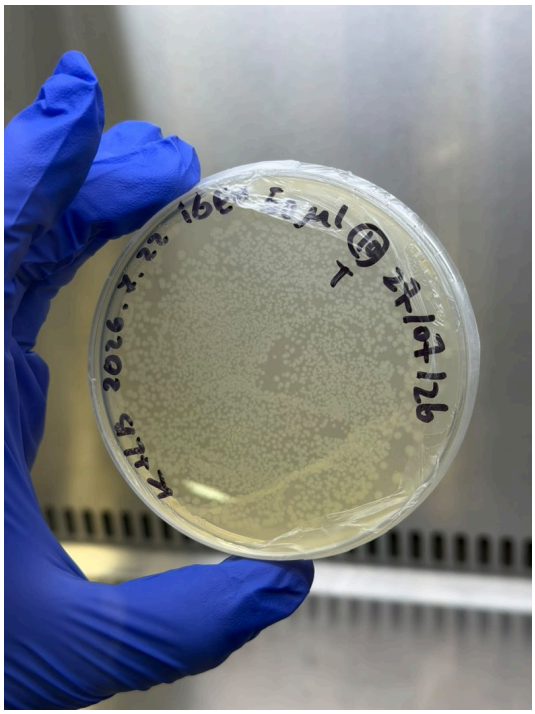
Construct ID.8 (50µL)



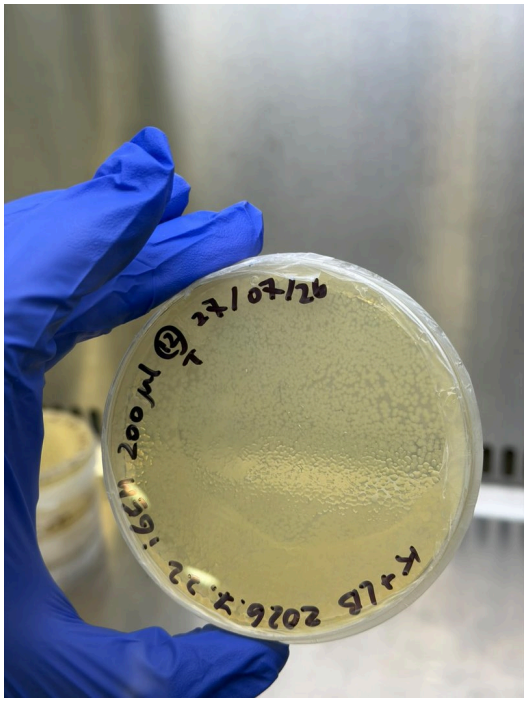
Construct ID.10 (200µL)



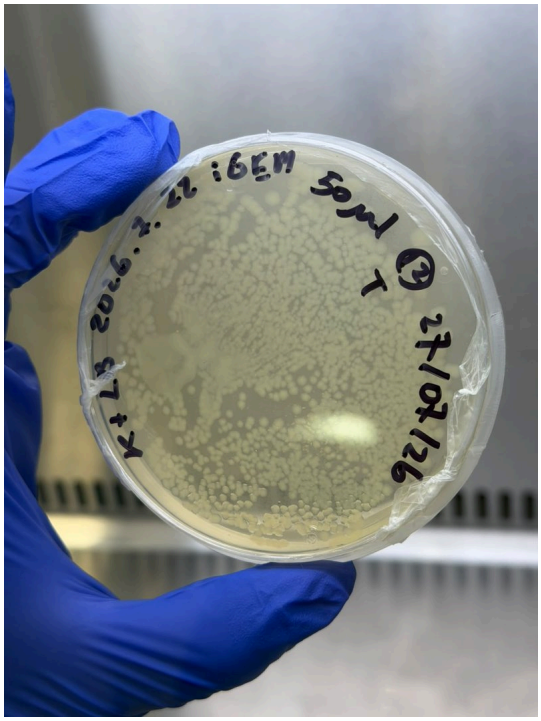
Construct ID.10 (50µL)



Construct ID.12 (200µL)



Construct ID.12 (50µL)



The plates seem to have grown more than we wished. Next time we should plate 5µL and 20µL

Objectives

- Prepare precultures

Procedure

(7.1A - Expression testing w. auto-induction TB , Day 1)

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

Supervisor

Debabrata Dutta

Plates

Project: iGEM 2026 Shared Project

Author: Mateo Martin-Nieto

Status: In progress

Entry Created On: 28 Jul 2026 11:30:10 UTC

Entry Last Modified: 28 Jul 2026 11:30:37 UTC

Export Generated On: 11 Oct 2026 18:42:25 UTC

TUESDAY, 28/07/2026

Plates

29/07/2026 - Wednesday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 29 Jul 2026 12:26:10 UTC
Entry Last Modified: 29 Jul 2026 19:53:56 UTC
Export Generated On: 11 Oct 2026 18:42:25 UTC

WEDNESDAY, 29/07/2026

Members

Hector, Mateo, Vaiva, Katie

Observations from 28/07/2026 - Tuesday

All 4 tubes that were incubated overnight looked cloudy.

Tubes incubated at 37 °C overnight



Objectives

- Prepare cultures for expression testing with auto-induction TB

The results from the spectrophotometer 600nm, blanked with auto-induction TB + Kanamycin

Culture ODs - Taken at 11:30h

	Construct	OD of 1:10 diluted sample	OD of undiluted sample
1	01 - HET-s	0.581	5.81
2	08 - Cap	0.526	5.26
3	10 - mCardinal-unit	0.817	8.17
4	12 - eGFP-cap	1.077	10.77

Protocol 7.1A - Expression testing w. auto-induction TB

To prepare the auto-induction TB solution (enough for all tubes), we added 360 mL TB, 40 mL 10X TB salt, 8 mL auto-induction TB, and 200 µL Kanamycin.

Protocol 7.1B - Expression testing w. IPTG TB

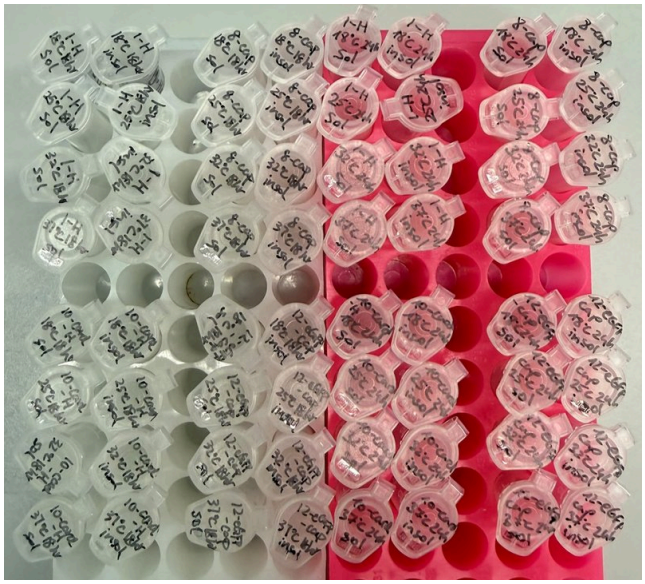
For each construct, we added 20 mL of the TB-autoinduction solution and the respective volume of the bacteria, such that the final concentration is 0.02 (01 → 70 µL, 08 → 80 µL, 10 → 50 µL, 12 → 40 µL). For each construct, 4 Falcon tubes were prepared, ready to be incubated at 37°C.

Tube labelling for the expression test 18h/24h

Nomenclature: Construct name (1 - H, 8 - cap, 10 - card-H, 12 - eGFP-cap) + temperature (18, 25, 32, 37 °C) + time incubated (18, 24 h) + sol/insol

Example: 1 - H, 18 °C, 18 h, sol

Labelled epp tubes



OD results at 16:15 h

The results from the spectrophotometer at 600nm, blanked with auto-induction TB + Kanamycin at 16:15 h, after incubation at 37 °C.

Culture ODs - Taken at 16:15 h

	Construct	OD
1	01 - HET-s	0.085
2	08 - Cap	0.162
3	10 - mCardinal-unit	0.073
4	12 - eGFP-cap	0.063

OD results at 18:30 h

The results from the spectrophotometer at 600nm, blanked with auto-induction TB + Kanamycin at 18:30 h, after incubation at 37 °C.

Culture ODs - Taken at 18:30 h

	Construct	OD
1	01 - HET-s	0.592
2	08 - Cap	0.855
3	10 - mCardinal-unit	0.510
4	12 - eGFP-cap	0.297

Since the 12 - eGFP-cap showed low OD, we took out all the samples, kept them at room temperature and incubated all 4 12 - eGFP-cap tubes for an additional ~30 mins at 37 °C.

12 - eGFP-cap OD results at 19:30 h

The results from the spectrophotometer at 600nm, blanked with auto-induction TB + Kanamycin at 19:30 h, after incubation at 37 °C.

12 - eGFP-cap ODs - Taken at 19:30 h

	Tube that will be incubated at a respective temp. later	OD
1	12 - eGFP-cap 18 °C	0.526
2	12 - eGFP-cap 25 °C	0.588
3	12 - eGFP-cap 32 °C	0.421
4	12 - eGFP-cap 37 °C	0.495

Next day we start induction at 19:30 h.

30/07/2026 - Thursday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 30 Jul 2026 15:22:57 UTC
Entry Last Modified: 31 Jul 2026 11:27:03 UTC
Export Generated On: 11 Oct 2026 18:42:25 UTC

THURSDAY, 30/07/2026

Members

Mateo, Vaiva, Katie, Vasily

Observations from 29/07/2026 - Wednesday

All tubes looked cloudy

Objectives

- Lyse the cells via soniation

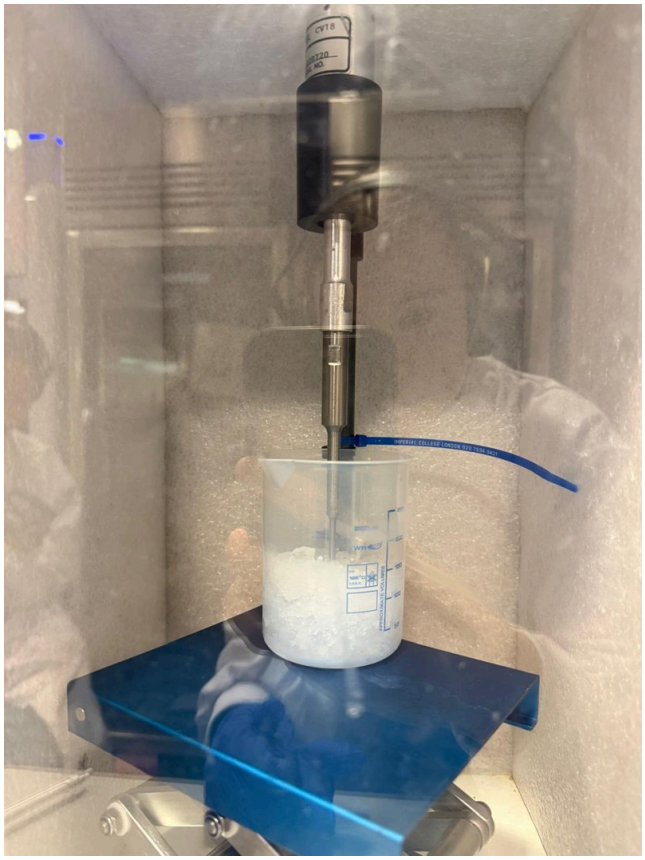
The OD measurments of 18 h samples, measured at 600 nm, blanked with auto-induction TB + Kanamycin.

18 h culture ODs - Taken at 13:30 h

	Construct	OD
1	01 - HET-s 18 °C	1.383
2	01 - HET-s 25 °C	1.656
3	01 - HET-s 32 °C	1.530
4	01 - HET-s 37 °C	1.336
5	08 - Cap 18 °C	1.567
6	08 - Cap 25 °C	1.480
7	08 - Cap 32 °C	1.430
8	08 - Cap 37 °C	1.289
9	10 - mCardinal-unit 18 °C	1.611
10	10 - mCardinal-unit 25 °C	1.535
11	10 - mCardinal-unit 32 °C	1.620
12	10 - mCardinal-unit 37 °C	1.712
13	12 - eGFP-cap 18 °C	1.727
14	12 - eGFP-cap 25 °C	1.761
15	12 - eGFP-cap 32 °C	1.782
16	12 - eGFP-cap 37 °C	1.781

Centrifuge 1 mL sample at 1200 rpm for 3 min and remove the supernatant. Resuspend the pellet with 400 µL PBS solution. Then sonicate for 5 mins: pulse on for 5 sec, pulse off for 10 sec and amplitude 30%. Keep the epp tubes in ice. Protocol 7.2 - Expression testing lysing.

Sonication setup with epp tube in ice



The OD measurments of 24 h samples, measured at 600 nm, blanked with auto-induction TB + Kanamycin.

24 h culture ODs - Taken at 19:20 h

	Construct	OD (1:5 dillution)	Actual OD
1	01 - HET-s 18 °C	0.444	2.22
2	01 - HET-s 25 °C	0.528	2.64
3	01 - HET-s 32 °C	0.435	2.175
4	01 - HET-s 37 °C	0.405	2.025
5	08 - Cap 18 °C	0.536	2.68
6	08 - Cap 25 °C	0.437	2.185
7	08 - Cap 32 °C	0.401	2.005
8	08 - Cap 37 °C	0.390	1.95
9	10 - mCardinal-unit 18 °C	0.564	2.82
10	10 - mCardinal-unit 25 °C	0.477	2.385
11	10 - mCardinal-unit 32 °C	0.513	2.565
12	10 - mCardinal-unit 37 °C	0.567	2.835
13	12 - eGFP-cap 18 °C	0.638	3.19
14	12 - eGFP-cap 25 °C	0.690	3.45
15	12 - eGFP-cap 32 °C	0.631	3.155
16	12 - eGFP-cap 37 °C	0.680	3.4

The 24 h samples were put in a -20 °C freezer for another day measurments.

31/07/2026 - Friday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 31 Jul 2026 11:23:14 UTC
Entry Last Modified: 31 Jul 2026 19:20:54 UTC
Export Generated On: 11 Oct 2026 18:42:26 UTC

FRIDAY, 31/07/2026

Members

Mateo, Vaiva, Katie, Vasily

Objectives

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

The same sonication procedure was performed as for the 18 h samples (see 30/07/2026 - Thursday and 7.2 - Expression testing lysing).

There was spillage in the 10 - Card 37 °C 24 h insol sample while vortexing (volume left in the Epp tube was 230 µL, instead of 400 µL). This sample was not used or run in SDS gel.

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

SDS-PAGE

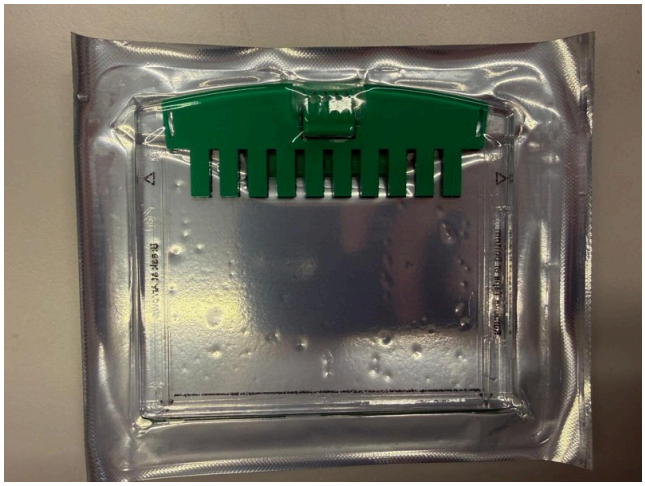
900 µL of Milli-Q water and 100 µL of Tris-Glycine SDS 10X were mixed for the running buffer. For the silver stain: 3 µL of sample, 3 µL of PBS (next time 2 µL should be loaded to make a final volume of 10 µL) and 5 µL of blue loading buffer. For the Coomassie stain: 15 µL of sample and 15 µL of blue buffer. The samples were briefly centrifuged to spin them down (and mix). The samples were then incubated at 100 °C for 10 mins on the heat block. Spin down briefly afterwards.

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

BioRad precast SDS gel



BioRad precast SDS gel

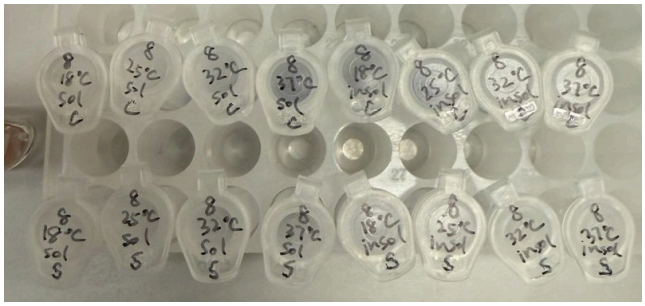


For loading the gel: take off the tape at the bottom, remove the comb, adjust the wells with a pipette tip. When putting in the gel, the smallergel must be towards the inside. After adding the running buffer, clean the wells by pipetting up and down.

When loading the ladder, it should be of the same volume as the sample volume. If any wells are left empty, load them with the same volume of PBS or water so that, when the samples are running, the weight across the gel is the same.

Gel loading seq. (left (1) → right (10))
MWL | 18 °C, sol | 25 °C, sol | ... | 18 °C, insol | 25 °C, insol | ... | PBS or water

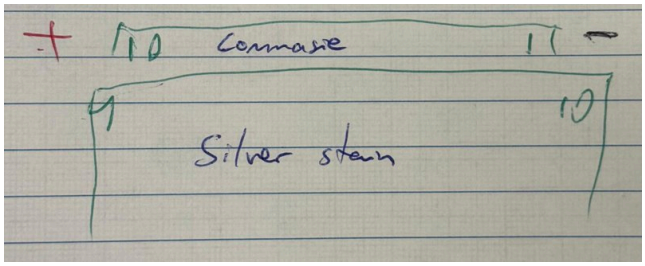
Screenshot_2026-07-31_at_13.32.04.png



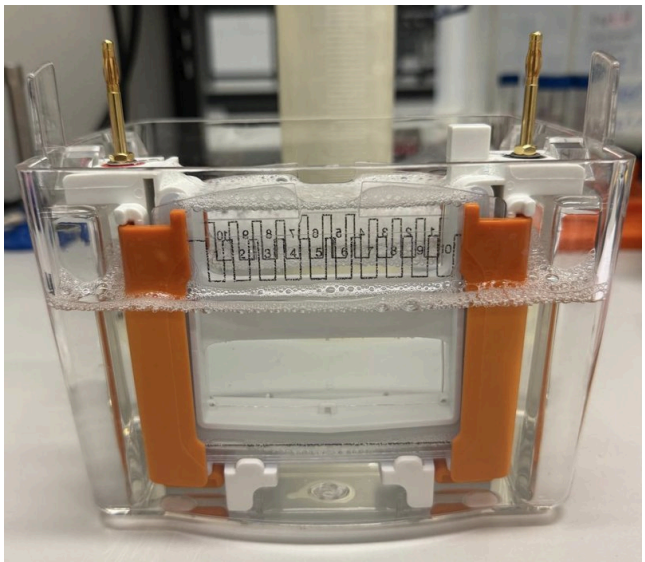
General SDS set-up photos

The gel closer to the tub edge is the silver-stained.

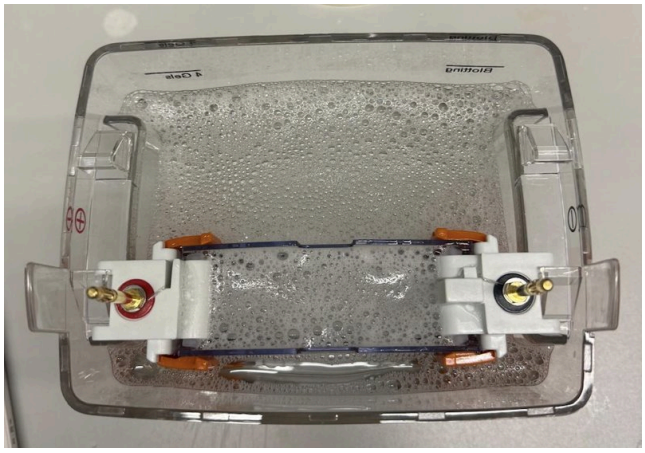
Gel Set-up



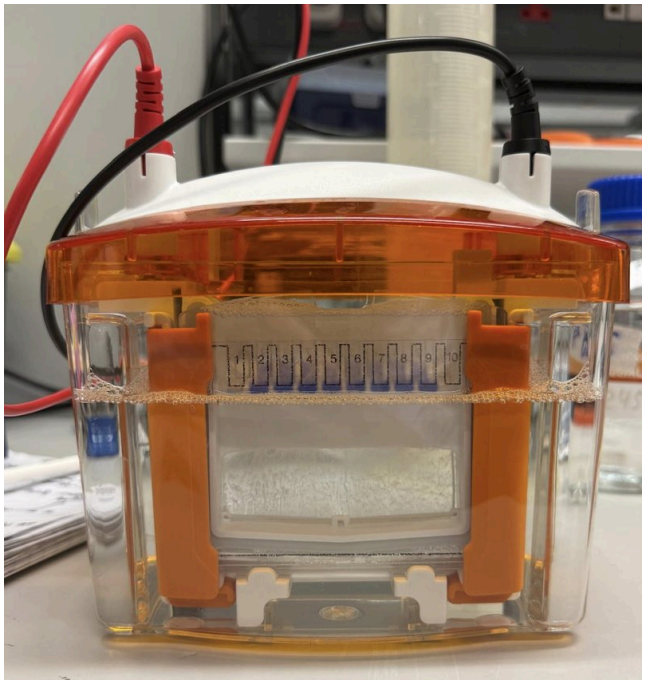
SDS set-up photo 1



SDS set-up photo 2



SDS set-up photo 3



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

Stop the gel when you see the blue stain just running off the gel. Then take the gel, break the plastic and cut away the wells.

For Coomassie stain: stain for 20 min while shaking. If the protein concentration is low, the gel can be kept overnight.

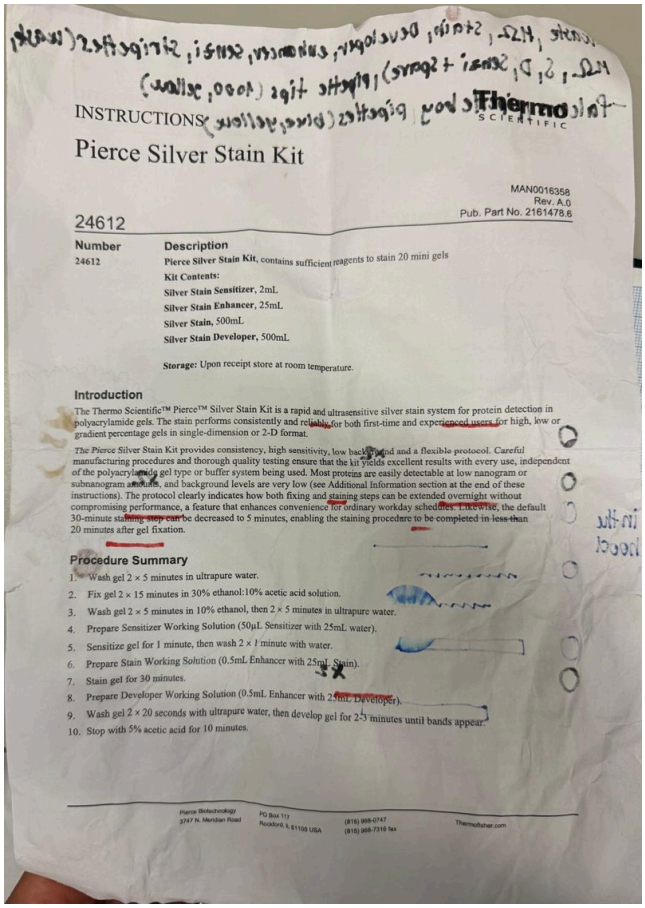
Coomassie staining



For silver stain (see the protocol below for more detail): xS Mili-Q for 5 min, x2 6:3:1 water:ethanol:acetic acid for 15 min, x2 10 % ethanolfor 5 min, x2 Mili-Q for 5 min, Sensitizer Working Solution for 1 min, x2 Mili-Q for 1 min, Stain Working Solution for 30 min, x2 Mili-Q for 20 sec, Developer Working Solution for 2-3 min.

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

Silver stain protocol (p. 1)



INSTRUCTIONS

Thermo SCIENTIFIC

Pierce Silver Stain Kit

MAN0016358
Rev. A.0
Pub. Part No. 2161478.6

24612

Number	Description
24612	**Pierce Silver Stain Kit**, contains sufficient reagents to stain 20 mini gels

Kit Contents:

Silver Stain Sensitizer, 2mL
Silver Stain Enhancer, 25mL
Silver Stain, 500mL
Silver Stain Developer, 500mL

Storage: Upon receipt store at room temperature.

Introduction

The Thermo Scientific™ Pierce™ Silver Stain Kit is a rapid and ultrasensitive silver stain system for protein detection in polyacrylamide gels. The stain performs consistently and reliably for both first-time and experienced users for high, low or gradient percentage gels in single-dimension or 2-D format.

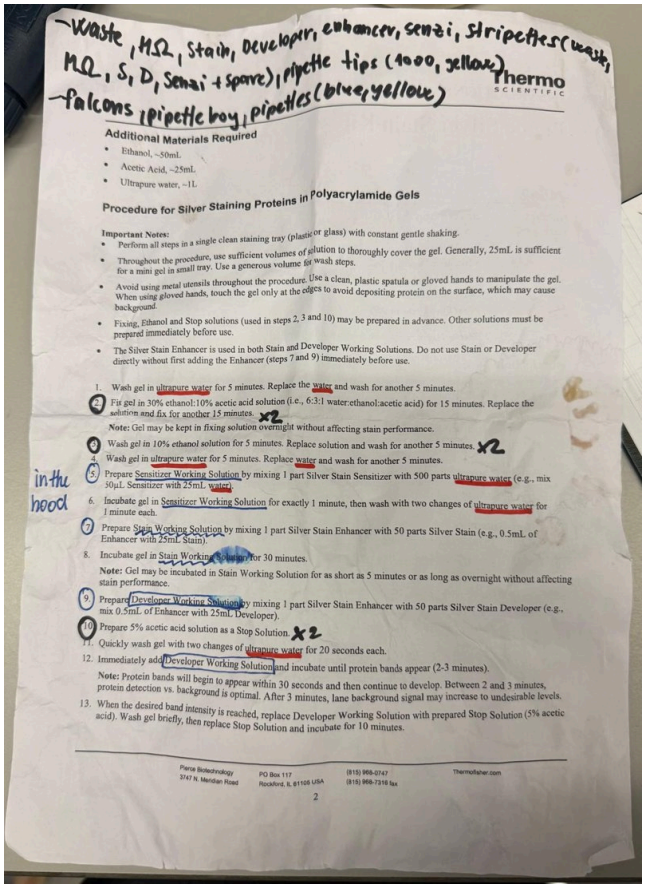
The Pierce Silver Stain Kit provides consistency, high sensitivity, low background and a flexible protocol. Careful manufacturing procedures and thorough quality testing ensure that the kit yields excellent results with every use, independent of the polyacrylamide gel type or buffer system being used. Most proteins are easily detectable at low nanogram or subnanogram amounts, and background levels are very low (see Additional Information section at the end of these instructions). The protocol clearly indicates how both fixing and staining steps can be extended overnight without compromising performance, a feature that enhances convenience for ordinary workday schedules. Likewise, the default 30-minute staining step can be decreased to 5 minutes, enabling the staining procedure to be completed in less than 20 minutes after gel fixation.

Procedure Summary

1. Wash gel 2 × 5 minutes in ultrapure water.
2. Fix gel 2 × 15 minutes in 30% ethanol:10% acetic acid solution.
3. Wash gel 2 × 5 minutes in 10% ethanol, then 2 × 5 minutes in ultrapure water.
4. Prepare Sensitizer Working Solution (50µL Sensitizer with 25mL water).
5. Sensitize gel for 1 minute, then wash 2 × 1 minute with water.
6. Prepare Stain Working Solution (0.5mL Enhancer with 25mL Stain).
7. Stain gel for 30 minutes.
8. Prepare Developer Working Solution (0.5mL Enhancer with 25mL Developer).
9. Wash gel 2 × 20 seconds with ultrapure water, then develop gel for 2-3 minutes until bands appear.
10. Stop with 5% acetic acid for 10 minutes.

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Silver stain protocol (p. 2)



-waste, MQ, Stain, Developer, enhancer, sensi, stripettes (wash, MQ, S, D, Sensi + spare), pipette tips (1000, yellow), falcons (pipette boy, pipettes (blue, yellow)

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Additional Materials Required

- Ethanol, ~50mL
- Acetic Acid, ~25mL
- Ultrapure water, ~1L

Procedure for Silver Staining Proteins in Polyacrylamide Gels

Important Notes:

- Perform all steps in a single clean staining tray (plastic or glass) with constant gentle shaking.
- Throughout the procedure, use sufficient volumes of solution to thoroughly cover the gel. Generally, 25mL is sufficient for a mini gel in small tray. Use a generous volume for wash steps.
- Avoid using metal utensils throughout the procedure. Use a clean, plastic spatula or gloved hands to manipulate the gel. When using gloved hands, touch the gel only at the edges to avoid depositing protein on the surface, which may cause background.
- Fixing, Ethanol and Stop solutions (used in steps 2, 3 and 10) may be prepared in advance. Other solutions must be prepared immediately before use.
- The Silver Stain Enhancer is used in both Stain and Developer Working Solutions. Do not use Stain or Developer directly without first adding the Enhancer (steps 7 and 9) immediately before use.

1. Wash gel in ultrapure water for 5 minutes. Replace the water and wash for another 5 minutes.
2. Fix gel in 30% ethanol:10% acetic acid solution (i.e., 6:3:1 water:ethanol:acetic acid) for 15 minutes. Replace the solution and fix for another 15 minutes. x2

   Note: Gel may be kept in fixing solution overnight without affecting stain performance.
3. Wash gel in 10% ethanol solution for 5 minutes. Replace solution and wash for another 5 minutes. x2
4. Wash gel in ultrapure water for 5 minutes. Replace water and wash for another 5 minutes.
5. Prepare Sensitizer Working Solution by mixing 1 part Silver Stain Sensitizer with 500 parts ultrapure water (e.g., mix 50µL Sensitizer with 25mL water). in the hood
6. Incubate gel in Sensitizer Working Solution for exactly 1 minute, then wash with two changes of ultrapure water for 1 minute each.
7. Prepare Stain Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain (e.g., 0.5mL of Enhancer with 25mL Stain).
8. Incubate gel in Stain Working Solution for 30 minutes.

   Note: Gel may be incubated in Stain Working Solution for as short as 5 minutes or as long as overnight without affecting stain performance.
9. Prepare Developer Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain Developer (e.g., mix 0.5mL of Enhancer with 25mL Developer).
10. Prepare 5% acetic acid solution as a Stop Solution. x2
11. Quickly wash gel with two changes of ultrapure water for 20 seconds each.
12. Immediately add Developer Working Solution and incubate until protein bands appear (2-3 minutes).

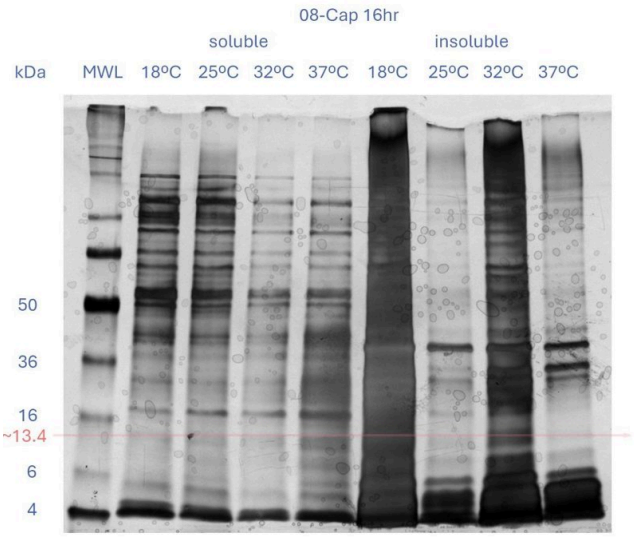
    Note: Protein bands will begin to appear within 30 seconds and then continue to develop. Between 2 and 3 minutes, protein detection vs. background is optimal. After 3 minutes, lane background signal may increase to undesirable levels.
13. When the desired band intensity is reached, replace Developer Working Solution with prepared Stop Solution (5% acetic acid). Wash gel briefly, then replace Stop Solution and incubate for 10 minutes.

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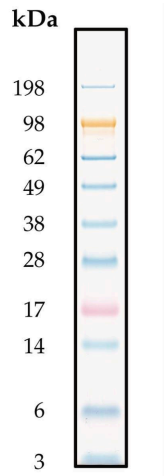
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SDS gel photos

SDS PAGE gel of 08-Cap 18 h stained with silver



Ladder



10 µL of SeeBlue® Plus2 Pre-Stained Protein Standard on a NuPAGE® 10% Bis-Tris Gel with MES SDS Running Buffer