01/09/26 - Tuesday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 01 Sep 2026 17:27:03 UTC
Entry Last Modified: 01 Sep 2026 18:06:09 UTC
Export Generated On: 11 Oct 2026 18:44:47 UTC

TUESDAY, 01/09/2026

Members

Mateo, Vaiva, Vasily

Objectives

- Purify the poisoned cap, cotA and concentrate them
- Prepare SDS loading buffer
- PCR

Protein purification

Protein purification of poisoned cap and cotA was performed following the 12/08/26 - Wednesday protocol. New imidazole solutions of 30 mM and 300 mM were prepared by adding 1 g and 10.2 g of imidazole into 500 mL of PBS, respectively. The solutions were filter sterilized.

The purified proteins were the concentrated following the 13/08/26 - Thursday protocol, but 10 kDa concentrators were used instead of 5 kDa.

SDS loading buffer (4X)

An SDS loading buffer (4X) was prepared with the following concentrations:

- Tris-HCl: 0.2 M
- SDS: 277 mM
- Bromophenol blue: 6 mM
- Glycerol: 4.3 M

The solution was made up to 10 mL with Milli-Q, vortexed, and placed in a water bath at 45 °C to dissolve the contents. The pH was adjusted with 1 M NaOH until it turned blue. Stored at -20°C.

PCR

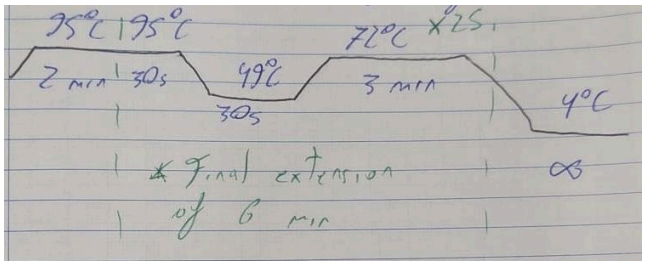
Resuspended IDT primers to 100 µM. Added the following:

- 5X QS reaction buffer
- 200 µM dNTPs
- 0.5 µM of each primer (pET28a (+) inverse PCR primers)
- 0.5 µL template DNA (03 - N-Tail cys, GenScript)

- 0.02 U/μL QS high fidelity DNAP
- Nuclease-free water (ddH_2O) to 25 μL

Thermocycler set of following conditions:

Thermocycler conditions



02/09/26 - Wednesday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 03 Sep 2026 08:36:17 UTC
Entry Last Modified: 07 Sep 2026 11:28:04 UTC
Export Generated On: 11 Oct 2026 18:44:48 UTC

WEDNESDAY, 02/09/2026

Members

Mateo, Vaiva

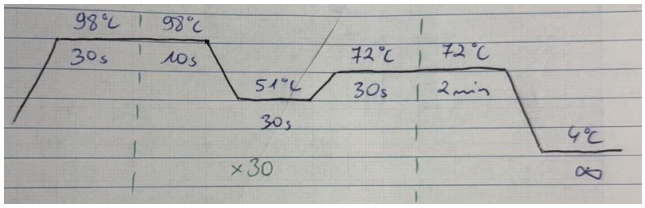
Objectives

- PCR for N-Tail-Cys, WT and His-tagged HET-s (TWIST)
- Run agaroe gel for PCR samples
- Run SDS gel for poisoned cap and cotA

PCR

PCR samples for N-Tail-Cys, WT and His-tagged HET-s (TWIST) were prepared following the 01/09/26 - Tuesday protocol. The thermocycler was set to the following conditions:

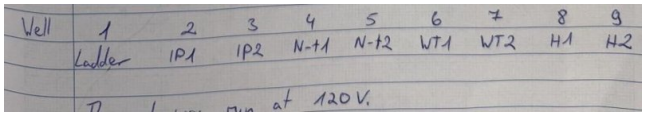
Thermocycler conditions



Agarose gel

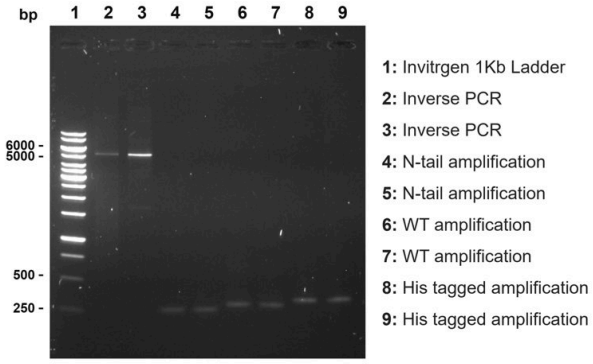
1% agarose gel was prepared by adding 1 g of agarose to 100 mL of 1X TAE, heating it up and adding 10 µL of SYBR stain. Samples of pET28a(+) inverse PCR (IP) (from 01/09/26 - Tuesday), N-Tail-Cys (N-t), 5GGS HET-s (WT) and 5GGS His-tagged HET-s (H) were prepared by mixing 1 µL of DNA, 1 µL of loading dye (6X) and 4 µL of Milli-Q. The loading order was as follows:

IMG_3624.jpg



The gel was run at 120 V.

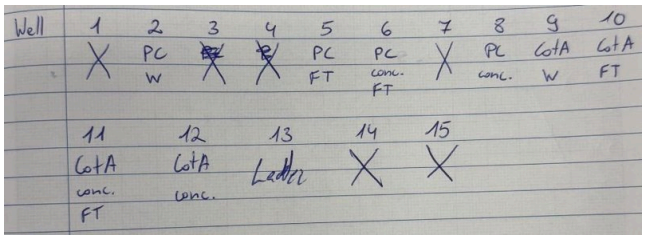
gel-annotation.png



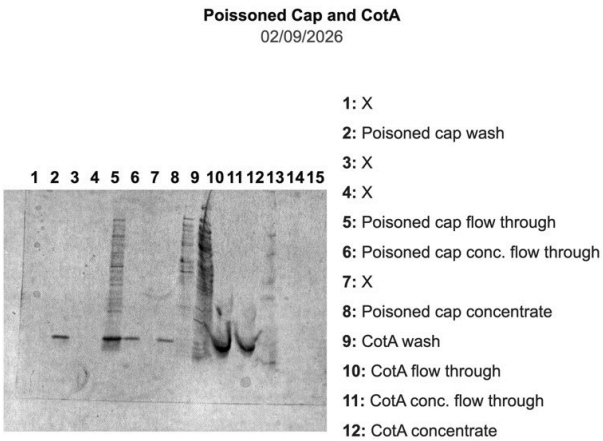
SDS

For poisoned cap flow through (PC FT), wash (PC W), concentrate flow through (PC conc. FT) and concentrate (PC conc.) refer to sample preparation protocol 17/08/26 - Monday. For cotA flow through (cotA FT), wash (cotA W), concentrate flow through (cotA conc. FT) and concentrate (cotA conc.)refer to 21/08/26 - Friday, but added 15 µL of sample + 15 µL of loading buffer. Tris-glycine gel was used, and the loading order was as follows:

IMG_3625.jpg



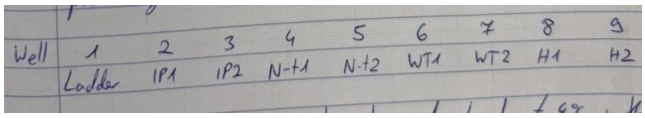
2026-09-02_Poissoned_Cap_and_CotA.png



Gel extraction

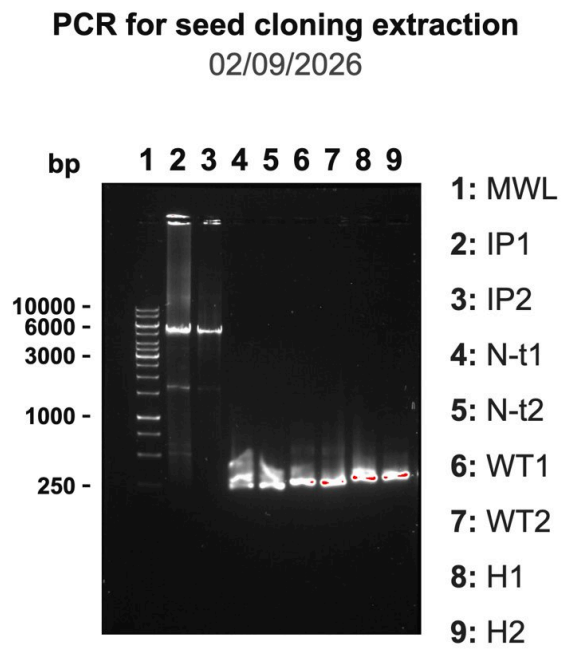
The same agarose gel protocol was followed as above, but this time samples were prepared by mixing 11 µL of DNA and 1 µL of loading dye (6X); the ladder was loaded as in previous gel. The loading order was as follows:

IMG_3626.jpg



The gel bands were cut out and stored at 4 °C in the cold room.

2026-09-02_PCR_for_seed_cloning_extraction.png



03/09/26 - Thursday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 03 Sep 2026 10:42:30 UTC
Entry Last Modified: 07 Sep 2026 11:28:41 UTC
Export Generated On: 11 Oct 2026 18:44:48 UTC

THURSDAY, 03/09/2026

Members

Mateo, Vaiva

Objectives

- Gel extraction
- Prepare TB cultures
- Precultures
- PCR
- Agarose gel
- Precultures

Gel (from 02/09/26 - Wednesday) extraction (NEB #T1120)

The excised DNA fragments from the agarose gel were weighed out nd Buffer BY was added, such that each sample had 4 volumes of buffer and 1 volume of gel slice. Samples were incubated at 50 °C for 5 min, until the gel slices dissolved. For each sample, the Spin Column S1A was inserted into the Spin Collection Tube, and samples were loaded (NB: the column volume is 800 µL). Spun down for 1 min at 13,000 rpm. Washed x2 with 200 µL of Buffer W2 and spun down for 1 min. Eluted with 10 µL of Buffer EY.

Biospectrometer readings of DNA samples

Running routine: dsDNA 1 mm; **1:10 dilution**

Table1

	Sample name	Concentration (µg/mL)	A260/1mm	A260/A280	A260/A230
1	Inverted PCR (IP)	2.9	0.007	3.56	0.41
2	WT	9.2	0.015	3.17	0.08
3	N-Tail-Cys (N-t)	4.5	0.007	3.69	0.43
4	His-tagged (H)	8.8	0.014	3.21	3.41

TB cultures

6 L of TB culture was prepared following the 26/08/26 - Wednesday protocol.

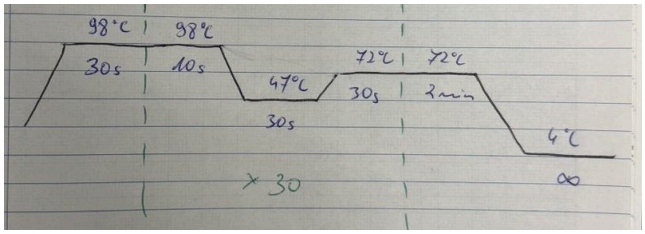
Precultures

Precultures for poisoned cap and WT were prepared by dipping a pipette tip into the glycerol stock of the respective culture and putting it into 9 mL of TB + 10X salt. Three tubes per construct were prepared and incubated at 37 °C overnight.

PCR#1

PCR for WT, N-t and H were prepared following the 01/09/26 - Tuesday, but the DNA templates were used from 02/09/26 - Wednesday and were diluted 1:100 with Mili-Q. Thermocycler set to the following conditions:

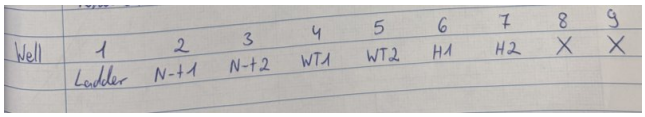
IMG_3633.jpg



Agarose gel

For gel preparation and sample loading refer to 02/09/26 - Wednesday protocol. The loading order was as follows:

IMG_3635.jpg

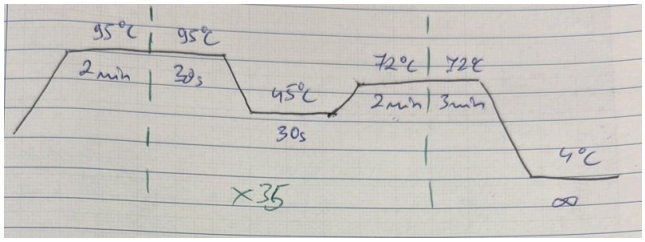


Same appearance as 02/09/26 - Wednesday

PCR#2

For IP, the thermocycler was set to teh following conditions:

IMG_3636.jpg



PCR#3

PCR samples for N-t, WT and H were prepared following 01/09/26 - Tuesday protocol, but double the volume (50 µL).

Thermocycler was set as in 02/09/26 - Wednesday.

04/09/26 - Friday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 04 Sep 2026 11:21:04 UTC
Entry Last Modified: 22 Sep 2026 13:28:58 UTC
Export Generated On: 11 Oct 2026 18:44:47 UTC

FRIDAY, 04/09/2026

Members

Mateo, Vaiva

Objectives

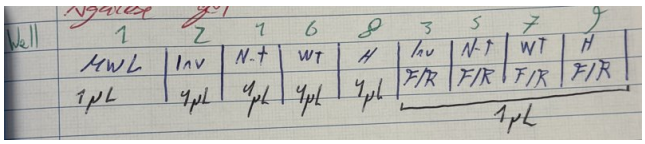
- Inoculate TB
- Do seed cloning and transform DH5-alpha
- NMR V1 with Ben

DpnI

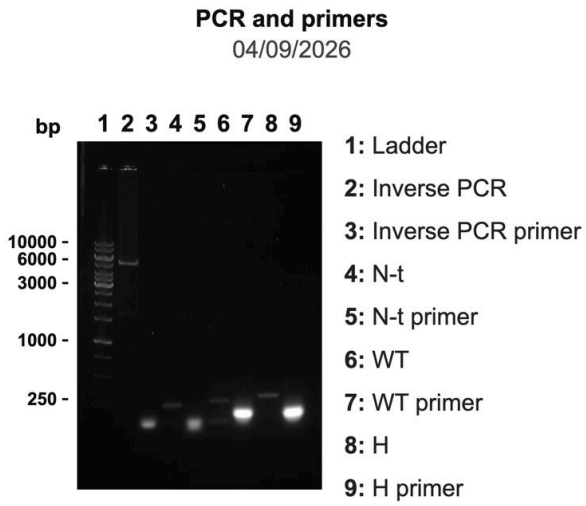
Digested the DNA original plasmid template by adding 6 µL 10X CutSmart, 1 µL DpnI, and 3 µL of ddH_2O to make a final volume of 60 µL. Incubated at 37 °C for 15 min.

Agarose gel

IMG_3642.jpg



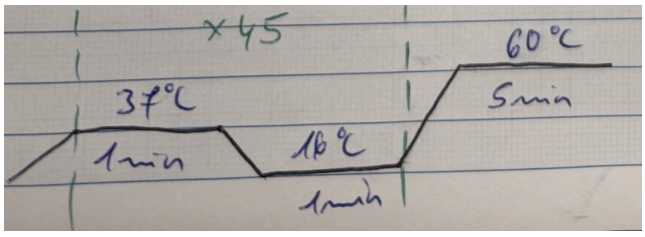
2026-09-04_PCR_and_primers.png



Assembly

The reaction was set up by adding 2.5 µL T4 DNA ligase buffer (10X), 2.5 µL (1000 U) T4 DNA ligase, 1.5 µL (30 U) BsaII-HFv2, 2 µL IP, 1 µL N-t, 1 µL H, 7 µL WT and made up with Milli-Q to 25 µL. Another reaction was set up the same way but excluding the vector (IP). Thermocycler set to the following conditions:

IMG_3644.jpg



Preculture

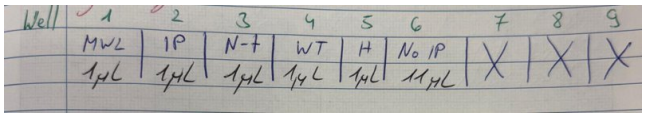
120 µL of TB salts (10X) and 20 µL of 50X autoinduction were added to each 1 L TB culture. 9 mL of precultures from 03/09/26 - Thursday were addded. Inoculated at 37 °C, 220 rpm.

PCR cleanup (Monarch #T1130)

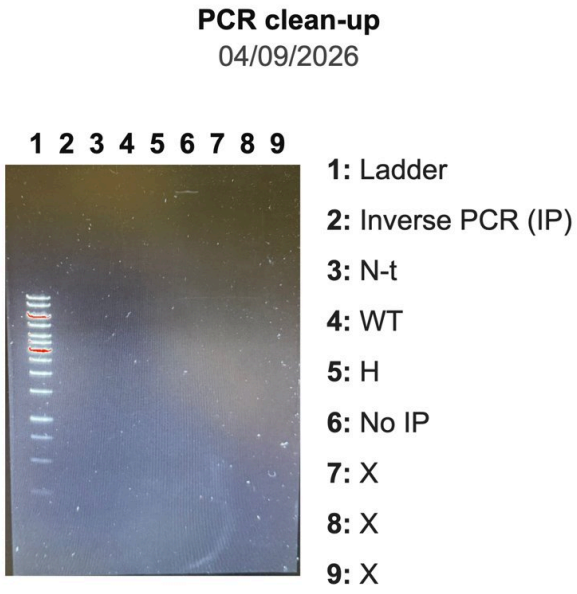
Refer to gel extraction protocol 03/09/26 - Thursday. PCR cleanup was performed for IP, N-t, WT and H samples.

Agarose gel

PHOTO-2026-09-04-17-10-19.jpg



2026-09-04_PCR_clean-up.png



NMR

One V1 aliquote was resuspend in 600µL of TBS+D2O and spun down at 4ºC for 5 min at 13000 rpm.

image.png

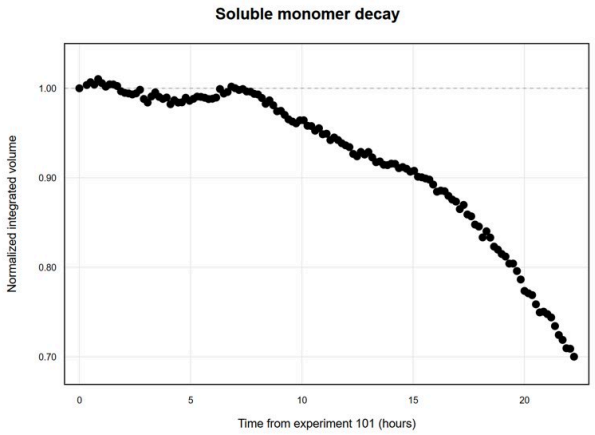
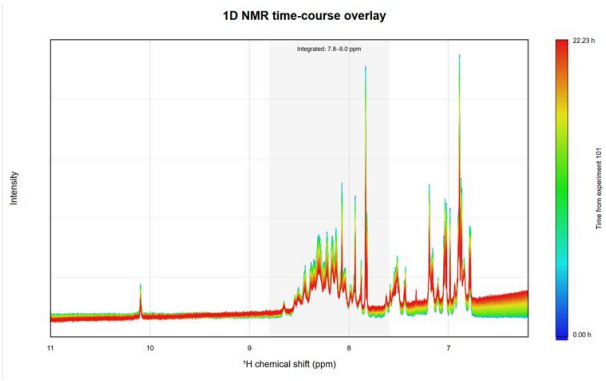


image.png



05/09/26 - Saturday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 07 Sep 2026 10:57:09 UTC
Entry Last Modified: 07 Sep 2026 11:12:41 UTC
Export Generated On: 11 Oct 2026 18:44:47 UTC

MONDAY, 07/09/2026

Members

Mateo

Objectives

- Spin down the cells
- Prepare Cap NMR sample

Spin down the cells

3 L cultures were centrifuged at 4000 rpm for 15 min, and the supernatant was removed. Cells harvested in 60mL of GuHCl lysis buffer, parafilmed and put on the roler overnight at 4ºC.

NMR

One cap aliquote was resuspend in 600µL of TBS+D2O and spun down at 4ºC for 5 min at 13000 rpm.

06/08/26 - Sunday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 07 Sep 2026 10:58:33 UTC
Entry Last Modified: 07 Sep 2026 11:25:27 UTC
Export Generated On: 11 Oct 2026 18:44:48 UTC

MONDAY, 07/09/2026

Members

Mateo

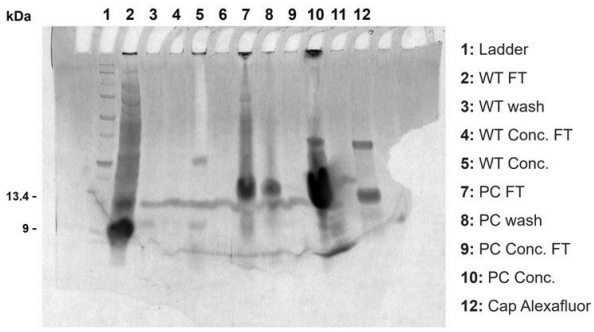
Objectives

- Purify WT and Poissoned Cap
- Concentrate WT and PC
- Lyophilise AlexaFluor Samples
- Prepare DC NMR

Purification

Binding for 1hr rocking. Around 7mL of resin used per construct. Flowthrough colected, washed three times and eluted with 45ml. Then all concentrated to 5mL. Flowthrough collected.

gel-annotation_1.png



Lyophilise AlexaFluor Samples

Alequoted in 500µL. Thrown in liquid nitrogen and lyophilised overnight.

DC NMR

One DC aliquote was resuspend in 600µL of TBS+D2O and spun down at 4ºC for 5 min at 13000 rpm.

07/09/26 - Monday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 07 Sep 2026 11:51:36 UTC
Entry Last Modified: 07 Sep 2026 18:31:51 UTC
Export Generated On: 11 Oct 2026 18:46:07 UTC

MONDAY, 07/09/2026

Members

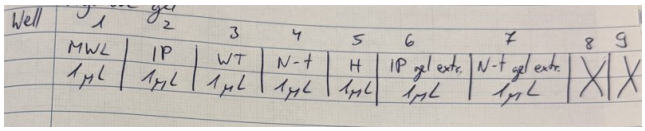
Hector, Mateo, Vaiva

Objectives

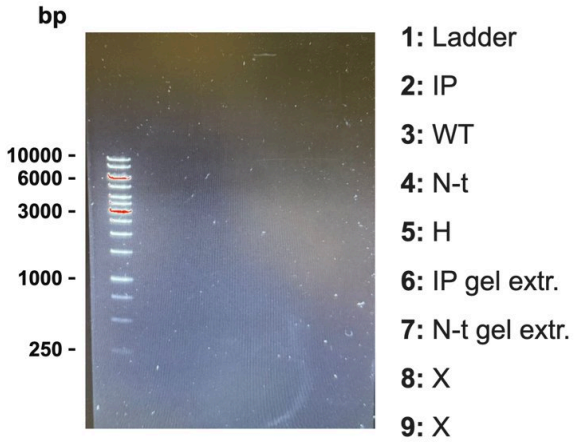
- Run DNA gel for gel extraction diagnosis
- Prepare TEM samples
- Purify and concentrate WT
- PCR
- Process NMR samples

Agarose gel

IMG_3667.jpg



2026-09-07_Gel_extraction_diagnosis.png



TEM sample preparation

TEM samples were prepared for WT, V1, DC and Cap following the 20/08/26 - Thursday protocol. Storage in a disc: 22 - WT, 23 - V1, 24 - DC, 25 - Cap.

Purification and concentration

Purification same process as @06/09/26. Concentration of WT was done following 13/08/26 - Thursday protocol.

PCR

PCR samples for IP, WT, N-t and H were prepared as in 03/09/26 - Thursday. Thermocycler conditions for WT, N-t and H were set as in 02/09/26 - Wednesday.

08/09/26 - Tuesday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 07 Sep 2026 18:34:23 UTC
Entry Last Modified: 09 Sep 2026 09:44:55 UTC
Export Generated On: 11 Oct 2026 18:46:08 UTC

TUESDAY, 08/09/2026

Members

Hector, Mateo, Vaiva

Objectives

- ~~Run inverse PCR~~
- ~~PCR Clean-up on 4 constructs w. gel~~
- ~~NMR sample on DC~~
- EM data collection on ~~WT, V1,~~ DC, Cap
- ~~Protein gel on WT purification~~
- ~~Sortase reaction test w. eGFP (100uM)~~
- Protocols for linker labelling & platereader assay
- ~~Golden gate assembly + gel check~~

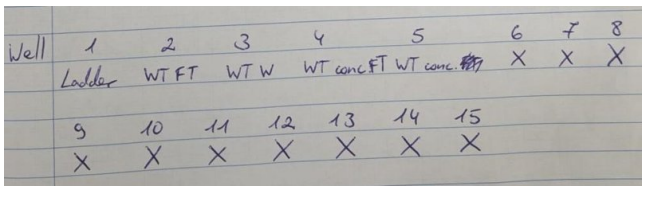
Inverse PCR

IP sample was run with thermocycler conditions set as in 01/09/26 - Tuesday.

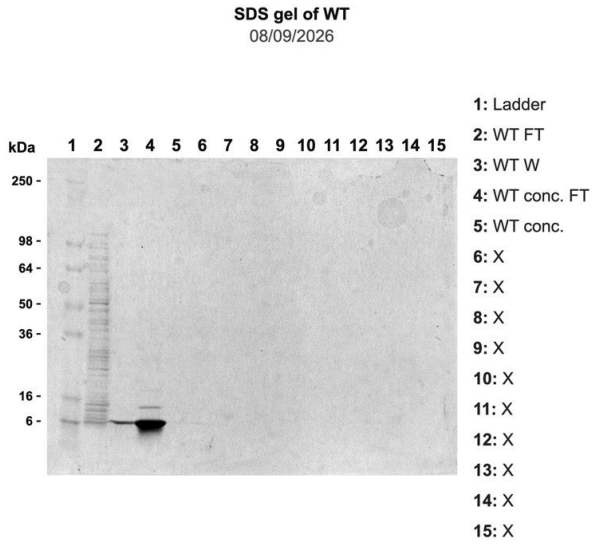
SDS gel

Samples for WT FT, WT W, WT conc., WT conc. from 07/09/26 - Monday were spun down, supernatant discarded and pellet resuspended in ~200-300 µL of PBS. The samples were then prepared following 21/08/26 - Friday protocol. The loading order was as follows:

PHOTO-2026-09-08-17-25-51_2.jpg



SDS_WT_08-09-26.png



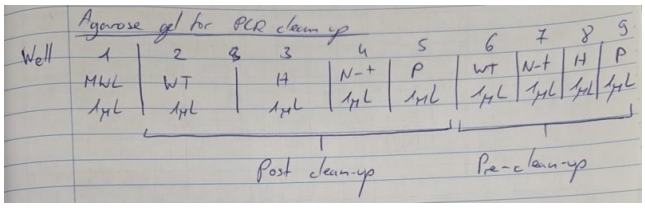
Reaction buffers for eSortA

10 mL of 10X Ca^{2+} dependent solution was prepared by adding 0.61 g Tris, 0.88 g NaCl and 0.11 g $CaCl_2$ to 10 mL of Milli-Q, so that final conc. is 500 mM, 1.5 M and 100 mM, respectively.

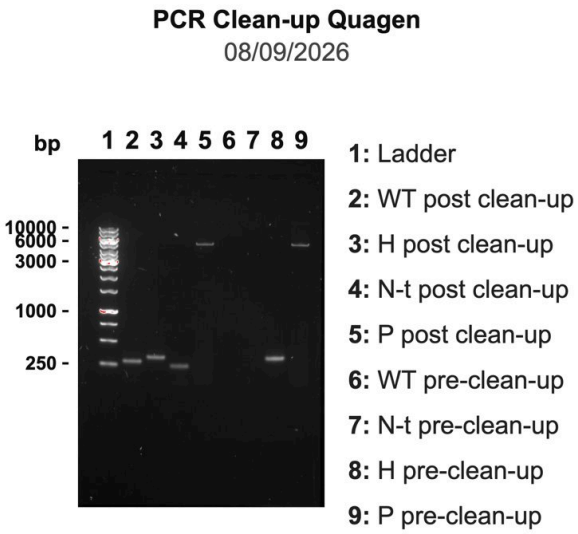
10 mL of 10X Ca^{2+} independent solution: 0.61 g g Tris, 0.88 g NaCl and 0.37 g EDTA, so the final conc. is 500 mM, 1.5 M and 100 mM, respectively. Both solutions adjusted to pH 8.

Agarose gel for PCR clean up

3706F3B8-4B5E-46C8-AFFF-9E4DB146DCE9_4_5005_c.jpeg



gel-annotation.png



Biospectrometer readings

Table1

	Construct	Conc., µg/mL	A260/1mm	A260/A280	A260/A230
1	WT	55.9	0.24	2.03	1.97
2	N-t	56.1	0.118	1.86	1.95
3	H	58	0.130	1.87	1.74
4	P	40.5	0.653	2.67	1.8

Cloning

Cloning reaction was set up as in 04/09/26 - Friday; one with P and one without. Volume of construct added: 4 µL WT, 0.5 µL N-t, 0.5 µL H and 4.5 µL P (in the one with).

eSortA reaction mixture

Table2

	A	eSort-5M +Ca2+	eSort-5M - Ca2+	eSort-7M +Ca2+	eSort-7M - Ca2+
1	eGFP (TEV)	37.5	37.5	37.5	37.5
2	HET-s (100 μM)	70	70	70	70
3	5 M (160 μM)	36	36	-	-
4	7 M (160 μM)	-	-	36	36
5	10X Ca2+ dep.	60	-	60	-
6	10X Ca2+ indep.	-	60	-	60
7	Total	600	600	600	600

Negative controls

- w/o TEV
- w/o eGFP
- w/o HET-s
- w/o SortA (5 M)

09/09/26 - Wednesday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 09 Sep 2026 09:40:52 UTC
Entry Last Modified: 09 Sep 2026 16:55:19 UTC
Export Generated On: 11 Oct 2026 18:46:08 UTC

WEDNESDAY, 09/09/2026

Members

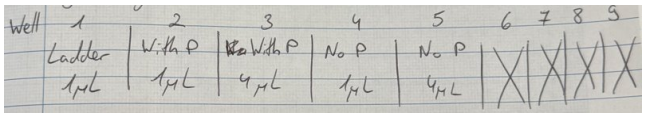
Hector, Mateo, Vaiva

Objectives

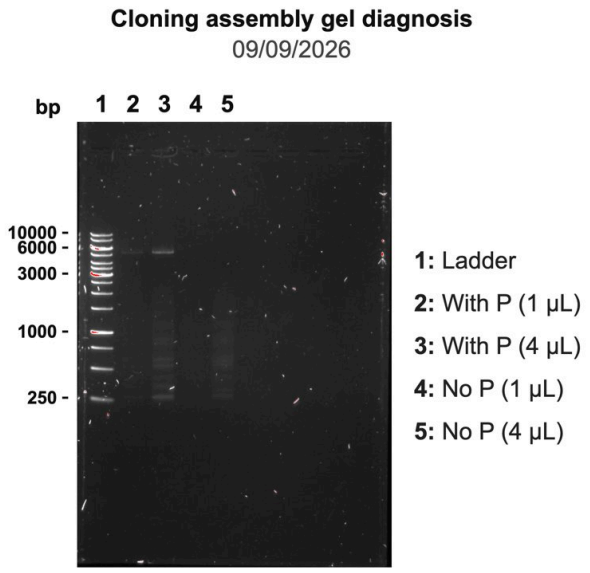
- ~~Run DNA gel of assembly~~
- ~~DpnI digest of Golden gate product~~
- ~~Transform DH5α cells~~
- BE/Lyophilise WT and PC
- Progress with SortA experiment
- Protocols for linker labelling & platereader assay

Agarose gel

IMG_3697.jpg



Cloning_assembly_gel_diagnosis_09-09-26.png

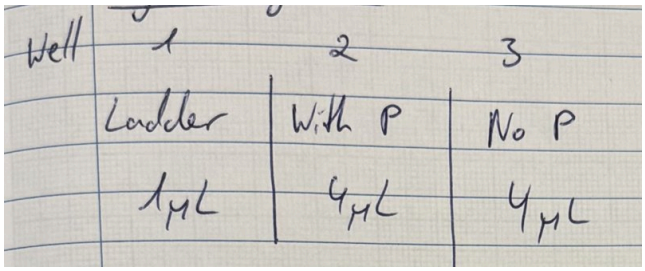


DpnI

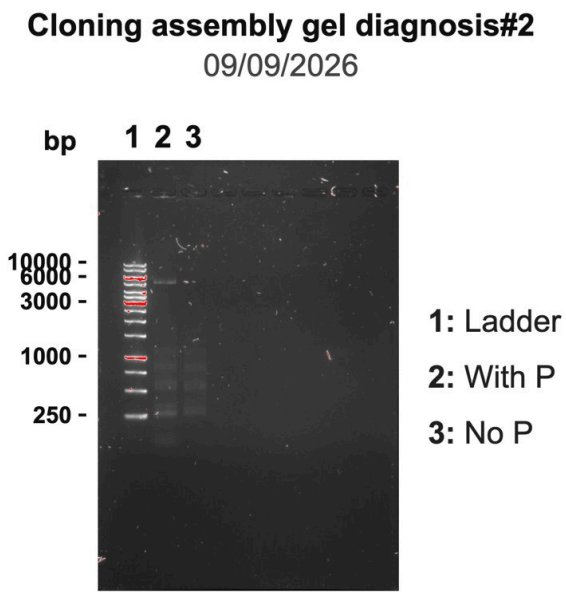
Was performed as on 04/09/26 - Friday

Agarose gel of the digested sample

IMG_3698.jpg



Cloning_assembly_gel_diagnosis2_09-09-26.png

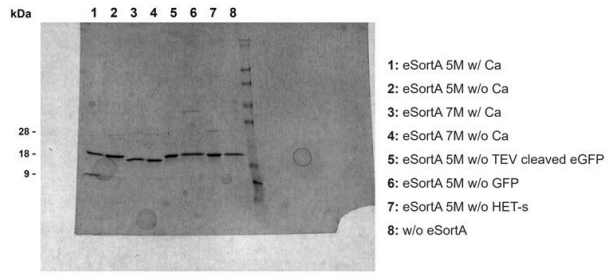


Transformation of the NEB10-beta competent *E. coli* cells

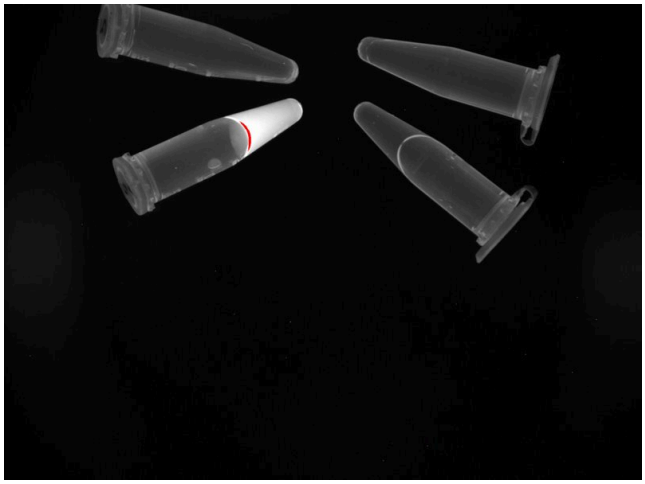
Added 10 µL of the assembly DNA mixture from 08/09/26 - Tuesday into 50 µL of cells, and flicked the tube to mix. Left on ice for 30 min. Heat-shocked at 42 °C for 30 sec. Ice for 5 min. Added 950 µL of LB and incubated at 37 °C, 250 rpm for 1 hr. Plated 50, 100, and 750 µL on LB + Kan plates and incubated overnight at 37 °C.

Sortase stuff

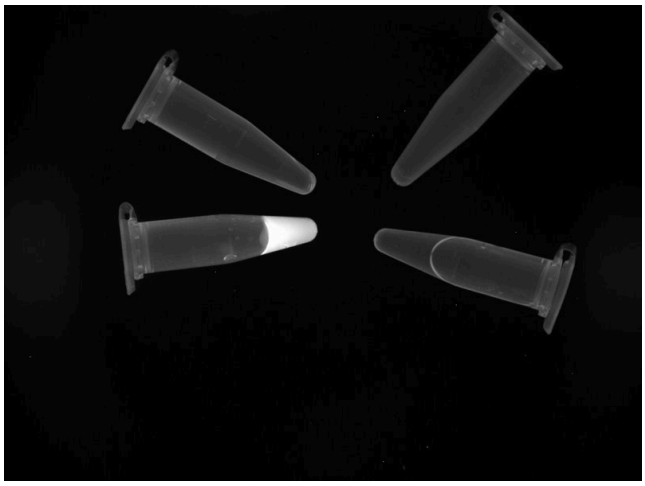
gel-annotation_2.png



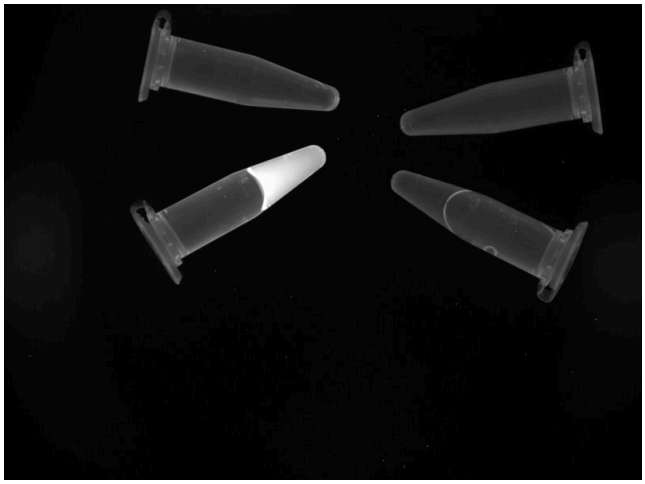
admin_2026-09-09_14hr_08min_eSortA_experiment_n5_and_n6.tif



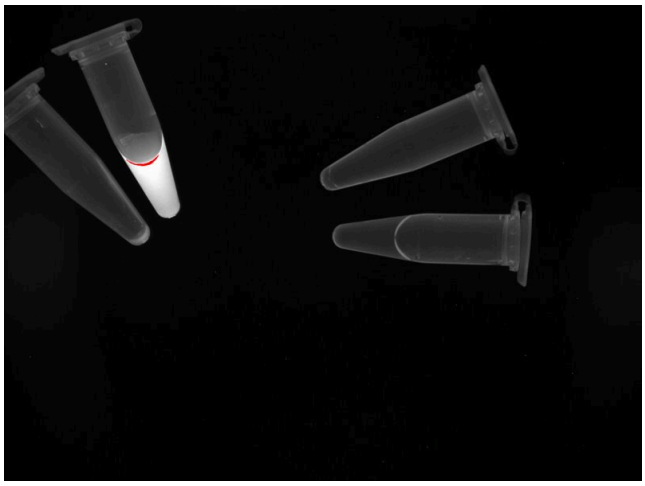
admin_2026-09-09_14hr_08min_eSortA_experiment_n7_and_n6.tif



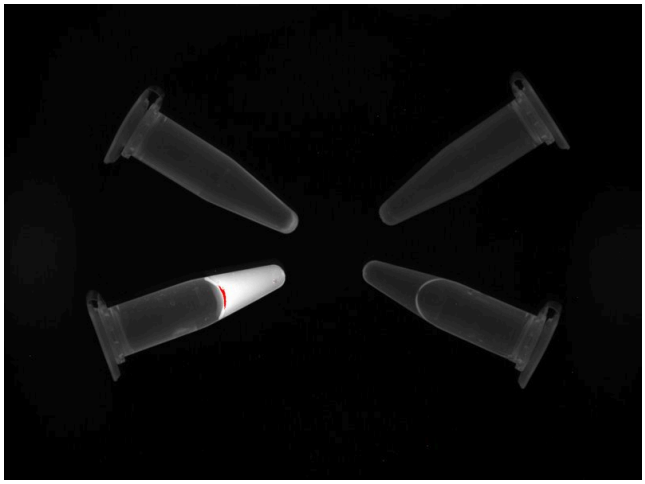
admin_2026-09-09_14hr_08min_eSortA_experiment_n8_and_n6.tif



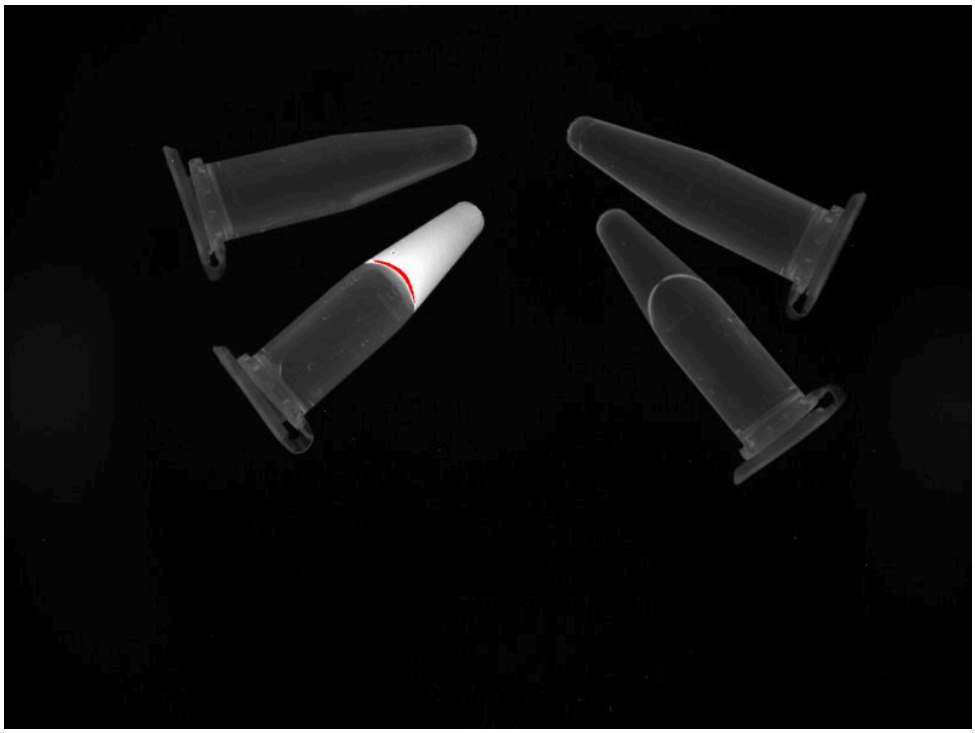
admin_2026-09-09_14hr_08min_eSortA_experiment_n1_and_n6.tif



admin_2026-09-09_14hr_08min_eSortA_experiment_n2_and_n6.tif



admin_2026-09-09_14hr_08min_eSortA_experiment_n4_and_n6.tif



10/09/26 - Thursday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 10 Sep 2026 11:31:05 UTC
Entry Last Modified: 11 Sep 2026 10:45:43 UTC
Export Generated On: 11 Oct 2026 18:46:07 UTC

THURSDAY, 10/09/2026

Members

Hector, Mateo, Vaiva

Objectives

- Colony PCR
- DNA gel for colony PCR samples

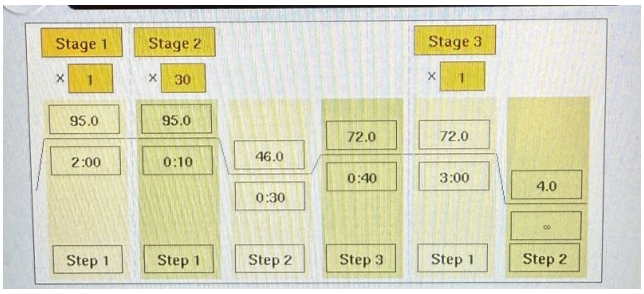
Colony PCR

Master mix for 30 smaples (25 µL per reaction):

- 5X Q5 reaction buffer: 150 µL
- 10 mM dNTPs: 15 µL
- 10 µM Fwrd. T7 primer: 37.5 µL -> 3.75 µL of 100 µM stock
- 10 µM Rev. T7 primer: 37.5 µL -> 3.75 µL of 100 µM stock
- Q5 High fidelity DNA pol.: 7.5 µL
- Nuclease free water: 502.5 µL

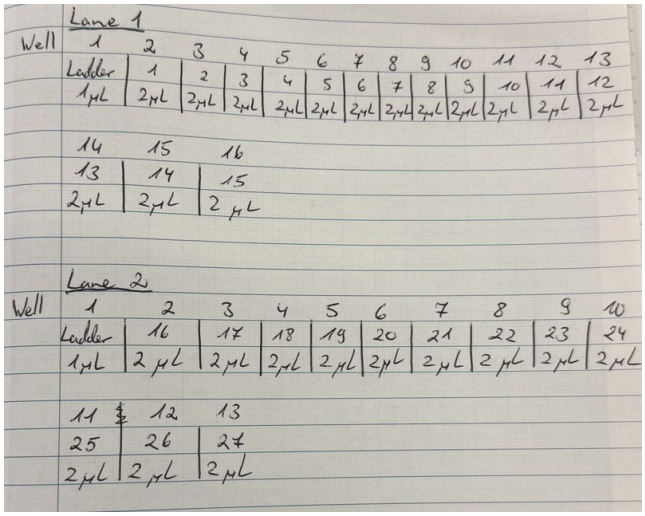
27 different colonies were selected (from 09/09/26 - Wednesday) and transferred to separate PCR tubes containing 25 µL of master mix. The thermal cycler was set to the following conditions:

IMG_3710.jpg

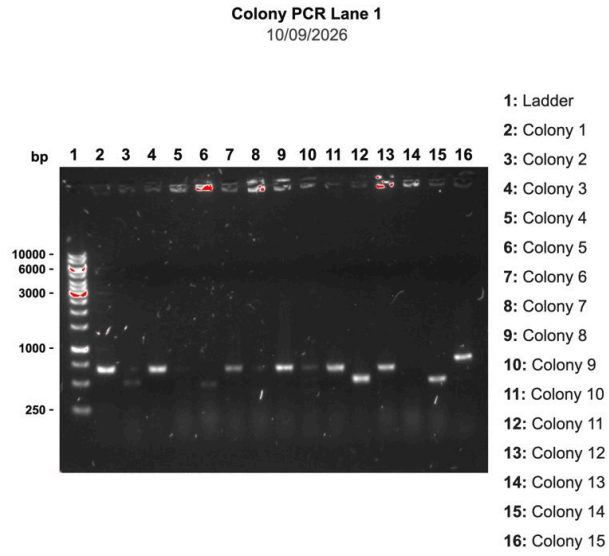


Agarose gel for colony PCR for seeds

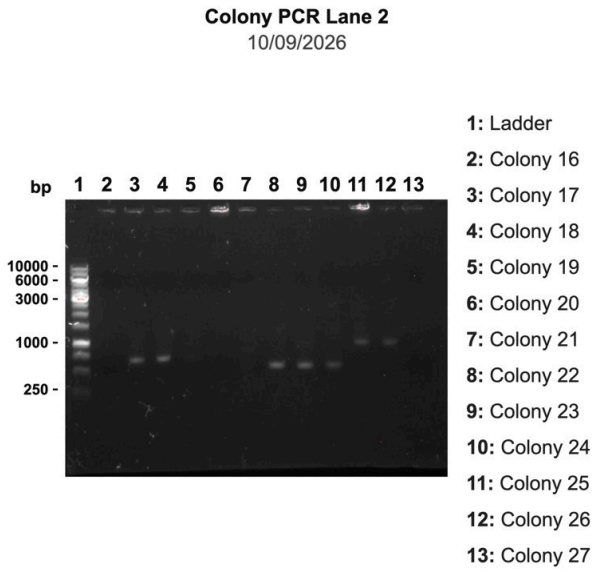
IMG_3712.jpg



ColonyPCR_Lane1.png



ColonyPCR_Lane2.png



11/09/26 - Friday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 11 Sep 2026 10:44:59 UTC
Entry Last Modified: 14 Sep 2026 08:57:52 UTC
Export Generated On: 11 Oct 2026 18:46:07 UTC

FRIDAY, 11/09/2026

Members

Hector, Mateo, Vaiva

Objectives

- PCR clean-up for colonies 1, 11 and 15
- DNA gel for PCR clean-up
- Do PCR for IP, WT, N-t and H

PCR clean-up

PCR clean-up was performed for colony PCR samples 1, 11 and 15 from 10/09/26 - Thursday using QIAquick PCR purification kit (28104).

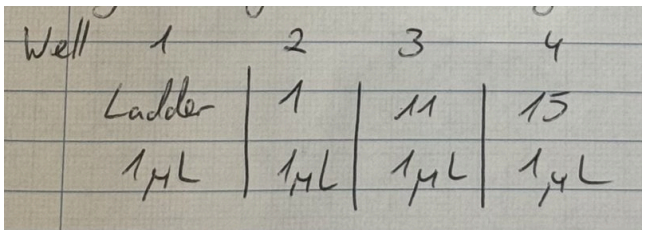
Biospectrometer readings of PCR clean-up

Table1

	Colony number	Conc., µg/mL	A260/1mm	A260/A280	A260/A230
1	1	17.7	0.035	1.91	1.35
2	11	13.1	0.027	1.81	1.11
3	15	9.2	0.018	1.82	1.26

Agarose gel of PCR colony clean-up samples

IMG_3720.jpg



PCR

PCR for IP, WT, N-t and H was performed as on 03/09/26 - Thursday with thermocycler conditions set for IP as on 01/09/26 - Tuesday and for WT, N-t and H as on 02/09/26 - Wednesday.

Full Circle Labs seq. (Bag code: 934RMY; Order number: #34282)

Tube 1 -> Colony 1; Tube 2 -> Colony 11, Tube 3 -> Colony 15

Measuring AlexaFluor 488 (N-term tail) and AlexaFluor 647 (Cap) efficiency

Lyophilised AlexaFluor samples were resuspended in both water and GuHCl and measured against protein absorbance (A280) using the µCuvette. Absorbance readings were consistent across both solvents.

Table2

	A	B	C	D	E	F	G	H
1	Solution	A280	A495	A650	Label ε (M-1cm-1)	Protein conc	Label conc	Label %
2	N-tail (AF488)	1.050	0.163	-	73000	0.0015021459	2.23287671232877e-5	1.4864579256
3	Cap (AF647)	0.344	-	1.087	270000	0.0004921316	4.02592592592593e-5	8.1805878553

Negative stain EM

Photos on OneDrive

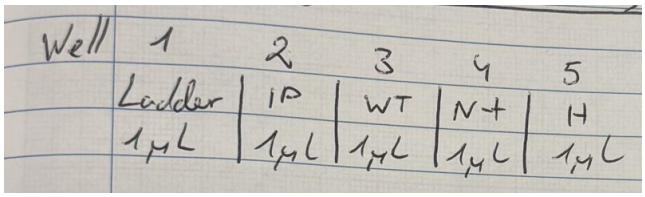
V1/DC - no fibrils negative result
Cap - irregular rise aggregated fibrils

PCR clean-up

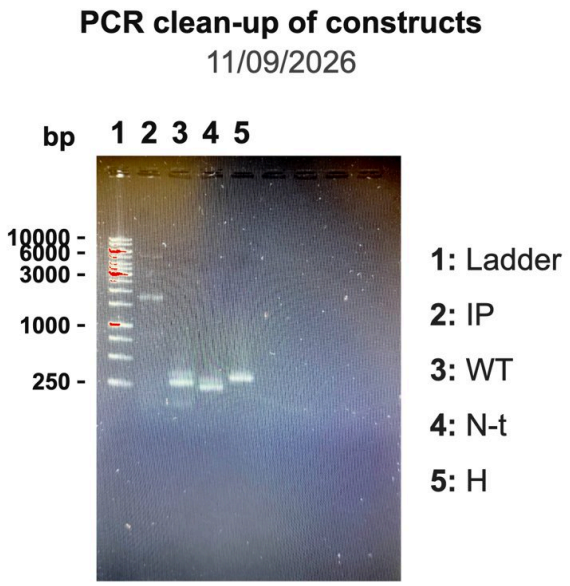
PCR clean-up for IP, WT, N-t and H was prepared as above. 1 µL of DpnI was added to each sample, incubated at 37 °C for 10 min and denatured at 80 °C for 20 min.

Agarose gel for IP, WT, N-t and H

IMG_3725.jpg



PCR_clean-up_of_constructsGel.png



Biospectrometer readings of PCR clean-up of constructs

Table3

	Construct	Conc., µg/mL	A260/1mm	A260/A280	A260/A230
1	WT	100.1	0.205	1.86	1.97
2	N-t	119.8	0.242	1.85	2.01
3	H	86.9	0.177	1.86	1.92
4	P	96.2	0.195	1.88	1.86

Cloning

Cloning was performed as on 04/09/26 - Friday with the following construct volumes added: 2.25 µL IP, 8 µL WT, 0.4 µL N-t, 0.3 µL H.

Setting up Fibrils

Fibrils set up using 10mM concentration of DTT. Resuspend fraction of lyophilize sample in 200uL of Growing Buffer w/ and w/o DTT.

Table4

	Condition	A280/1mm
1	WT DTT	0.059
2	V1 DTT	0.123
3	V2 DTT	0.128
4	DC DTT	0.081
5	Seed DTT	0.091
6	Cap DTT	0.140
7	PC DTT	0.304
8	V1	0.132
9	V2	0.119
10	DC	0.085
11	PC	0.190

Left incubating at 25ºC at 300 rpm.

Data generation next week

- Sortase + fibrils repeat (Monday)
- Repeat Golden gate assembly of seed + colony PCR (Monday-Tuesday)

- AlexaFluor labelled WT trial (Today-tomorrow)
- ThT vs free ThT on end labelled fibrils (Monday)
- Setup TIRF samples (Monday

12/09/2026 - Saturday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 14 Sep 2026 08:46:56 UTC
Entry Last Modified: 14 Sep 2026 09:02:32 UTC
Export Generated On: 11 Oct 2026 18:46:07 UTC

MONDAY, 14/09/2026

Members

Mateo

Objective

Spin down fibrils for 5 min at 4ºC to observed pellet.

Table1

	Pelleted	No pellet
1	WT DTT	V1
2	V1 DTT	V2
3	V2 DTT	DC DTT
4	Cap DTT	DC
5	PC DTT	Seed
6	PC	

The pellets of the caps look morphologically distinct from the rest, more cloudy:

Cap DTT pellet



V1 DTT Pellet



14/09/26 - Monday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 14 Sep 2026 08:48:20 UTC
Entry Last Modified: 15 Sep 2026 17:07:48 UTC
Export Generated On: 11 Oct 2026 18:47:24 UTC

MONDAY, 14/09/2026

Members

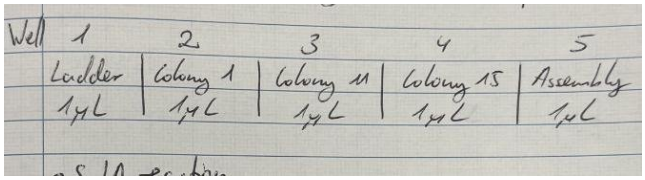
Hector, Mateo, Vaiva

Objectives

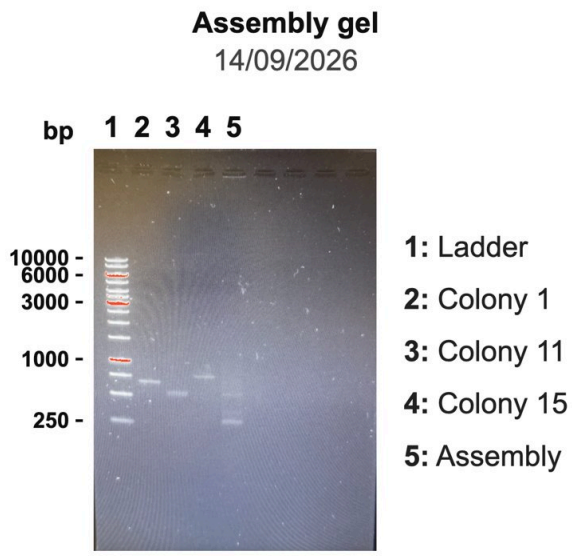
- ~~Run DNA gel for assembly~~
- ~~Setup sortase reaction triplicates~~
- ~~AlexaFluor labelling of seed and poisoned cap~~
- ~~Neg stain weekend fibrils~~
- ~~Make 1mM ThT stocks~~
- ~~Setup initial ThT anisotropy plate~~
- ~~Run PCR & transform new seed assembly~~

Agarose gel for assembly from 11/09/26 - Friday

IMG_3778.jpg



assemblyGel.png



eSortA reaction mixture

eSrtA reactions were repeated following the 08/09/26 - Tuesday protocol with the following volumes added.

Table2

	A	eSort-5M +Ca2+	eSort-5M - Ca2+	eSort-7M +Ca2+	eSort-7M - Ca2+
1	eGFP (TEV)	3 µL	3 µL	3 µL	3 µL
2	HET-s (100 µM)	87.5 µL	87.5 µL	87.5 µL	87.5 µL
3	5 M (160 µM)	40 µL	40 µL	-	-
4	7 M (160 µM)	-	-	40 µL	40 µL
5	10X Ca2+ dep.	60 µL	-	60 µL	-
6	10X Ca2+ indep.	-	60 µL	-	60 µL
7	Total	600 µL	600 µL	600 µL	600 µL

Negative controls

- w/o eGFP (TEV)
- w/o eGFP
- w/o HET-s
- w/o SortA

AlexaFluor-647 Labelling in PBS

1. Resuspend in PBS w. AF647 and dilute to 100uM
2. Wrap in aluminum foil and leave shaking at 37C for 3 hours
3. Make up to 2.5mL and BE into 30% Acetonitrile
4. Dilute 2x with 100% Acetonitrile and incubate at RT for 10 mins
5. Snap freeze & lyophilse

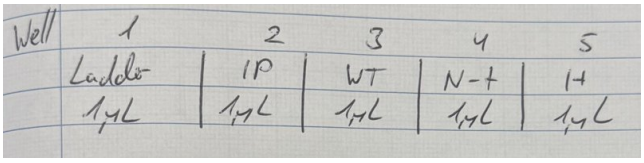
PCR

PCR for IP, N-t, WT and H were performed as on 02/09/26 - Wednesday and 03/09/26 - Thursday for inverse PCR (IP), but with different T_m temperatures:

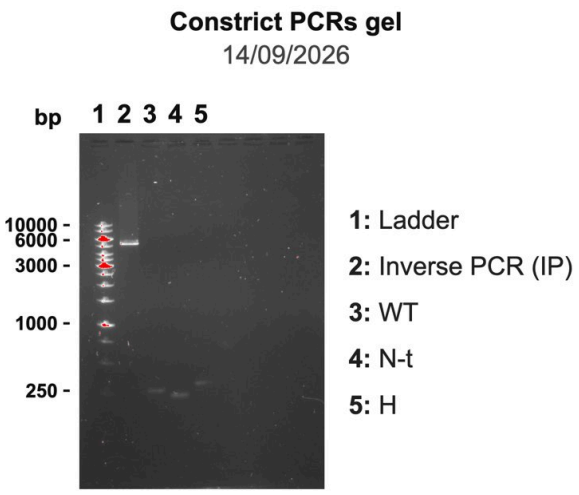
- Inverse PCR: T_m = 56 °C
- N-tail: T_m = 67 °C
- WT and H: T_m = 59 °C

Agarose gel for construct PCRs

IMG_3783.jpg



constructPCRgel.png



15/09/26 - Tuesday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 15 Sep 2026 13:08:00 UTC
Entry Last Modified: 15 Sep 2026 17:38:37 UTC
Export Generated On: 11 Oct 2026 18:47:23 UTC

TUESDAY, 15/09/2026

Members

Hector, Mateo, Vaiva

Objectives

- PCR clean-up
- Assembly
- Prepare LB + Kan plates
- eSortA experiment
- Agarose gel for the assembly
- Troubleshoot ThT platereader
- Collect AF647 Seed+PC from platereader

PCR clean-up

PCR clean-up for constructs from 14/09/26 - Monday was performed as on 11/09/26 - Friday using QIAquick kit, and treated with DpnI afterwards as on 11/09/26 - Friday.

Biospectrometer readings (blanked with elution buffer)

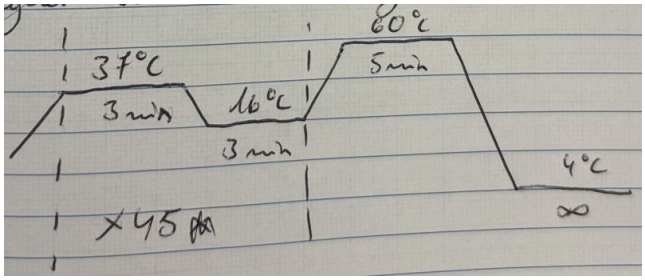
Table1

	Sample name	Concentration (µg/mL)	A260/1mm	A260/A280	A260/A230
1	Inverted PCR (IP)	127.6	0.281	1.84	2.06
2	N-Tail-Cys (N-t)	74.3	0.157	1.87	1.74
3	WT	71.2	0.163	1.86	1.84
4	His-tagged (H)	64.6	0.159	1.87	2.08

Assembly

Reaction mixture was set-up by adding: 2.5 µL T4 DNA ligase buffer (10X), 2.5 µL T4 DNA ligase, 1.5 µL BsaI-HFv2, 2.9 µL IP, 0.3 µL N-t, 4.8 µL WT, 0.4 µL H and made up to 25 µL with Milli-Q. Thermalcycler set to the following conditions:

IMG_3794.jpg



LB + Kan plates

LB + Kan plates were prepared as on 22/07/2026 - Wednesday.

eSortA experiment

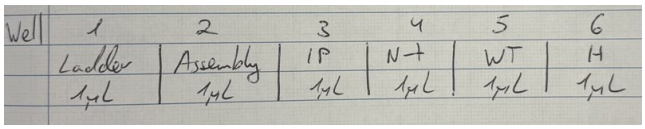
200 µL aliquots spun down at 4 °C for 10 min. Seperated supernatant from the pellet. Pellet resuspended in 60 µL of Growth B-F.

Plated 50 µL:

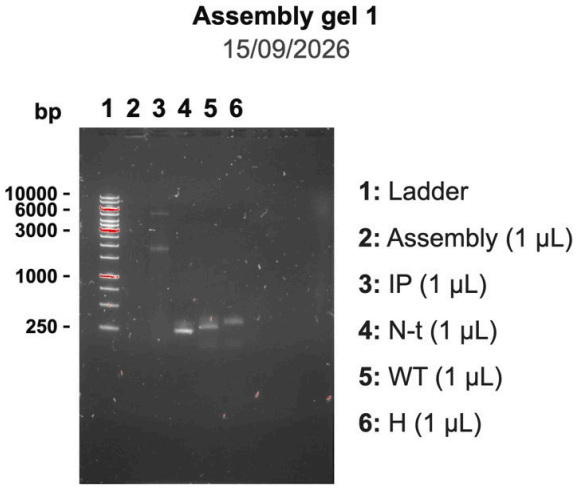
- Lane G: Pellets (in triplicates)
- Lane H: Supernatants (in triplicates)

Agarose gel for the assembly

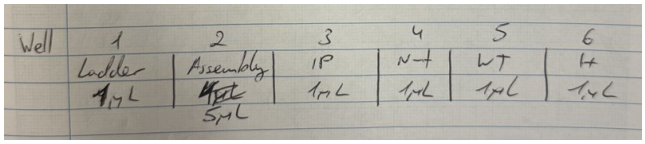
IMG_3795.jpg



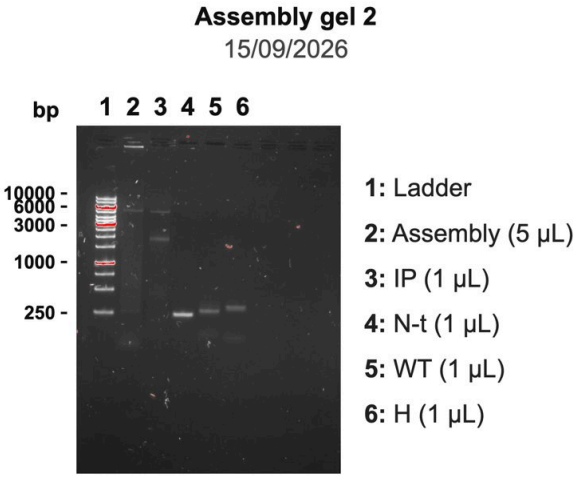
AssemblyGe1.png



IMG_3796.jpg



gel-annotation.png



16/09/26 - Wednesday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 15 Sep 2026 18:38:43 UTC
Entry Last Modified: 18 Sep 2026 10:07:16 UTC
Export Generated On: 11 Oct 2026 18:47:23 UTC

TUESDAY, 15/09/2026

Members

Hector, Mateo, Vaiva, Katie

Objectives

- ~~Transform gibson mix~~
- AlexaFluor FP assay
- ~~Setup TIRF samples~~
- ~~AF-488 V1/V2 fibrils~~
- ~~EM: V1-DTT, V2-DTT, PC-DTT, Seed-DTT~~

Transformation

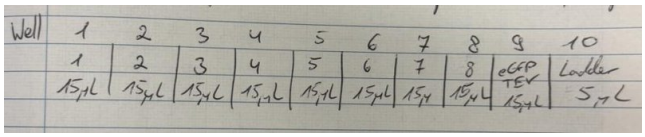
Transformation was performed as on 09/09/26 - Wednesday, using an assembly mixture from 15/09/26 - Tuesday. Plated 50 µL and 200 µL on LB + Kan plates and incubated overnight at 37 °C.

SortA stats

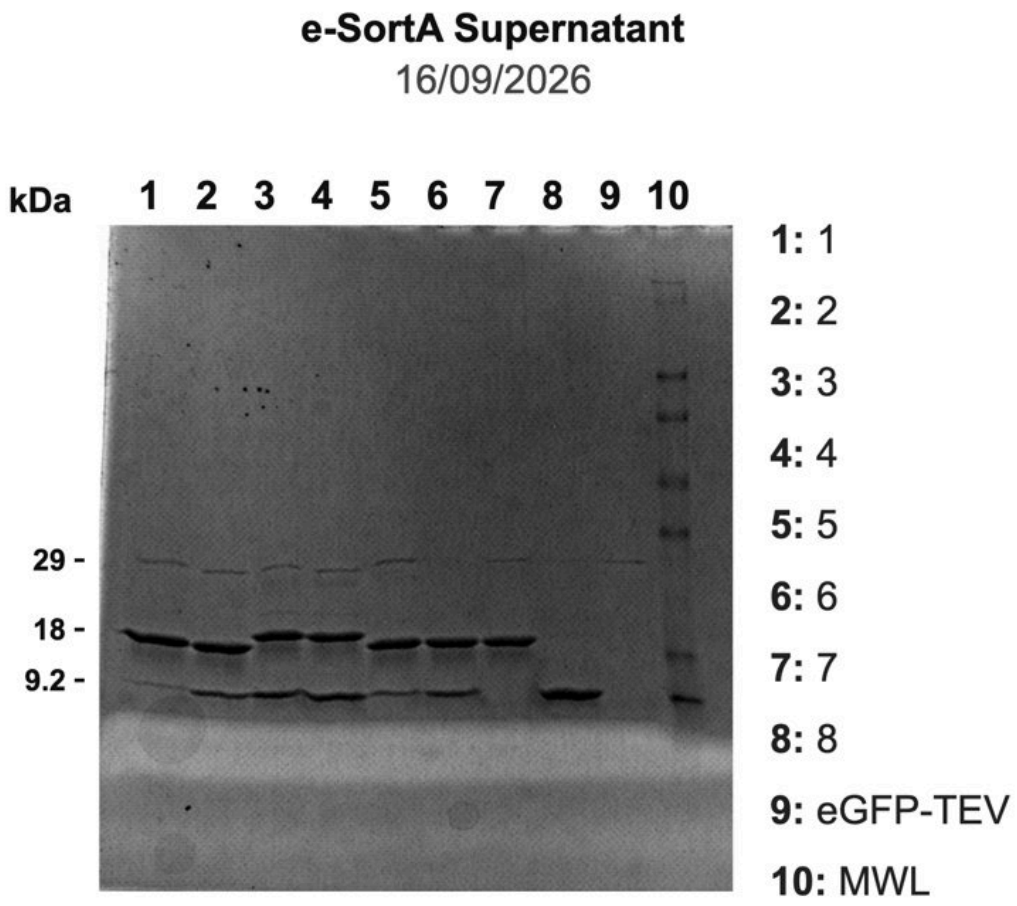
Dilution series of supernadant measure with plate reader 150uL. Linear relationship proved. Labeling efficiency 0.3% for both 7M and 5M with Calcium.

SDS gel for supernatants from eSortA experiment from 15/09/26 - Tuesday

IMG_3803.jpg



e-SortA_supernatant_2026-09-16.png



EM samples

taken EM samples of V1, V2, Seed and PC all in DTT

TIRF sample setup

Table1

	A	B	C	D	E	F	G	H	I
1	Solution	A280	A495	A650	Label ε (M-1cm-1)	Protein ε (M-1cm-1)	Protein conc (uM)	Label conc (uM)	Label %
2	N-tail (AF488)	1.884	0.372	-	73000	6990	269.5278969957	5.095890411	1.8906727162
3	Cap (AF647)	2.433	-	2.814	270000	6990	348.0686695279	10.4222222222	2.9943005891
4	Seed (AF647)	1.570	-	18.12	270000	27960	56.1516452074	67.1111111111	119.5176220807
5	Poisoned cap (AF647)	2.52	-	42.64	270000	6990	360.5150214592	157.9259259259	43.805643739

For AF for TIRF we want an effective concentration between 10pM to 10nM with the following reaction conditions spiked in alongside their unlabelled equivalent. We also AF488 label preformed V1 & V2 fibrils overnight and wash with PBS to remove excess AF488 before TIRF imaging tomorrow.

Table2

	Base	AF488	AF647
1			
2	WT	WT N-tail	Cap
3	WT	WT N-tail	Poisoned cap
4	WT	WT N-tail	Seed
5	V1 fibrils	Label ON	
6	V2 fibrils	Label ON	

Following measurements we made the seed and poisoned cap up to 2.5mL GuHCl lysis buffer and concentrated at 4200xg

Table3

	Sample	A280	A650	Protein ε (M-1cm-1)	Label ε (M-1cm-1)	Protein concentration (uM)	Label concentration (uM)	Labelling percentage
1	Concentrated Seed	0.109	0.098	27960	270000	3.8984263233	0.362962963	9.3104994903
2	Seed FT1	0.021	0.372	27960	270000	0.7510729614	1.3777777778	
3	Seed FT2	-0.054	0.035	27960	270000	0	0.1296296296	
4	Seed FT3	0.000	0.022	27960	270000	0	0.0814814815	
5	Concentrated PC	0.055	0.541	6990	270000	7.8683834049	2.0037037037	25.4652525253
6	PC FT1	-0.036	0.442	6990	270000	0	1.637037037	
7	PC FT2	-0.067	0.118	6990	270000	0	0.437037037	
8	PC FT3	-0.029	0.024	6990	270000	0	0.0888888889	

Fibrillation reaction conditions for 1000uL reaction volume to get an AF conc of 50nM

Table4

	Base	Added protein	Unlabelled base (50uM)	N-tail	Labelled protein (uL)	F	G
1	WT	Cap	980	10	10		
2	WT	Poisoned cap	950	10	40		
3	WT	Seed	890	10	100		
4							
5							

V1 and V2 fibrils in DTT where washed with 300uL PBS three times and resuspended with 500uL of PBS. 2uL of 5mM 488 AlexaFluor where added to each. Incubated at 25C overnight.

eSortA experiment

Set up the same experiment as 14/09/26 - Monday but using free monomeric HET-s to test if the labeling efficiency will increase. Left at 25ºC in the heatblock.

17/09/26 - Thursday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 17 Sep 2026 08:16:05 UTC
Entry Last Modified: 18 Sep 2026 10:07:07 UTC
Export Generated On: 11 Oct 2026 18:47:24 UTC

THURSDAY, 17/09/2026

Members

Hector, Mateo, Vaiva, Katie

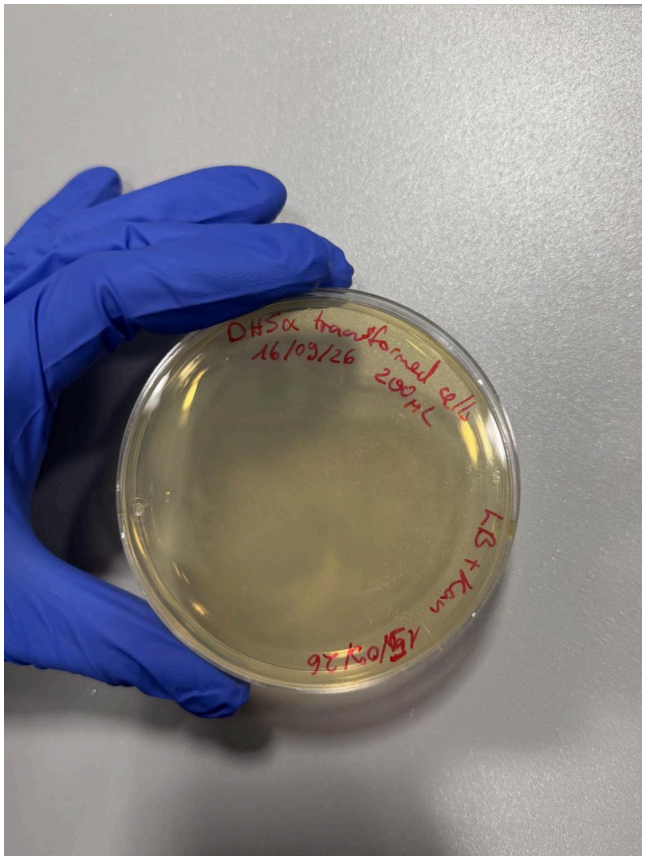
Objectives

- Run colony PCR (using 10/09/26 - Thursday colonies)
- Run DNA gel for colony PCR samples

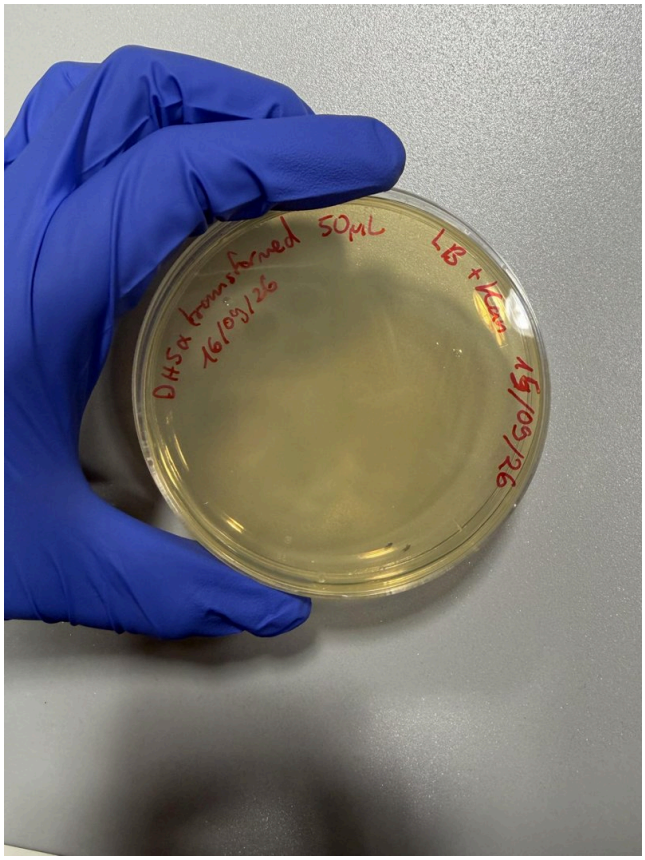
Cloning plate results

No colony was observed on both plates

IMG_1315.jpeg



IMG_1314.jpeg



Transformation

Transformation was performed as on 16/09/26 - Wednesday with the assembly from 15/09/26 - Tuesday. Control was prepared by transforming the cells with 1 µL of plasmid DNA from GenScript. 50 µL of cells were plated on LB + Kan plates and incubated at 37 °C. The unplated cells were incubated overnight at 37 °C, 250 rpm.

Photo 1: DH5-alpha cells before the 1hr incubation
(Left: Assembly transformation; Right: control)

Photo1.jpg

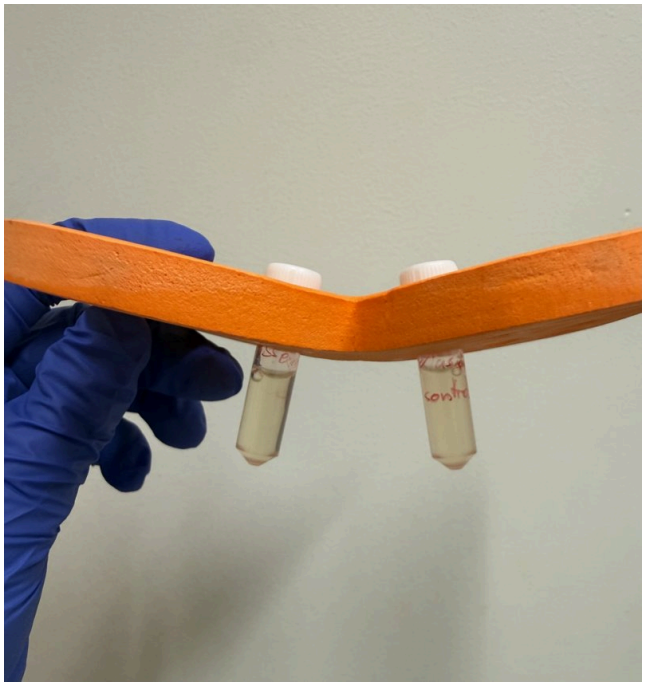


Photo 2: DH5-alpha cells after the 1hr incubation
(Left: Assembly transformation; Right: control)

IMG_3820.jpg

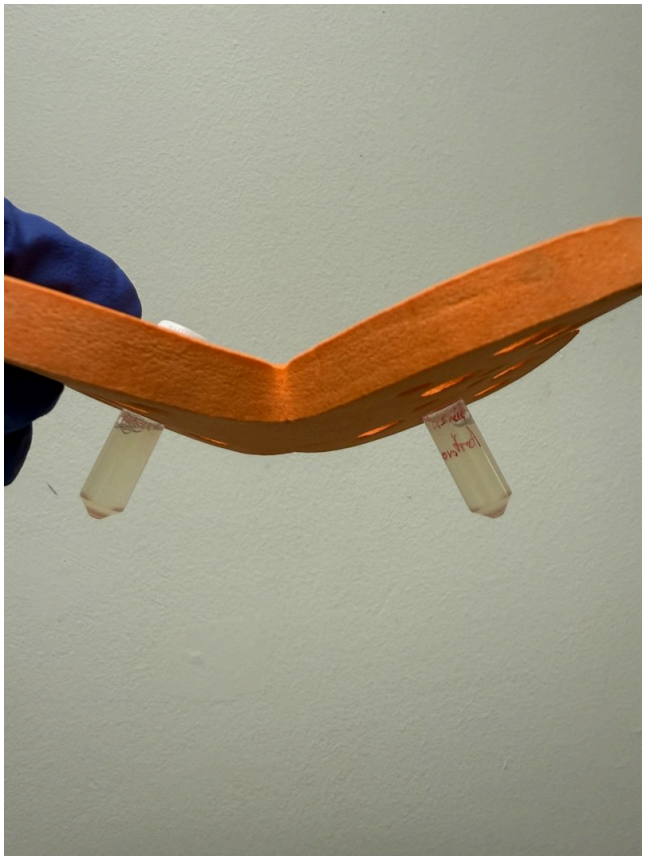
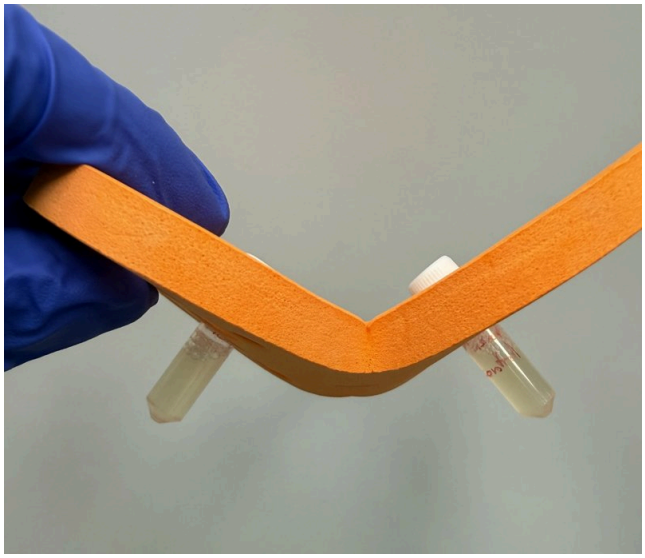


Photo 3: DH5-alpha cells after the 24h incubation
(Left: Assembly transformation; Right: control)

IMG_3829.jpg



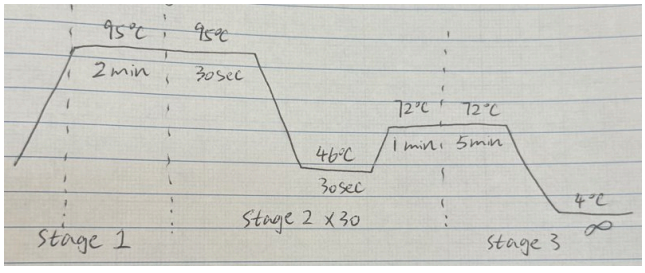
Colony PCR

Prepare a PCR master mix with a total volume of 1500 µL (48 colonies and 30 µL per reaction) with the following components:

- 750 µL DreamTaq PCR Master Mix (2X)
- 15 µL T7 forward primer
- 15 µL T7 reverse primer
- 720 µL Milli-Q

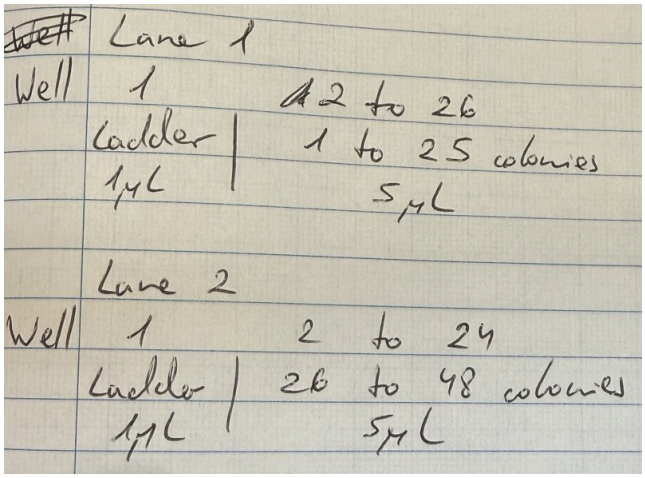
Pick 48 colonies (labelled red on the plates) from seed cloning (10/09/26 - Thursday) 50 µL and 200 µL plates, and add them to individual PCR tubes with 30 µL aliquot of the master mix. Spin the PCR tubes and run the thermal cycler:

IMG_3822.jpg



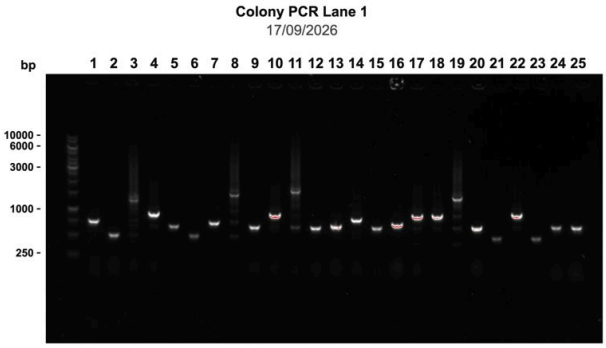
Agarose gel for colony PCRs

IMG_3823.jpg



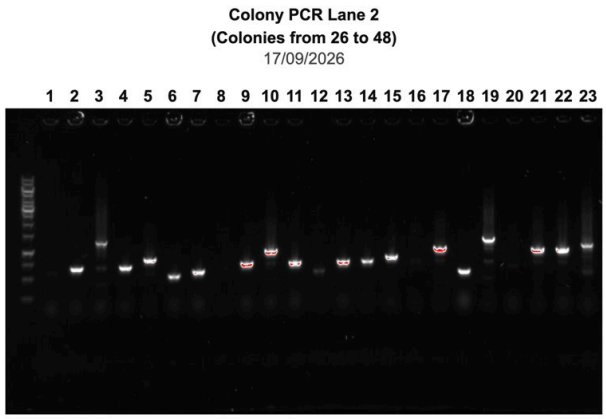
Lane 1 (Colonies 1 to 25)

Lane1.png



Lane 2 (Colonies 26 to 48)

Lane2.png



Colony PCR #2

Colonies (from 10/09/26 - Thursday, labelled blue): 4, 5, 7, 13, 16, 19, 20, 21, 27; and colonies (from 10/09/26 - Thursday, labelled red): 26, 33, 41, 45, were selected for another round of PCR following the protocol above with thermalcycler conditions set as above, but with 3 min of extention instead of 1 min.

V1 & V2 Fibril washes before TIRF to eliminate free Alexafluor

Samples were spun down at x17,000g for 20 minute cycles and washed repeatedly with growing buffer.

Table1

	Sample	Wash 1	Wash 2	Wash 3	Wash 4
1	V1-AF488	0.672	0.172	0.025	-0.012
2	V2-AF488	0.623	0.132	0.031	-0.067
3					

Trial of AF488 Anisotropy

Run Fluorescence polarisation on the VantaStar using 5uM AF488 to set the Gain/Focus against 10uM of N-tail labelled Het-s monomer

18/09/26 - Friday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 18 Sep 2026 09:47:04 UTC
Entry Last Modified: 04 Oct 2026 09:44:38 UTC
Export Generated On: 11 Oct 2026 18:47:23 UTC

FRIDAY, 18/09/2026

Members

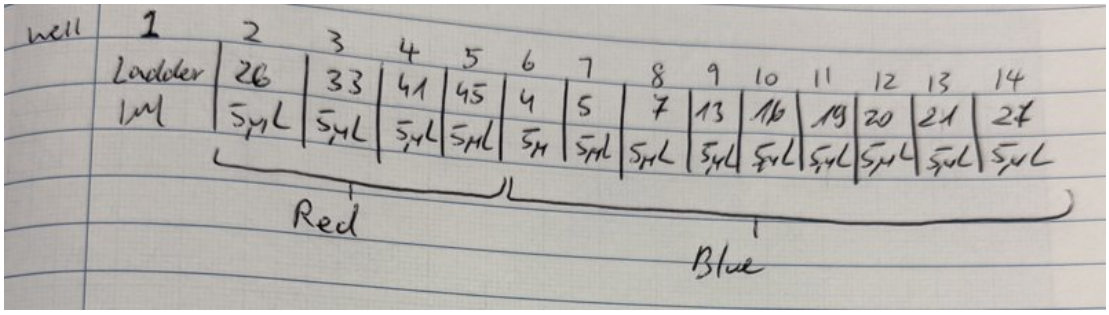
Hector, Mateo, Vaiva, Katie

Objectives

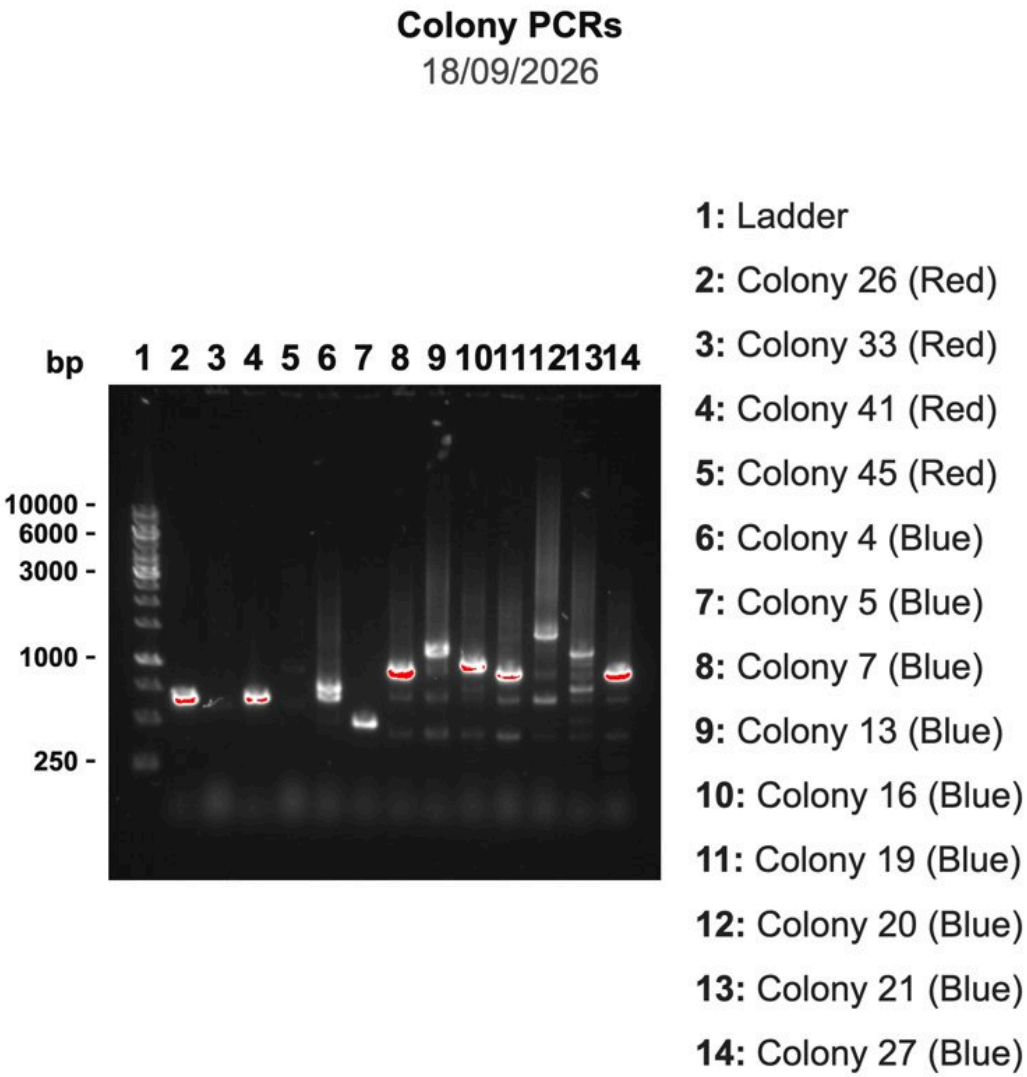
- Run DNA gel for Colony PCRs
- Do gel extraction
- Send samples for Full Circle seq.

Colony PCR gel from 17/09/26 - Thursday samples

Loading order



gel-annotation.png



Platereader results of AF488 ON

Partially observe the AF488 plateau as free AF488 is benchmarked as 35mP and the starting polarisation is ~60mP which rises to ~80mP. Measuring the endpoint labelled TIRF samples which are AF488 labelled V1 fibrils had polarisation ~180mP.

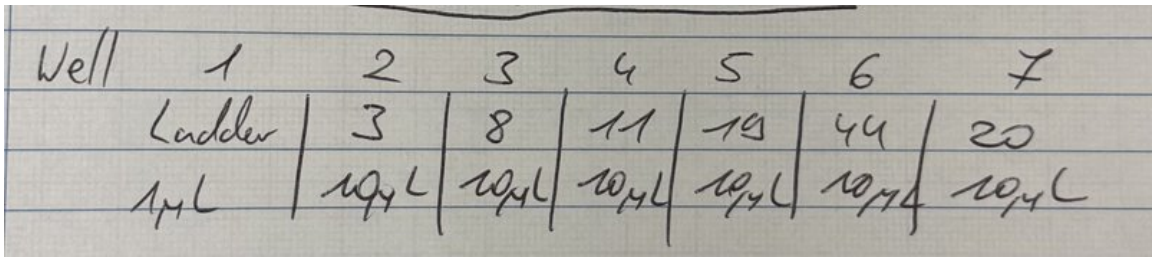
Labelling V1 in GuHCl at RT for 2 hours

1. Dissolve tube of V1 (Final conc 400uM in GuHCl lysis buffered to pH 7 with 1mM TCEP (stock from Liz) and 5uL 5mM AF488
2. Incubate at 25 degrees shaking 200RPM for 2 hours
3. Take time zero sample 25uL aliquot into ethanol & freeze at -80

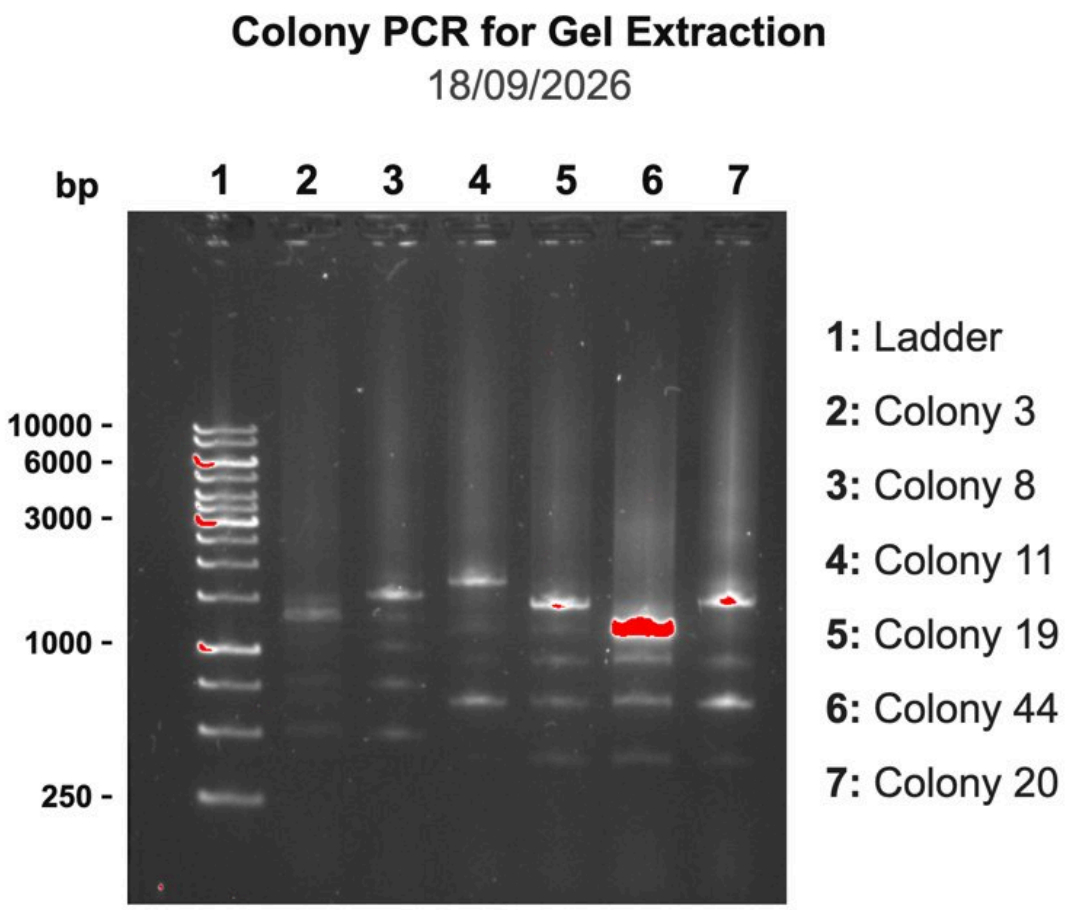
4. Take gel samples for 0hr, 1hr, 2hr endpoint for imaging gel with excitation+coomassie
5. Buffer exchange into Tris growing buffer should give 3.5mL in growing buffer
6. Pre-plate serial dilutions 1:2, 1:4, 1:8, 1:16
7. Run overnight alongside prelabelled fibril control

Agarose gel for the selected colonies

IMG_3835.jpg



Colony_PCR_for_Gel_Extraction_2026-09-18.png

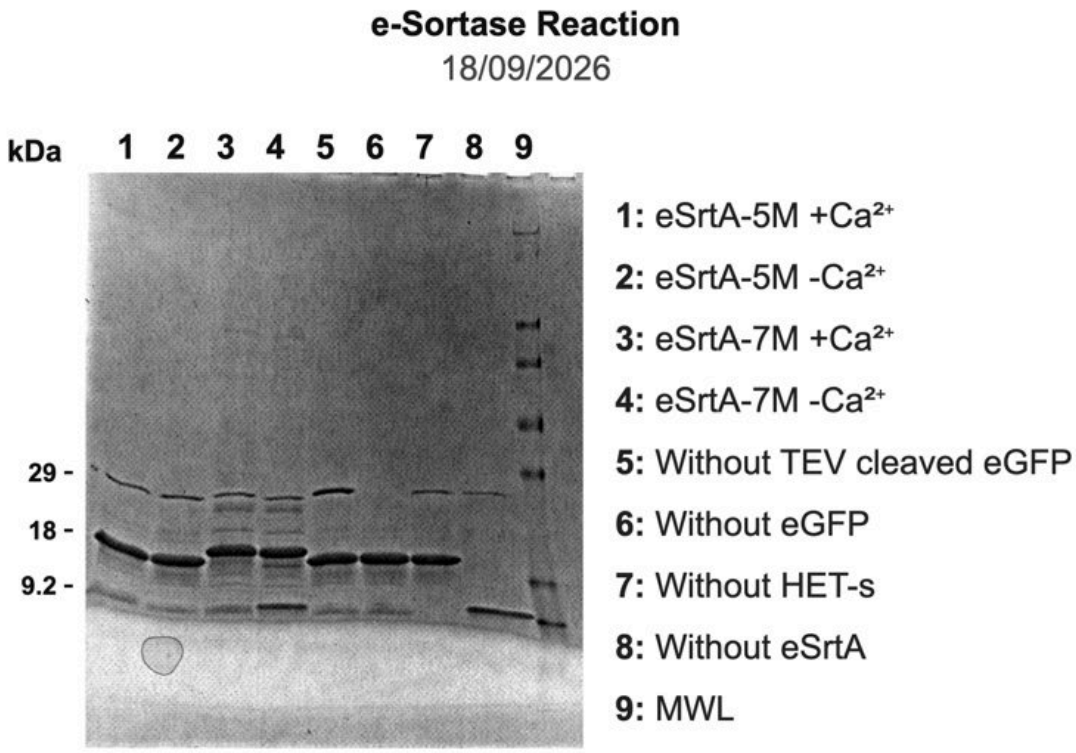


Gel extraction and Full Circle seq.

Gel extraction for colonies (labelled red): 3, 8, 11, 19, 44, and colonies (labelled blue): 20, was performed as on 11/09/26 - Friday. The colonies were then sent for Full Circle sequencing (Bag code: Z4TWCB; Order number: #34521): Tube 1 -> Colony 3; Tube 2 -> Colony 8; Tube 3 -> Colony 11; Tube 4 -> Colony 19; Tube 5 -> Colony 44; Tube 6 -> Colony 20.

e-Sortase reaction gel result

e-Sortase_Reaction_2026-09-18.png



Trial of V1-AF488 Fluorescence polarisation trial

Plate layout: stock was 40uM following BE

Table1

	A	B	C	D	E	F	G	H	I	J	K	L
1	Free AF488	Free AF488	Free AF488	Preformed V1	Preformed V1	Preformed V1	Undilute	Undilute	Undilute	1:2	1:2	1:2
2	1:5	1:5	1:5	1:10	1:10	1:10	1:20	1:20	1:20	Growing buffer	Growing buffer	Growing buffer
3												

21/09/26 - Monday

Project: iGEM 2026 Shared Project
Author: Vaiva Knabikaite
Status: In progress
Entry Created On: 21 Sep 2026 12:04:57 UTC
Entry Last Modified: 22 Sep 2026 17:32:32 UTC
Export Generated On: 11 Oct 2026 18:48:33 UTC

MONDAY, 21/09/2026

Members

Hector, Mateo, Vaiva, Katie

Objectives

- Do assembly
- Run agarose gel for the assemnbly
- Do transformation
- Setup EM fibrils concentrations screen
- Redo AF488 Anisotropy on V1 & V2
- Setup sortase free monomer 40uM screen

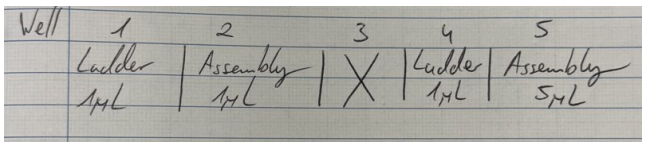
Assembly

Refer to 04/09/26 - Friday for assembly detail, volume of fragment and vector added are as follows:

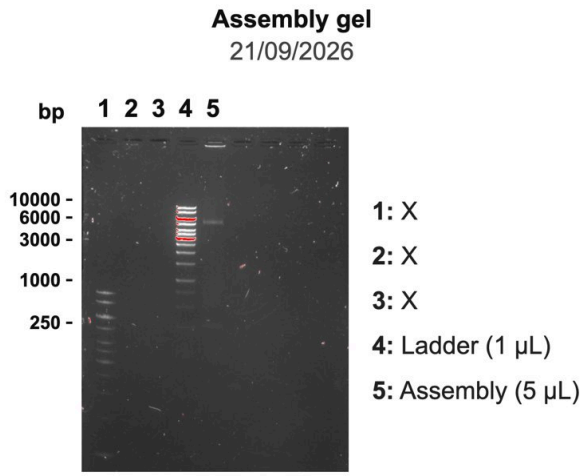
- Vector (IP): 2 µL
- N-t: 1 µL
- H: 1 µL
- WT: 6 µL

Agarose gel for the assembly

IMG_3864.jpg



gel-annotation.png



Transformation

NEB 5-alpha Competent *E. coli* cells were transformed as on 04/09/26 - Friday. 50, 200 and 750 µL were then plated on LB + Kan plates.

V1/V2-AF488 Platereader layout

Table1

	A	B	C	D	E	F	G	H	I	J	K	L
1	Free AF488	Free AF488	Preformed V1	Preformed V1	Preformed V2	Preformed V2	Undilute V1	Undilute V1	Undilute V1	1:1	1:1	1:1
2	1:2	1:2	1:2	1:5	1:5	1:5	1:10	1:10	1:10	Growing buffer	Growing buffer	Growing buffer
3	Undilute V2	Undilute V2	Undilute V2	1:1	1:1	1:1	1:2	1:2	1:2	1:5	1:5	1:5
4	1:10	1:10	1:10									

22/09/26 - Tuesday

Project: iGEM 2026 Shared Project
Author: Hector Cross
Status: In progress
Entry Created On: 22 Sep 2026 09:15:50 UTC
Entry Last Modified: 23 Sep 2026 15:17:13 UTC
Export Generated On: 11 Oct 2026 18:48:33 UTC

TUESDAY, 22/09/2026

Members

Hector, Mateo, Vaiva, Katie

Objectives

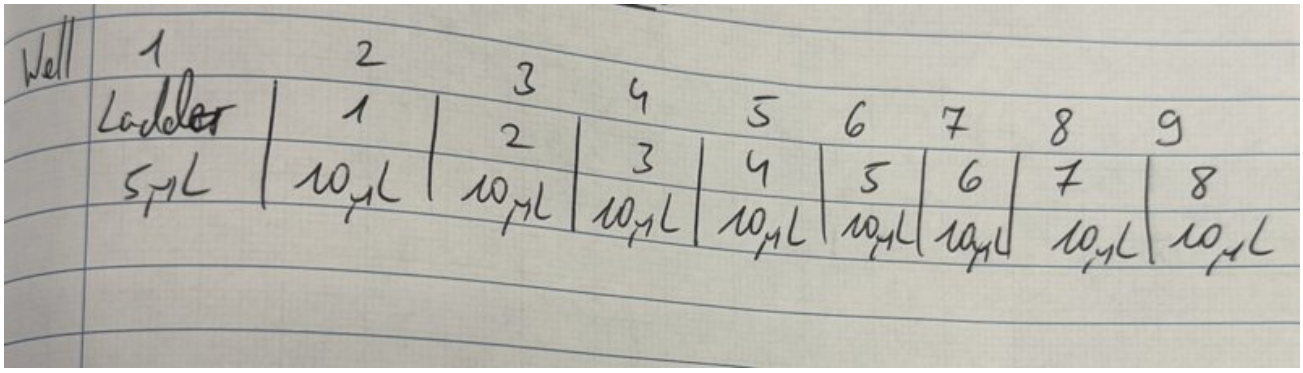
- ~~Spin down & measure sortase on the microplate reader~~
- ~~EM: Dilution series (5 samples)~~
- ~~EM: Pellet + supernatant (just 100uM, 50, 20 & 10uM)~~
- EM: Platereader
- Setup combos for EM
- Make TB for CotA expression test
- ~~Make fresh Kan plates~~
- ~~Plate assembly transformation~~
- Organise platereader recording samples

Transformation

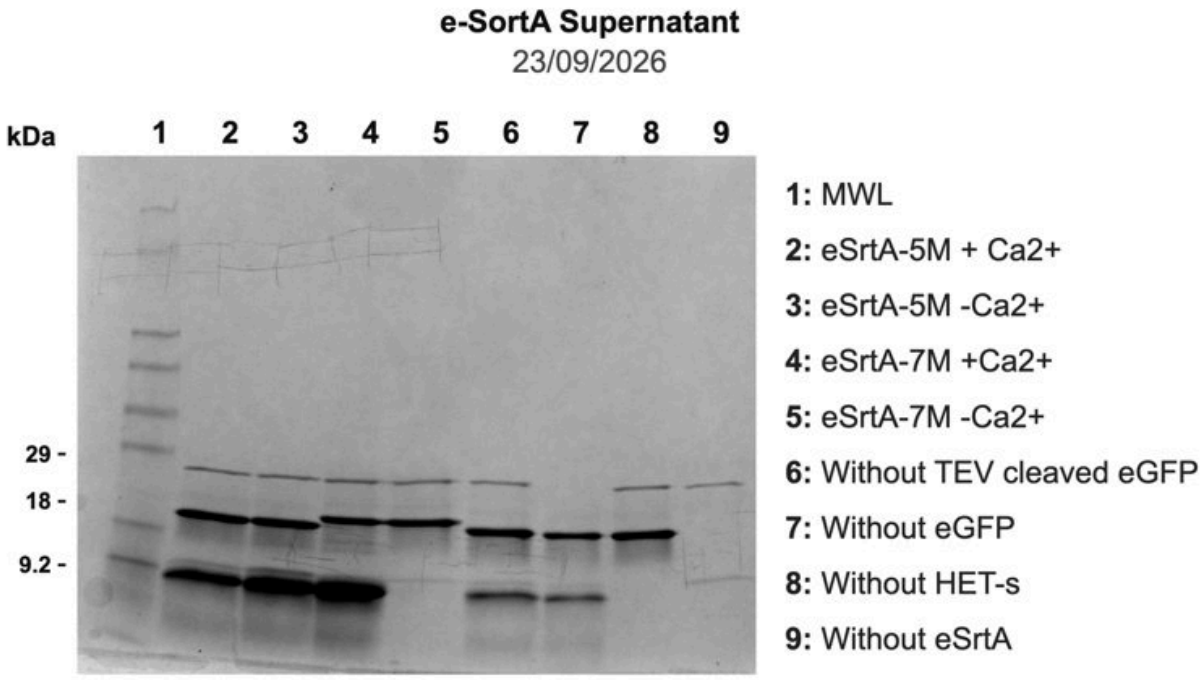
NEB 10-beta Competent *E. coli* cells were transformed as on 09/09/26 - Wednesday using the assembly from 21/09/26 - Monday.

SDS gel for supernatants

IMG_3879.jpg



e-SortA_Supernatant_2026-09-22.png



23/09/26 - Wednesday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 22 Sep 2026 09:28:04 UTC
Entry Last Modified: 23 Sep 2026 15:39:25 UTC
Export Generated On: 11 Oct 2026 18:48:33 UTC

TUESDAY, 22/09/2026

Members

Hector, Mateo, Vaiva, Katie

Objectives

- EM: Platereader
- Setup combos for EM
- Make TB for CotA expression test
- Organise platereader recording samples
- GuHCl label V1 and platereader dilute out plus spin down

CotA expression

20mL of culture were grown in 50mL falcon tubes to desiered ODs, shaking 140rpm. Add IPTG and CuSO4. Then for cultures 1, 2 and 4; 12mL of culture where transefered to an semi-closed 15mL tube and left staticaly overnight at the desireded temperatures.

Table1

	A	B	C	D	E	F	G	H	I
1	**Tube**	**Paper**	**Medium**	**Pre-induction growth**	**Cu salt & conc.**	**IPTG**	**Post-switch temp**	**Microaerobic**	**Aeration profile**
2	1	Durão et al. 2008 Brander, Mikkelsen & Kepp 2014	LB	30°C to OD_{600} 0.6	$CuCl_2$, 0.25 mM sub with CSO4	0.1 mM	25°C	yes	4 h shaking → 20 h static
3	2	Mohammadian et al. 2010	LB	37°C to OD_{600} 0.6	$CuSO_4$, 2.0 mM	0.1 mM	18°C	yes	4 h shaking (120 rpm) → static 20 h
4	3	Koschorreck, Schmid & Urlacher 2009	TB	37°C to OD_{578} 1.8	$CuSO_4$, 2.0 mM	0.25 mM	18°C	no	Shaking (140 rpm) overnight
5	4	Koschorreck, Schmid & Urlacher 2009 in microaerobic	TB	37°C to OD_{578} 1.8	$CuSO_4$, 2.0 mM	0.25 mM	18°C	yes	4 h shaking (140 rpm) → 20 h static

Negative stain EM

Imaged 5uM, 10uM, 20uM, 50uM and 100uM of WT HET-s.

Imaged supernadand and pellet of 10uM and 100uM. Spun down for 5 min and pellet resuspended in 600uL of growth buffer, vortex to mix.

Colony PCR

Prepare a master mix for 20 samples (20 uL per sample) of a total volume of 400 uL with:

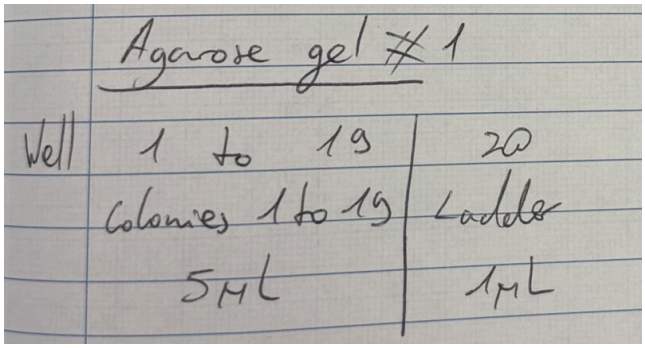
- 200 uL DreaTaq PCR master mix (2X)
- 4 uL Fwrd. Primer
- 4 uL Rev. Primer
- 192 uL Milli-Q

Colonies form 22/09/26 - Tuesday were selected from 50 uL Petri dish. Thermal cycler set as on 17/09/26 - Thursday.

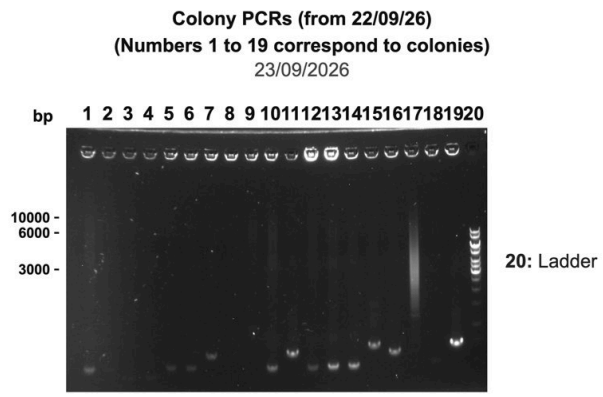
Agarose gel for the colony PCR

First gel was run at 135 V

IMG_3880.jpg

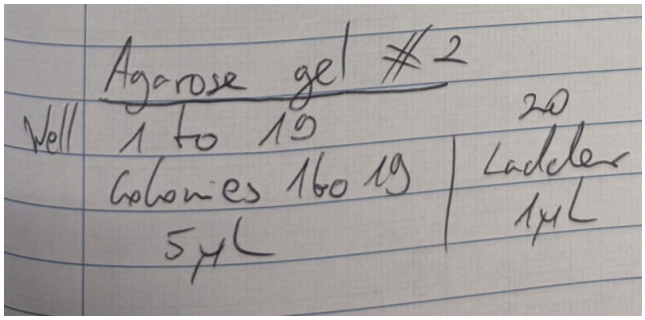


gel-annotation.png

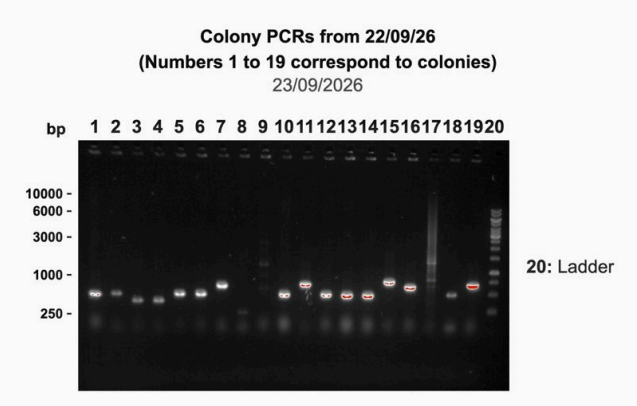


The same gel was repeated again (run at 100 V)

IMG_3880_2.jpg



49E21BF6-F972-454A-9F9C-B7A388BE63FA_1_201_a.jpeg



24/09/26 - Thursday

Project: iGEM 2026 Shared Project
Author: Katie Wei
Status: In progress
Entry Created On: 24 Sep 2026 10:42:22 UTC
Entry Last Modified: 24 Sep 2026 10:52:41 UTC
Export Generated On: 11 Oct 2026 18:48:33 UTC

THURSDAY, 24/09/2026

Members

Hector, Mateo, Vaiva, Katie

Objectives

- CotA expression test

CotA expression test

- Four 20 ml cultures of LB and TB was prepared and different amount of overnight bacterial preculture from 23/09/26 - Wednesday was added to each culture to ensure that the starting OD600 is 0.05
- The OD600 absorbance reading of the precultures were as follow:

Table1

	Tube Number	OD600 (1:10)	OD600
1	1	0.283	2.83
2	2	0.177	1.77
3	3	0.203	2.03
4	4	0.188	1.88

- Cultures were incubated at 30 °C and 37 °C for 3 hours and the OD600 absorbance was measured

Monday

Project: iGEM 2026 Shared Project
Author: Mateo Martin-Nieto
Status: In progress
Entry Created On: 28 Sep 2026 12:04:20 UTC
Entry Last Modified: 28 Sep 2026 14:20:05 UTC
Export Generated On: 11 Oct 2026 18:49:21 UTC

MONDAY, 28/09/2026

Members

Mateo, Vaiva, Katie

Objectives

- Set up SortA reactions DTT vs no DTT
- Make stock of Biotin-PEG-Maleimide (BPM) and alequote
- Label fibrils with BPM
- Label V1 monomer with BPM and run on a gel to see labeling

SortA DTT vs no DTT

DC and V1 stock liophilized protein where resuspended in growth buffer. Spun down for 5 min at 4ºC and supernatant was collected and measured A280.

The following reactions where set up:

Table1

	Component	V1 +DTT	V1 -DTT	DC +DTT	DC -DTT
1	eGFP, TEV-cleaved (67 µM)	12 µL	12 µL	12 µL	12 µL
2	V1 (127 µM)	315 µL	315 µL	-	-
3	Double cys (200 µM)	-	-	200 µL	200 µL
4	eSortA-5M (150 µM)	60 µL	60 µL	60 µL	60 µL
5	DTT (1 M)	6 µL	-	6 µL	-
6	10x Ca-dependent mmixture	60 µL	60 µL	60 µL	60 µL
7	Growth Buffer	147 µL	153 µL	262 µL	268 µL
8	**Total**	600 µL	600 µL	600 µL	600 µL

Reactions where incubated at 25ºC shaking at 300 rpm.

Biotin-PEG-Maleimide (BPM) stock

Dissolved the 100mg of BPM in 5 mL of DMSO to make a 10 mM stock. Allequoted the stock into 250 µL eppendorfs and stored at -80ºC.

Label fibrils with BPM

Fibrils where spun down for 10 min at max speed and resuspended in PBS. TCEP was added to a final concentration of 1mM and 6 µL of 10 mM BPM was added to have a 600 excess with the amount of protein. Incubated at 25ºC shaking for 2.5hrs.

Label V1 monomer with BPM

Took 200 µL from a stock of V1 at 127 µM in growth buffer. TCEP was added to a final concentration of 1mM and 2 µL of 10 mM BPM was added to have a ~600 excess with the amount of protein.

Run gel

Added 20% BME to the 4x loading buffer. Mixed 15 µL of the labelled and unlabelled V1 monomer with 5 µL of loading buffer. Run on 12% gel at 150V until dye reached the bottom. Stained using coomasie for 10 min and destained overnight.