Week 30 - Overview

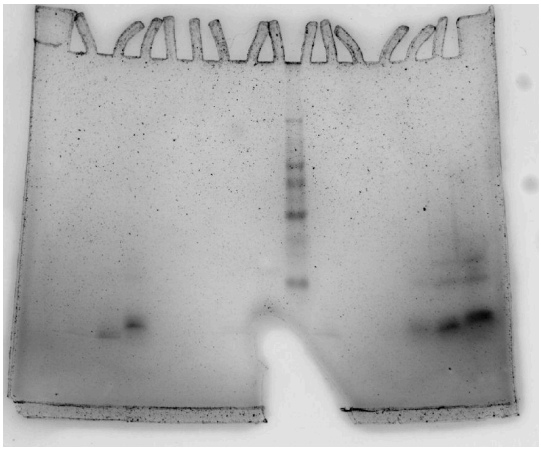
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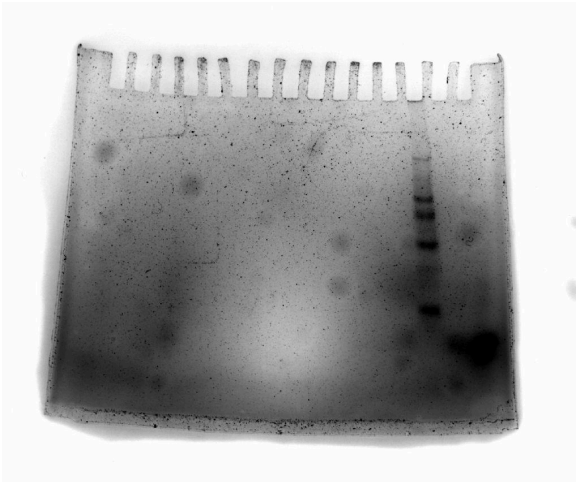
Weekly summary:

During Week 30, we continued attempting to transform *E. coli* TG1 with our remaining gene constructs, successfully transforming AKR1D1-His into *E. coli* TG1. Protein expression of ci2350-His and ci2350-MBP was carried out at 17ºC to maximise the yield, followed by protein purification using an IMAC column. Glycerol stocks of *E. coli* TG1 and BL21(DE3) carrying ci2350-His and ci2350-MBP were made for storage. We also began digesting pET-BLANK containing ci2350-MBP, to obtain the empty vector, since it is more reliable than digesting pET-11a.

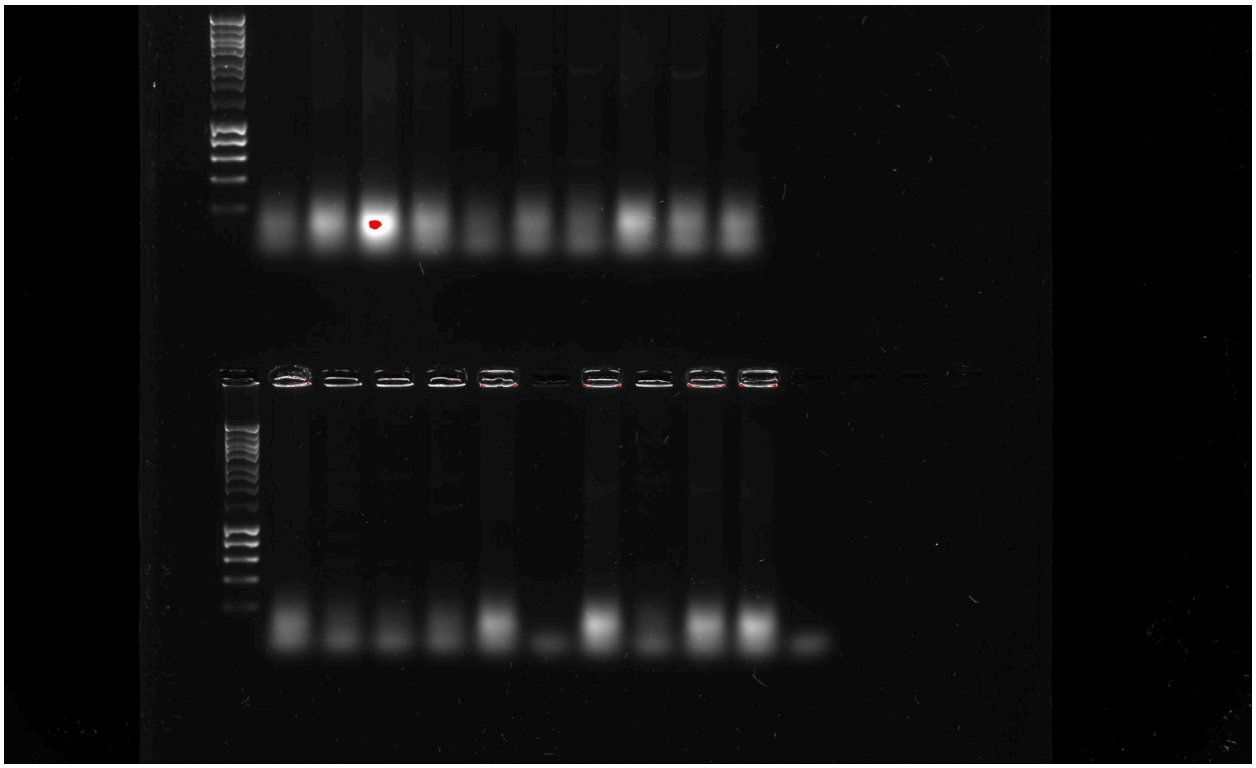
Main results of the week:

SDS-PAGE analysis of ci2350-His and ci2350-MBP after protein purification was performed. The gels had been left in the fridge in water over the weekend, as we did not have time to stain them on Friday. After staining and visualizing the gels, we noticed they had a few bands, with most having diffused out of the gel. Due to this storage error, no bands were present in samples for ci2350-His, and only a few visible for ci2350-MBP, with visible band swelling and abnormal migration. Purification was repeated using aliquots of samples stored in the -80ºC freezer.

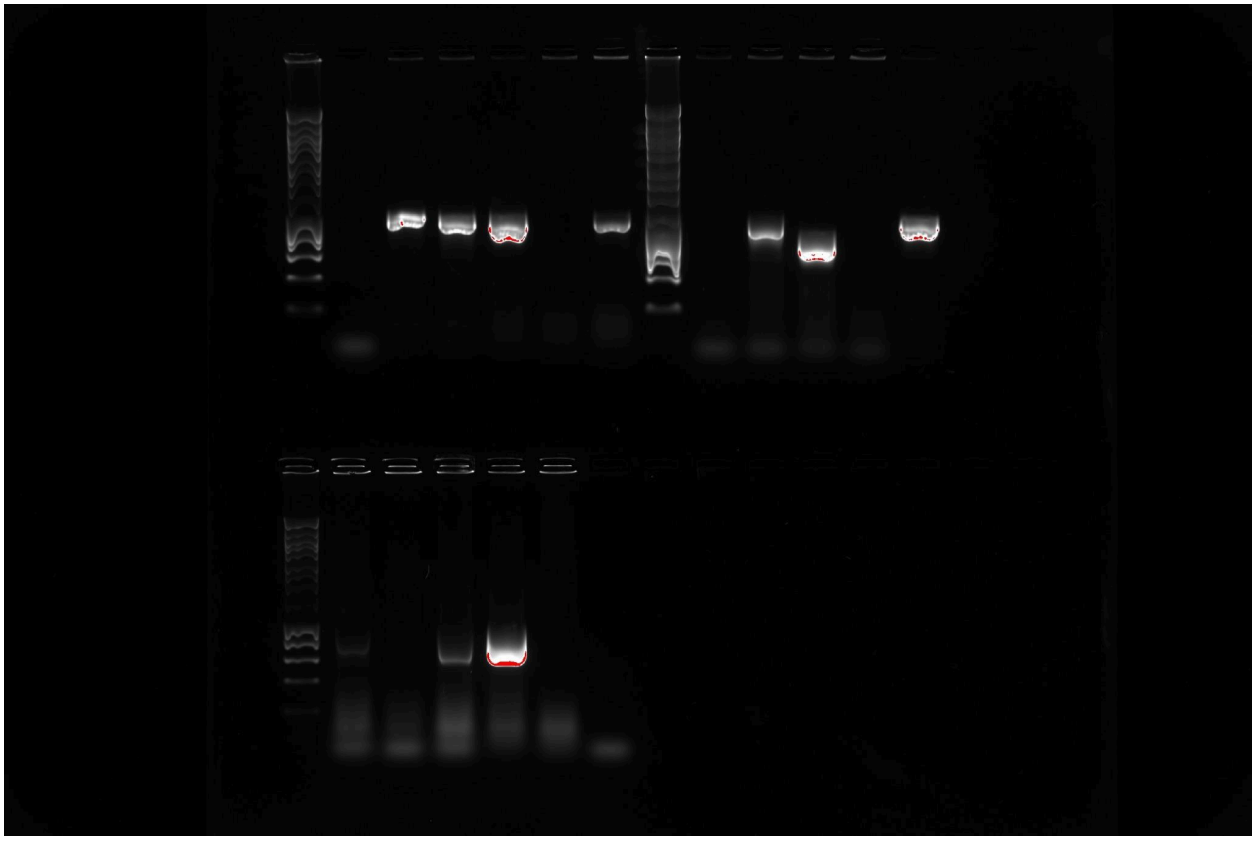




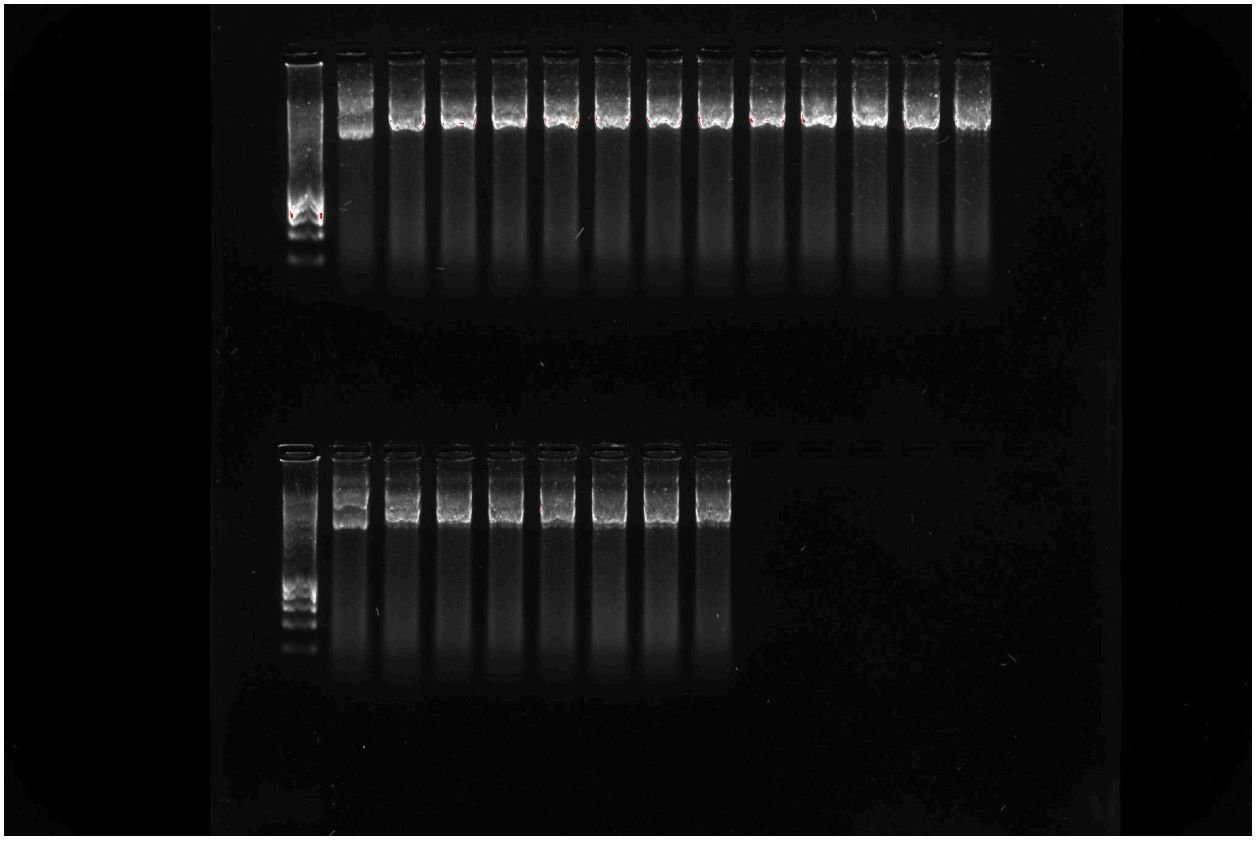
Due to the unusually high numbers of colonies on all plates, including the negative ligation control, indicating religated vectors, we decided to test only 4 different plates, containing the AKR1D1-His and AKR1D1-SUMO inserts. Agarose gel electrophoresis of colony PCR products from the transformed colonies showed that the colonies had not been transformed with our inserts, but with religated pET-11a.



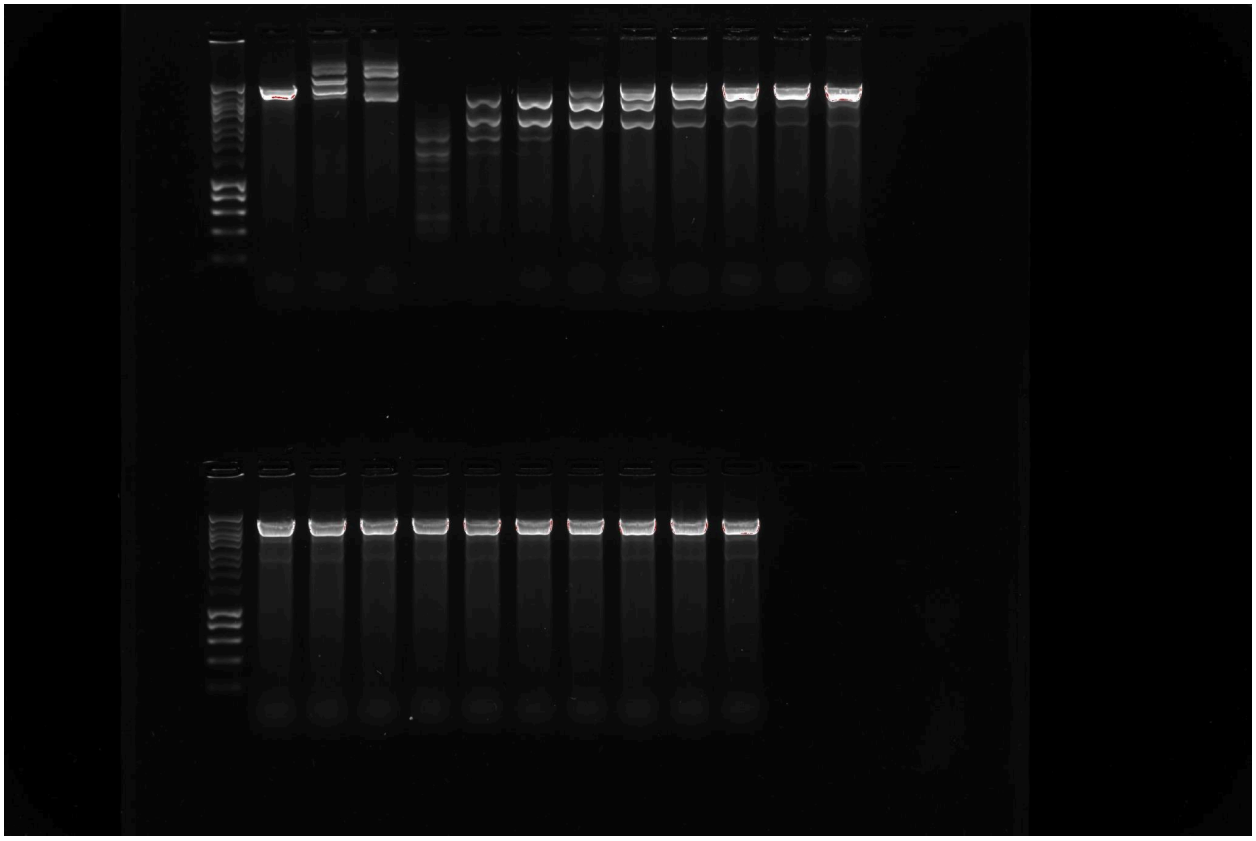
To avoid restarting the whole transformation experiment from start (including plasmid miniprep and related steps), we decided to redo the digestion of already digested pET-11a, by incubating it again with NdeI, followed by another ligation reaction using only AKR1D1-His insert, to avoid wasting other inserts. Another round of transformation into *E. coli* TG1 was performed using this construct. Colony PCR showed that some colonies were positive, and sequencing identified a correct AKR1D1-His sequence.



Following our supervisor Camille's suggestion, we decided to digest pET-11a with NdeI only, allowing us to distinguish between single-digested and non-digested plasmid and extract it from the gel more easily. BamHI digestion, which is more reliable, would then follow separately. After visualizing the NdeI digestion products, the gel appeared very grainy, probably due to the agarose not fully dissolving in the microwave.

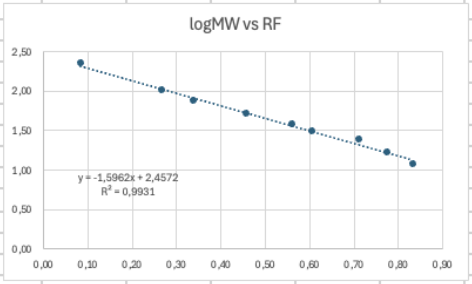


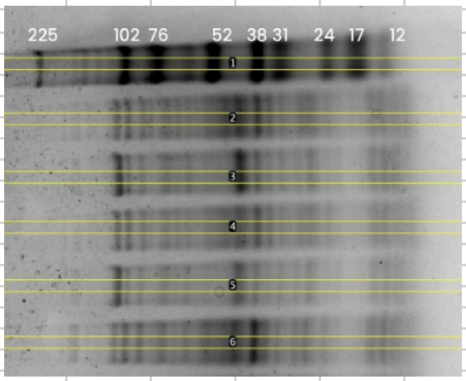
To follow up on last week's idea, we decided to digest pET-BLANK containing ci2350-MBP with NdeI and BamHI, to distinguish between single and double-digested plasmid, and extract only the double digested band from the gel, preventing religation of single-cut plasmid and false-positive transformations. Agarose gel electrophoresis of the pET-BLANK digestion gave some positive results, with a few bands being good candidates for gel extraction. However, the gel also showed a trend in which digestion strength decreased steadily across the well, from heavily digested plasmid to undigested plasmid. The most likely explanation is that restriction digestion mastermix was not properly mixed before aliquoting, where the first aliquots taken contained the most enzyme, while later ones contained progressively less, until some aliquots had essentially no enzyme at all, explaining the undigested wells. Going forward, we will ensure the mastermix is fully mixed before aliquoting, to prevent this from happening again.



To confirm that we had expressed ci2350-MBP, we analysed the SDS-PAGE gel to determine the molecular weight of the overexpressed protein. The results showed a molecular weight of ~113 kDa, close to the expected ~112 kDa of ci2350-MBP, confirming we had successfully expressed our desired protein.

BAND	LABEL	X	Standard MW (kD)	Rf	logMW	MW of unknowns
1	Standard	54,00	225,00	0,08	2,35	
2	Standard	172,50	102,00	0,27	2,01	
3	Standard	218,50	76,00	0,34	1,88	
5	Standard	296,50	52,00	0,46	1,72	
6	Standard	362,50	38,00	0,56	1,58	
7	Standard	391,50	31,00	0,61	1,49	
8	Standard	459,50	24,00	0,71	1,38	
9	Standard	500,50	17,00	0,77	1,23	
10	Standard	538,50	12,00	0,83	1,08	
11	Lane2	161,50		0,25	2,06	114,34
12	Lane3	165,50		0,26	2,05	111,77
13	Lane4	162,50		0,25	2,06	113,69
14	Lane5	163,50		0,25	2,05	113,05
15	Lane6	157,50		0,24	2,07	116,98
16	DyeFront	646,1				





eq (y)						
m	-1,5962			ci2350+mp	110.89	
b	2,4572			b expected		
x	Rf					

Use the equation of the standard curve to calculate the log(MW) of the four bands using in the Rf value for each band as the X value in the equation (you will be solving for Y, the log MW). The inverse log [=10^(cell#)]of that number will be the molecular weight of that band.

Date	23/07/26

Day 25: [July 20, 2026], Monday

Weekly responsible: Patrik, Eleonora, Giulia
Lab participants: Hema, Hamsini, Cecilia, Rytis

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Checking of inserts amplification with agarose gel + restriction of inserts + PCR cleanup		PCR samples in freezer
Nanodrop concentration measurement and purity of gel extracted restriction of pET-11a		Samples in freezer
Visualization and analysis of SDS gel from Friday		
Ligation of restricted plasmid + inserts and transformation		TRANSFORMATION NOT DONE
Glycerol stock preparation of ci2350his and ci2350his-MBP BL21 cultures		
inoculation of transformed E.coli BL21 for starter culture		

Results and interpretation

Checking of inserts amplification with agarose gel

Gel performed using 10 µL of the PCR tubes for each reaction.

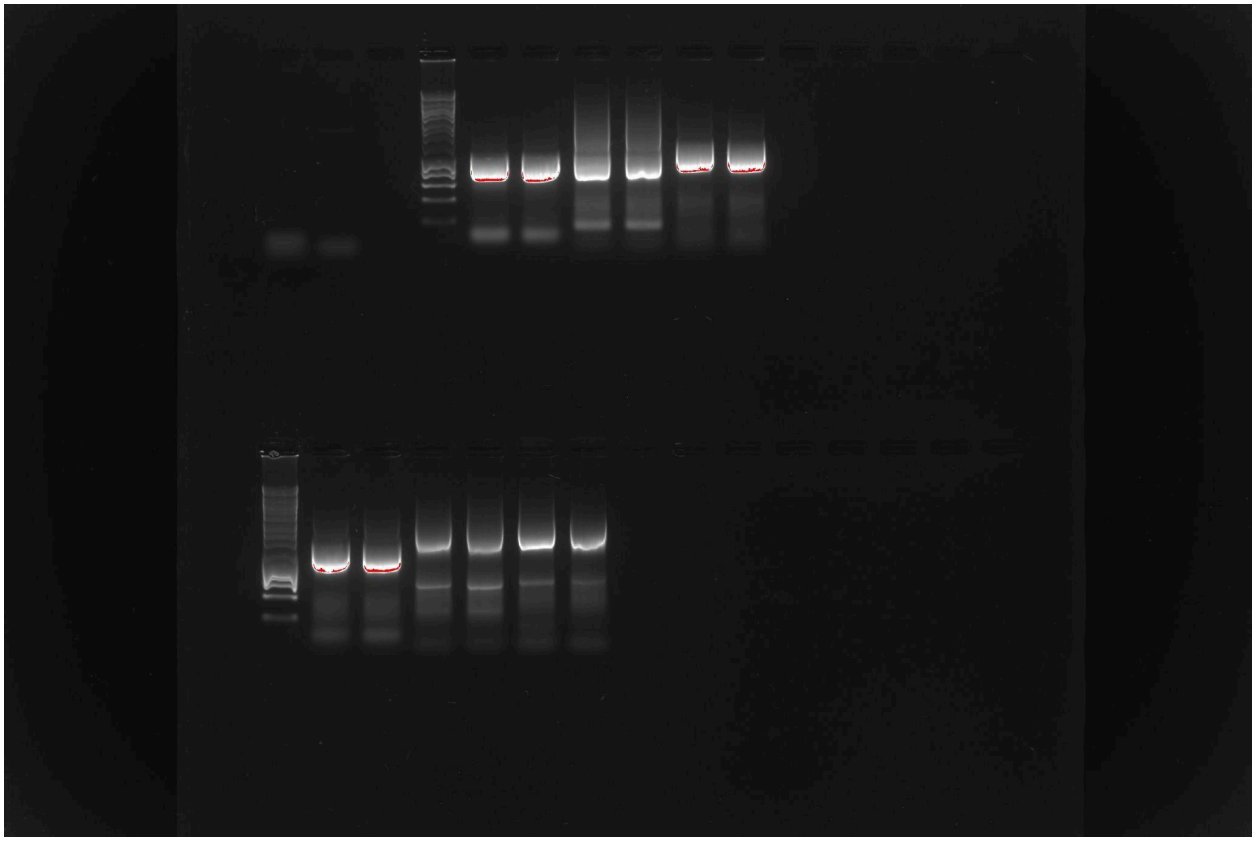
Scheme:
- 10 µL DNA*
- 2 µL water
- 3 µL 5x Gel red

Total: 15 µL loaded on the gel

*important: previous time only 1 µL was loaded and amplifications were not visible (also too low primer concentration used). → 10 µL is ok amount with 50 µL PCR reaction

- First row: Control AKR1D1, control Ci2350, ladder 1.1 µL, AKR1D1 1, AKR1D1 2, AKR M1, AKR M2, AKR T1, AKR T2

- Second row: ladder, AKS1, AKS2, CI S1, CI S2, CI T1, CI T2



Inserts were correctly amplified, so digestion was performed, followed by PCR cleanup.

Digestion was set up directly with the amplified inserts, adding 1 µL of each restriction enzyme to the remaining 40 µL PCR samples, and incubated in the PCR machine with the Digestion program 37 °C for 45 minutes. The digestion program can be found in the IGEM folder in the machine.

For PCR cleanup, initially Nucleospin kit was used, and binding buffer was added to akr1d1 control and AKR1D1his 1 and 2. However a component of the kit is missing, therefore Cytiva kit was used instead from that point on with 2x25 µL warmed up elution buffer used.

Nanodrop measurements after cleanup

Sample	**Concentration (ng/µL)**	**Abs 260/280**	**Abs 260/230**
AKR-His A	52.7	1.860	0.414
AKR-His B	37.8	1.854	1.652
AKR-TrxA	60.6	1.877	0.206
AKR-TrxB	49.7	1.887	1.923
AKR-SumA	31.8	1.939	0.552
AKR-SumB	58.2	1.887	0.437
AKR-MBP-A	47.2	1.939	0.455
AKR-MBP-B	41.3	1.844	0.454
ci-TrxA	15.4	1.938	0.261
ci-TrxB	25.3	1.962	0.345
ci-SumA	28.8	1.933	0.725
ci-SumB	39.3	1.881	0.303

Inserts were pooled and concentration measured again for ligation:

AKR1D1	50.7
AKR TRX	61.6
AKR SUMO	57.7
AKR MBP	67.6
CI TRX	34.8

CI SUMO	38.3

FOR FUTURE REFERENCE → USE CYTIVA KIT FOR PCR CLEANUP

Plasmid nanodrop measurement and pooling

Plasmids concentration and purity was checked with nanodrop, samples 14 and 15 were excluded, the rest was pooled.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
1	6.958	1.750	0.161
2	6.461	1.857	0.141
3	24.9	1.563	0.110
4	15.9	1.600	0.119
5	10.9	1.692	0.044
6	6.958	1.750	0.070
7	5.964	1.714	0.117
8	8.449	1.700	0.850
9	7.455	1.667	0.147
10	8.946	2.000	0.029
11	6.461	1.857	1.182
12	4.970	2.000	0.152
13	5.467	1.833	0.440
14	1.491	1.500	0.071
15	0.497	1.000	0.033
Pooled Plasmid	**7.455**	**1.500**	**0.074**

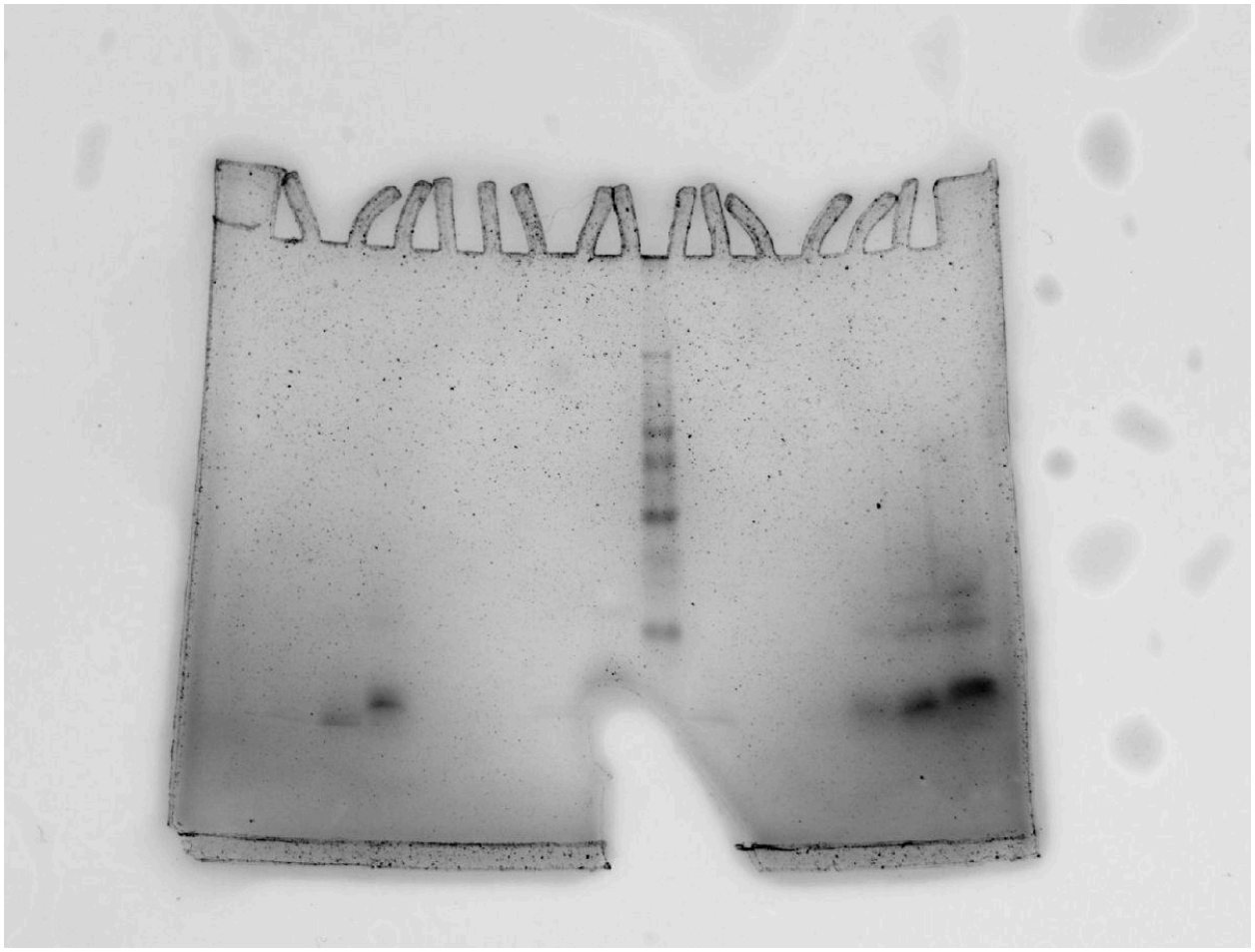
119.789/13 = 9.21 ng/µL theoretical concentration

SDS gel staining and visualization

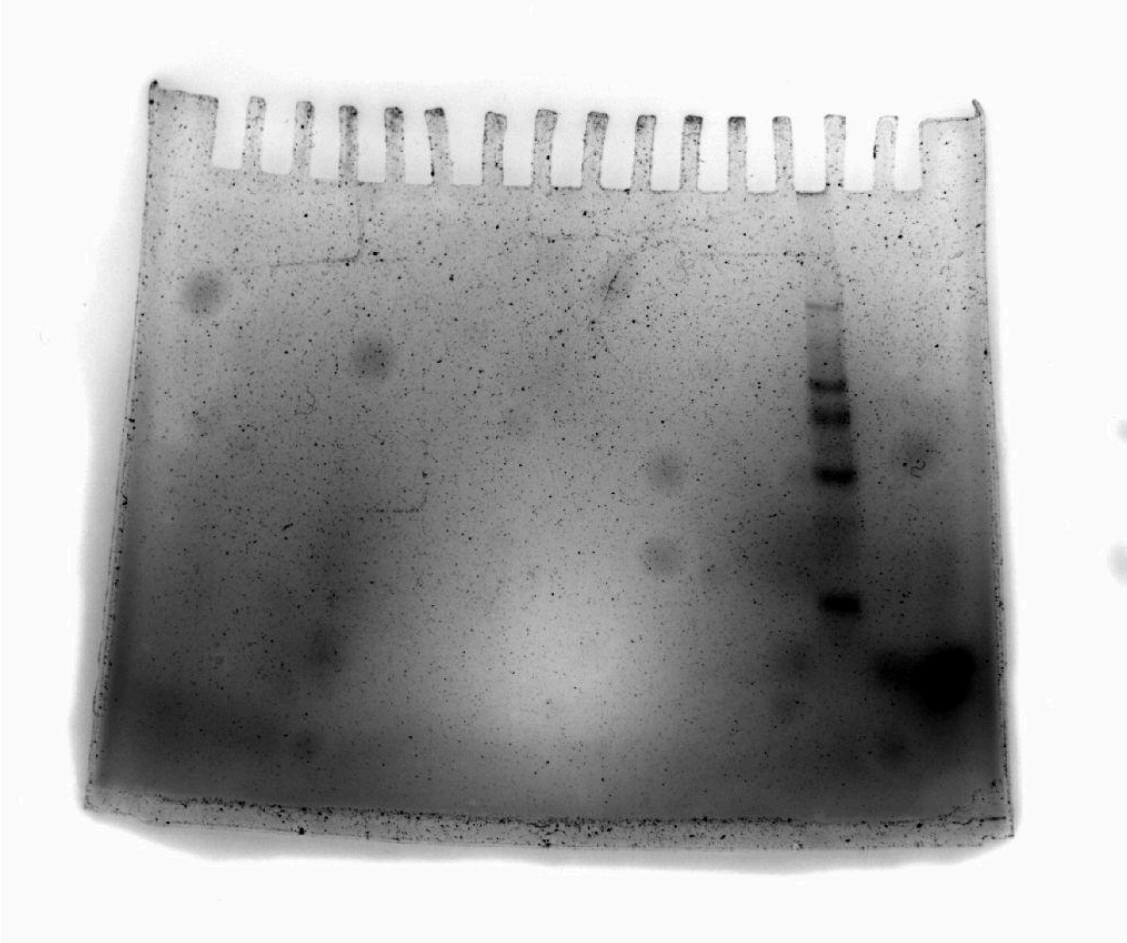
IMPORTANT!!!: after running it on friday, the gel was conserved in the fridge in water over the weekend. However, after running the gel needs to be fixed before storage, or the proteins will leak out. Store in staining solution (which contains 10%acetic acid and 40% methanol). Once proteins are fixed (by acetic acid) then it can be stored in pure water.

Due to error with gel storing, no bands are present in samples from wild type ci2350his, and few are visible in ci2350his-MBP, with evident swelling and travelling of the bands as well. Purification will be attempted again on Tuesday with a leftover sample.

Ci2350his-MBP SDS gel



Ci2350his



TB broth and LB broth preparation and autoclaving, buffer preparation

300 mL prepared of LB medium → 60 tubes autoclaved
900 mL solution A + 100 mL solution B prepared of TB medium → autoclaved and stored to be mixed at time of expression work.

For sample purification master NaPho buffer was prepared + 200 NaCl. It was then used to prepare the required buffers with appropriate Imidazol concentration.

Buffer and imidazol C	Imidazol	MQ
Lysis buffer (5 mM)	0.25 mL	24.75 mL

Washing 1 (20 mM)	1 mL	24 mL
Washing 2 (50 mM)	2.5 mL	22.5 mL
Elution (500 mM)	25 mL	0

Ligation reaction

Inserts were diluted to reach 0.02 pmol concentration adding MQ water:

AKR1D1	281 uL
AKR TRX	250 uL
AKR SUMO	236 uL
AKR MBP	147 uL
CI TRX	20.6 uL
CI SUMO	34 uL

Ligation mixtures were prepared with 1:1, 1:3 and 1:5 ratio plasmid/insert for all constructs:

Ratio	Samples	H2O (µL)	Ligase buffer (µL)	70 ng of vector (µL)	Insert (µL)	Ligase (µL)
1:1	All constructs	9	2	7.8	1	0.2
1:3	All constructs	7	2	7.8	3	0.2
1:5	All constructs	5	2	7.8	5	0.2

After the mixtures were made, they were incubated at room temperature for about an hour, then put into the fridge (+4C) overnight. Tomorrow morning they were taken out of the fridge at the same time with the competent cells, for an additional incubation period.

Glycerol stock preparation of ci2350his and ci2350his-MBP BL21 cultures and Inoculation of starter culture for ci2350 his and ci2350 his-MBP

7 LB-ampicilline tubes were inoculated, following the scheme shown in the table below, and incubated at 37°C for overnight cultivation.

2 tubes	E. coli BL21 with ci2350+His for expression and glycerol stock
2 tubes	E. coli BL21 with ci2350+MBP for expression and glycerol stock
1 tube	E. coli BL21 with empty pET-11a for expression and glycerol stock
1 tube	E. coli TG1 with ci2350+His for glycerol stock
1 tube	E. coli TG1 with ci2350+MBP for glycerol stock

ci2350his TG1 originally expressed was re-plated as it was getting old in case other colonies are needed for future work.

Day 26: [July 21, 2026], Tuesday

Weekly responsible: Patrik, Eleonora, Giulia
Lab participants: Hema, Hamsini, Cecilia, Rytis

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Transformation of E. coli TG1 competent cells with overnight ligation mixtures		
Preparing the ligation control		It was forgotten the day before
Starting of new expression cycle, conditions based on last week results, ci2350 and ci2350_MBP →		For both construct testing: 0.4, 0.8 [IPTG], 37° degrees at 4h
Protein Purification of leftover samples (ci2350 WT (0.25M IPTG, 3h) and ci2350_MPB (0.25M IPTG, 3h))	Protein purification vi…	0.1 M NaPho + 0.2 M NaCl buffer, single 500mM imidazole Elution, 2 wash conditions (20mM and 50mM imidazole)
Preparation of glycerol stocks	Glycerol Stock Prepa…	See Monday 20th July table.
Preparation of samples for SDS and Bradford assay		

Transformation of E. coli TG1 competent cells with overnight ligation mixtures

Ligation controls were forgotten on 20th July, so a control mixture was prepared on 21th July.

Reaction mix

Samples	H2O (µL)	Ligase buffer (µL)	70 ng of vector (µL)	Insert (µL)	Ligase (µL)
Ligation control	10	2	7.8	no	0.2

Incubation at 10 a.m. for 2 hours. 10 µL of ligation samples and controls were used to transform 200 µL of competent cells each, for a total of 20 samples. After incubation on ice for 50 minutes, transformants underwent heat shock at 42°C followed by addition of 400 µL of LB medium to each transformation tube. After incubation at 37°C, 150 rpm for 45 minutes, tubes were centrifuged at maximum speed for 1 minute and resuspended according to the transformation protocol. Finally all transformed cells were plated and incubated overnight at 37°C.

Preparation of glycerol stocks

Two tubes of 1 mL glycerol stocks were made for each sample, then put into a -80C freezer.

Samples:

E. coli BL21 with ci2350+His
E. coli BL21 with ci2350+MBP
E. coli BL21 with empty pET-11a
E. coli TG1 with ci2350+His
E. coli TG1 with ci2350+MBP

Cultivation of E.coli BL21 with ci2350+His and ci2350+MBP for protein expression.

4 main cultures + 2 uninduced controls (one per construct) were set up in 100 mL TB medium. Inoculum volume was determined from OD measurement of pooled overnight E.coli BL21 starter culture.

0.5 mL inoculates 100 mL of main culture at OD 3.

Pooled ci2350+His OD = 1.45 → 1.034 mL
Pooled ci2350+MBP OD = 3.78 → 0.396 mL

Construct	IPTG (mM)	T of incubation	TB medium volume (mL)	Inoculum (mL)
ci2350+His	0.4	37°C	100	1.034
ci2350+MBP	0.4	37°C	100	0.396
ci2350+His	0.8	37°C	100	1.034
ci2350+MBP	0.8	37°C	100	0.396
Uninduced control ci2350+His	– –	37°C	100	1.034
Uninduced control ci2350+MBP	– –	37°C	100	0.396

OD measurements will be taken after 2 hours and then every 30 minutes to determine time of induction (required OD 1.5). All data is available in the protein expression logging sheets: Protein expression logging sheets

E.coli BL21 with ci2350+His culture was initially mistakenly kept growing at 21°C instead of 37°C. After 2 hours the culture was then set at 37°C until induction and then kept at 17°C overnight.

Protein purification of samples from previous week cultivation (ci2350 WT and ci2350_MPB)

Bacterial pellets of these samples were stored in -80C freezer in 50 mL Falcon tubes. They were resuspended in 10 mL of Buffer (20 mM sodium phosphate and 500 mM NaCl), then centrifuged at ~4700 g for 10 minutes. Supernatant was discarded, and the pellet was resuspended in 5 mL lysis buffer (0.4 g of pellet gets 5 mL of lysis buffer). Samples were lysed according to the protocol (20% amplitude, 3 s ON/OFF, 2 min, repeated 3 times). Samples were collected into 15 mL Falcon tubes and then centrifuged (20 147 g, 1h 30 min, 4C). Supernatant was collected and protein purification was performed following the Protein purification via Sepharose beads (Camille's protocol)

NOTE: The flowthrough collected after the first protein loading in “ci-His A FT1” was accidentally mixed with ~750 µL of the Lysate supernatant fraction. Due to this, the protein loading on Sepharose-Ni^{2+} beads for “ci-His” was continued by taking the remaining 270 µL Lysate supernatant fraction and 730 µL of the mixed flowthrough. Thus, the final protein fraction is expected to be slightly lesser.

ci2350 WT was named as “ci-His” and ci2350_MPB was named as “ci-MBP”, SN - supernatant, FT- flowthrough. Wash A - had 20 mM imidazole, Wash B - had 50 mM imidazole.

Ci2350 - His Tag (WT)					
Sample	**Absorbance (y)**	**[Protein] µg/mL**	**Washing Buffer**		
E.A	1,884	7,32433891	20mM [Imidazole]	Elution 1 - [Imidazole]	500 mM
E.B	1,928	7,561791689	50mM [Imidazole]		
Sup WT	>2.5	N/A	N/A		

Ci2350 - MPB (TAG)					
Sample	**Absorbance (y)**	**[Protein] µg/mL**	**Washing Buffer**		
E.A	1,693	6,293577982	20mM [Imidazole]	Elution 1 - [Imidazole]	500 mM
E.B	1,72	6,439287642	50mM [Imidazole]		
Sup TAG	>2.5	N/A	N/A		

Equation	x= (y-0,5268)/0,1853
Parameter	Value
m	0,1853
b	0,5268
Equation	x= (y-0,5268)/0,1853

Preparation of samples for SDS and Bradford

2 mL samples at time of induction and after 3 hours were taken for all E. coli BL21 ci2350+His cultures. After OD measurement and centrifugation of the remaining volumes, the pellet was resuspended in MQ water to reach 20 OD for later use in SDS and Bradford assay.

At time of induction	Sample	OD	MQ water for resuspension (uL)
	ci2350+His no induction	1.45	137.75
	ci2350+His 0.4 IPTG	1.37	130.15
	ci2350+His 0.8 IPTG	0.96	91.2
	Sample	OD	MQ water for

After 3 hours			resuspension (uL)
	ci2350+His no induction		
	ci2350+His 0.4 IPTG		
	ci2350+His 0.8 IPTG		

Day 26: [July 22, 2026], Wednesday

Weekly responsible: Patrik, Eleonora, Giulia
Lab participants: Hema, Hamsini, Cecilia

Daily overview of experiments

Activity	***Protocol File***	***Comments***
SDS and Bradford Assay for expressed protein	Protein Quantificatio… SDS-PAGE	
Restriction digestion	Simple Restriction Di…	Digested pET11-a was re-incubated with Nde1. AKR1D1-His insert was digested with BamH1 and Nde1.
Ligation of pET11 + AKR1D1 insert	Ligation with pET-11…	Two ratios were done (1:1 and 1:3)
Transformation into E.coli TG1 + plating on LB-Amp plate	E. coli Transformatio…	
Colony PCR of overnight transformants	Colony PCR.docx	
Agarose gel electrophoresis for colony PCR samples	Gel electrophoresis.d…	
Autoclave	Autoclaving.docx	Waste, new pipet tips, eppis, flasks
Prepare 20% EtOH for column purification	-	1L prepared from 70% EtOH; 715 mL MQ + 285 mL EtOH (70%)
Inoculating LB media with E. coli TG1-pET11-a for plasmid extraction	-	Ten times 5mL of LB media + 5µL ampicillin + inoculation with TG1-pet11. Overnight incubation at

		37C° and 150 rmp

Results and interpretation

Colony PCR of overnight transformants

Due to large number of plates and large number of colonies in ligation control, it was decided to test only 4 plates (AKR1D1 (different plasmid:insert ratios -> 1:1 and 1:3), AKR1D1-SUMO (1:1 and 1:3)), and 5 colonies of each plate PCR mastermix was done, with AKR1D1 reverse and T7 forward primers. A negative control was added.

Component	Volume per 1 reaction (µL)	Volume for 23 reactions (µL)
Red Load Taq Master (5x)	4	92
T7 promoter forward primer (10 µM)	0.8	18.4
AKR1D1 reverse primer (10 µM)	0.8	18.4
PCR Grade Water	14.4	331.2
Total	20	460

The annealing temperature was set at 56°C → 61°C-5
The extension time was set at 1 min 30 sec as AKR1D1 SUMO fragment length is 1.3 kb, given the rule of thumb 1 min per kb of DNA.

Agarose Gel electrophoresis for colony PCR samples

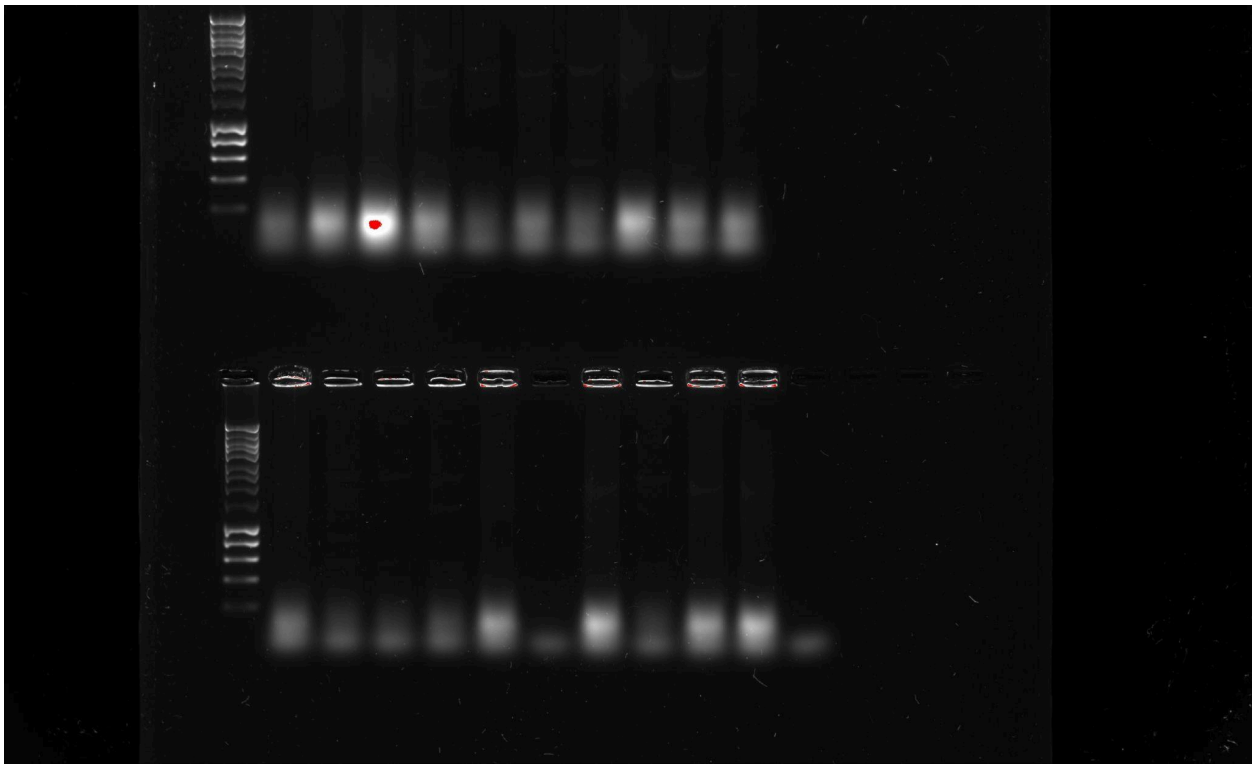
Big Agarose gel was made (150 mL), with 1% agarose. It was divided in two sections (2x15 wells), as all of our samples could not fit into one row. Sample volume was 20 µL and the Ampliqon High Range Ladder was 1.5 µL.
As negative control, a 20µL sample was mixed together without adding the cells.

Sample loading order:

Upper / Lower Gel	**Lane**	**Sample**

Upper Gel	1	Marker
	2-6	AKR1D1-His 1:1
	7-11	AKR1D1-His 1:3
Lower Gel	1	Marker
	2-6	AKR1D1 + SUMO 1:1
	7-11	AKR1D1 + SUMO 1:3
	12	Negative control

No amplification in the correct size was visible, as already suspected by looking at the ligation mix negative control, which showed colonies (see pictures below).



Restriction digestion

To reuse the already digested pET-11a, it was incubated again with NdeI enzyme, due to suspicion it had not cut all the plasmid.

Reagent	MasterMix (µl)	AKR1D1 (µL)

FD Green Buffer	20	4
NdeI	10	2
BamHI	-	2
DNA	170	36
MilliQ H2O	0	0
Total Volume	200	44

The reactions were incubated at 37ºC for 45 minutes, followed by PCR cleanup by Cytiva Kit.

After nanodrop measurement, a ligation reaction was set up, with 1:1 and 1:3 ratio + control. AKR1D1-His was used as the insert.

Reagent	1:1	1:3	Control
Ligation buffer (µL)	2	2	2
Ligase (µL)	0.2	0.2	0.2
DNA (µL)	11.2	11.2	11.2
Insert (µL)	2.03	6.1	0
MilliQ H2O (µL)	4.57	0.5	6.6
Total Volume (µL)	20	20	20

Ligation was set up at 16°C for 2 hours in the thermocycler and was followed by transformation by heat shock.

Transformation of ligation reactions into E.coli TG1

10 µL of each ligation reaction was used to transform 200 µL aliquots of E. coli TG1 competent cells. Additional control was added for transformation, where 10 µL of MilliQ H2O was used.
In total 4 reactions were made; 1 transformation control (MQ H2O), 1 ligation control (digested vector only, no insert) and 2 transformations with AKR1D1 inserts (one for 1:1 ratio and one for 1:3 ratio).
Before plating, 550 µL of supernatant was removed from all four reactions leaving ~60µL liquid for resuspension, followed by plating on Amp-LB agar plates. Plates were incubated overnight at 37°C.

Bradford Assay

Samples were prepared as follows and data collected, later () a new standard curve was generated where mq water was used as a buffer.
Samples were either CI2350 with His Tag only or His Tag + MBP solubility Tag, different IPTG conditions were tested before and after induction.

Sample ID	Before/After Induction	[IPTG]	Construct	Absorbance (y)	[Protein] µg/mL	Diluition Factor	[Protein] µg/mL
a	B	0	His	>2.5	N/A	N/A	N/A
b	B	0.4	His	1.017	2.645	25	66.136
c	A	0.8	His	1.147	3.347	25	83.675
d	B	0	MBP	1.012	2.618	25	65.461
e	B	0.4	MBP	1.173	3.487	25	87.183
f	A	0.8	MBP	1.168	3.460	25	86.508
g	A	0	His	1.121	3.207	25	80.167
h	A	0.4	His	1.096	3.072	25	76.794
i	B	0.8	His	1.259	3.951	25	98.786
l	A	0	MBP	0.936	2.208	25	55.208
m	A	0.4	MBP	0.839	1.685	25	42.121
n	B	0.8	MBP	1.027	2.699	25	67.485
			Equation	x= (y-0,5268)/0,1853			
			Parameter	Value			
			m	0.1853			
			b	0.5268			
			Equation	x= (y-0,5268)/0,1853			

	Before Induction	After Induction	Variation
[IPTG]	HIS Tag - Soluble Fraction		
0	N/A	N/A	N/A
0.4	66.13599568	76.79438748	16.11587107
0.8	98.78575283	83.67512142	-15.2963671
[IPTG]	MBP TAG - Soluble Fraction		
0	65.46141392	55.20777118	-15.6636438
0.4	87.18294657	42.12088505	-51.6867842
0.8	67.4851592	86.50836481	28.18872451

Day 27: [July 23, 2026], Thursday

Weekly responsible: Patrik, Eleonora, Giulia
Lab participants: Hema, Hamsini, Cecilia

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Colony PCR	Colony PCR .docx	The samples are AKR1D1 (1:1 and 1:3) and Ligation control
Gel electrophoresis of Colony PCR	File	
Inoculation of positive transformants	File	
SDS-PAGE Data Analysis		
Plasmid miniprep of pET-11a	E.Z.N.A protocol	
Restriction Digestion of pET-11a + nanodrop	Simple Restriction Di…	NdeI digestion only
Agarose gel of digested plasmid		There was a mistake with the gel preparation, therefore the bands were not sharp and gel needed to be discarded
1x TAE Buffer Preparation		1960 mL MQ + 40 mL 50x TAE buffer
Medium Preparation	Media preparation.docx	LB Agar Preparation.
Terrific Broth Preparation	TB-medium.doc	1000 mL of Solution A and 111.1 mL of Solution B was made

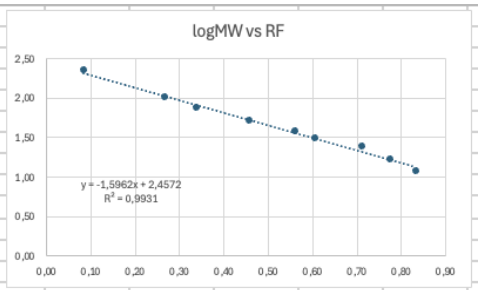
Results and interpretation

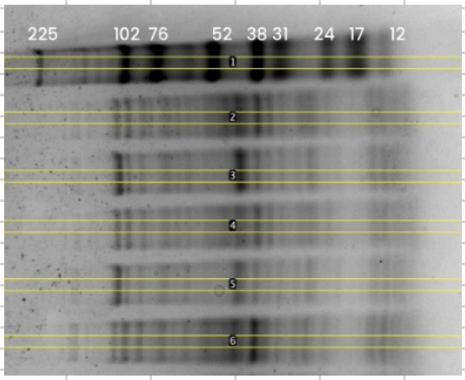
Data Analysis and Molecular Weight Determination of Unknown from SDS-PAGE gel from July 15th

BAND	LABEL	X	Standard MW (kD)	Rf	logMW	MW of unknowns
1	Standard	54,00	225,00	0,08	2,35	
2	Standard	172,50	102,00	0,27	2,01	
3	Standard	218,50	76,00	0,34	1,88	
5	Standard	296,50	52,00	0,46	1,72	
6	Standard	362,50	38,00	0,56	1,58	
7	Standard	391,50	31,00	0,61	1,49	
8	Standard	459,50	24,00	0,71	1,38	
9	Standard	500,50	17,00	0,77	1,23	
10	Standard	538,50	12,00	0,83	1,08	
11	Lane2	161,50		0,25	2,06	114,34
12	Lane3	165,50		0,26	2,05	111,77
13	Lane4	162,50		0,25	2,06	113,69
14	Lane5	163,50		0,25	2,05	113,05
15	Lane6	157,50		0,24	2,07	116,98
16	DyeFront	646,1				
eq (y)						
m	-1,5962			ci2350+mp	110.89	
b	2,4572			b expected		
x	Rf					

Use the equation of the standard curve to calculate the log(MW) of the four bands using in the Rf value for each band as the X value in the equation (you will be solving for Y, the log MW). The inverse log [=10^(cell#)]of that number will be the molecular weight of that band.

Date	23/07/26





Data analysis was made according the attached procedures, in order to check actual molecular weight of the unknown sample

Plasmid Miniprep of pET-11a

It was done with EZNA kit, and in total 10 samples were miniprepped. Elution was done 2x25 uL, preheated elution buffer to 60C. Washing of the column was done twice, as advised in the protocol.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
1	56.7	1.781	1.629
2	36.3	1.825	2.086
2b (second measurement)	36.8	1.850	2.056

3	62.1	1.866	2.119
4	46.2	1.824	2.114
5	69.6	1.842	2.121
6	75.5	1.831	2.171
7	46.7	1.808	1.918
8	50.7	1.821	2.125
9	71.6	1.823	2.087
10	68.6	1.816	2.190
Pooled	53.7	1.831	2.118

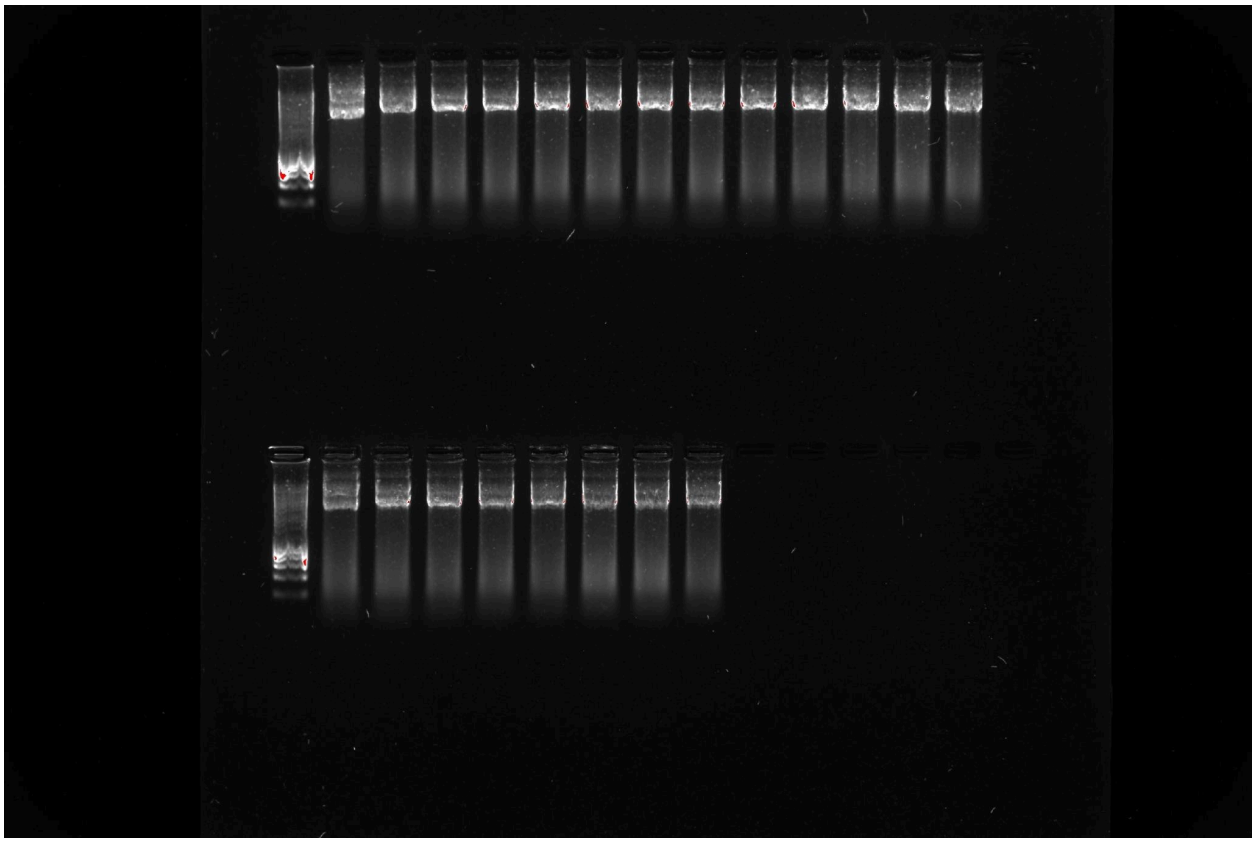
Restriction digestion of pET-11a

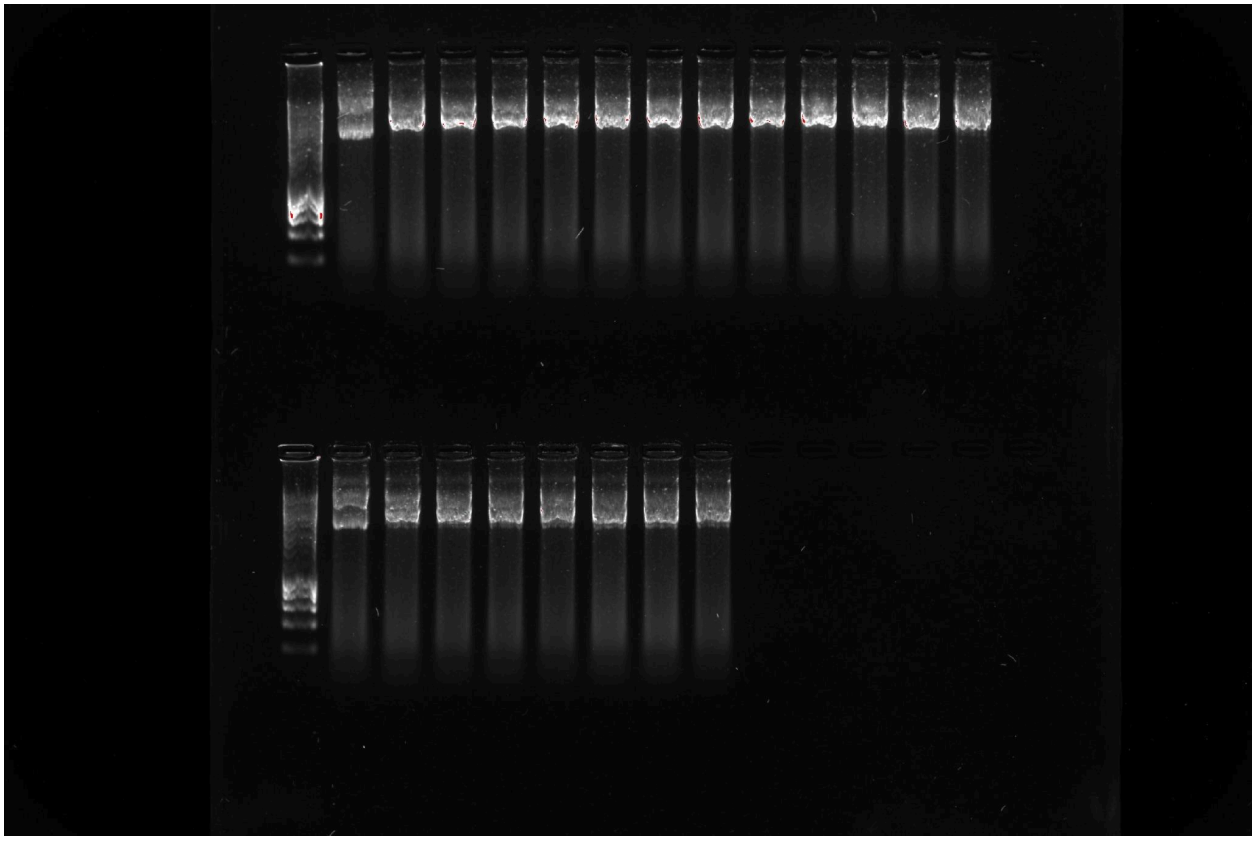
Restriction digestion was done with only NdeI, as Camille suggested, to visualize and extract from the gel only the linearized plasmid, as it is possible to differ between NdeI digested and undigested if using only one restriction enzyme. Fast AP was added, to prevent religation. Incubation was done for 40 minutes at 37C.

Reagent	(µl)
FD Green Buffer	2
NdeI	1
Fast AP	1
DNA	16
MilliQ H2O	0
Total Volume	20

Agarose gel electrophoresis

Following the digestion, samples were analysed on 1% agarose gel. Gel was visualized after 10min at 60V, then ~50min at 90V. After seeing the first gel, it was put back onto the gel machine, for another 20 minutes.





Analysing the picture, it was found that it is very grainy, probably due to not fully dissolving agarose in the microwave. This gel was not usable for gel extraction, and will be repeated the next day.

Terrific Broth Preparation

Accidently added 1000 mL of water instead of 900 mL so the amount was recalculated:
- 1000mL of Solution A and 111.1 mL of Solution B as the ratio was 9:1 for Solution A:B.

For solution A:

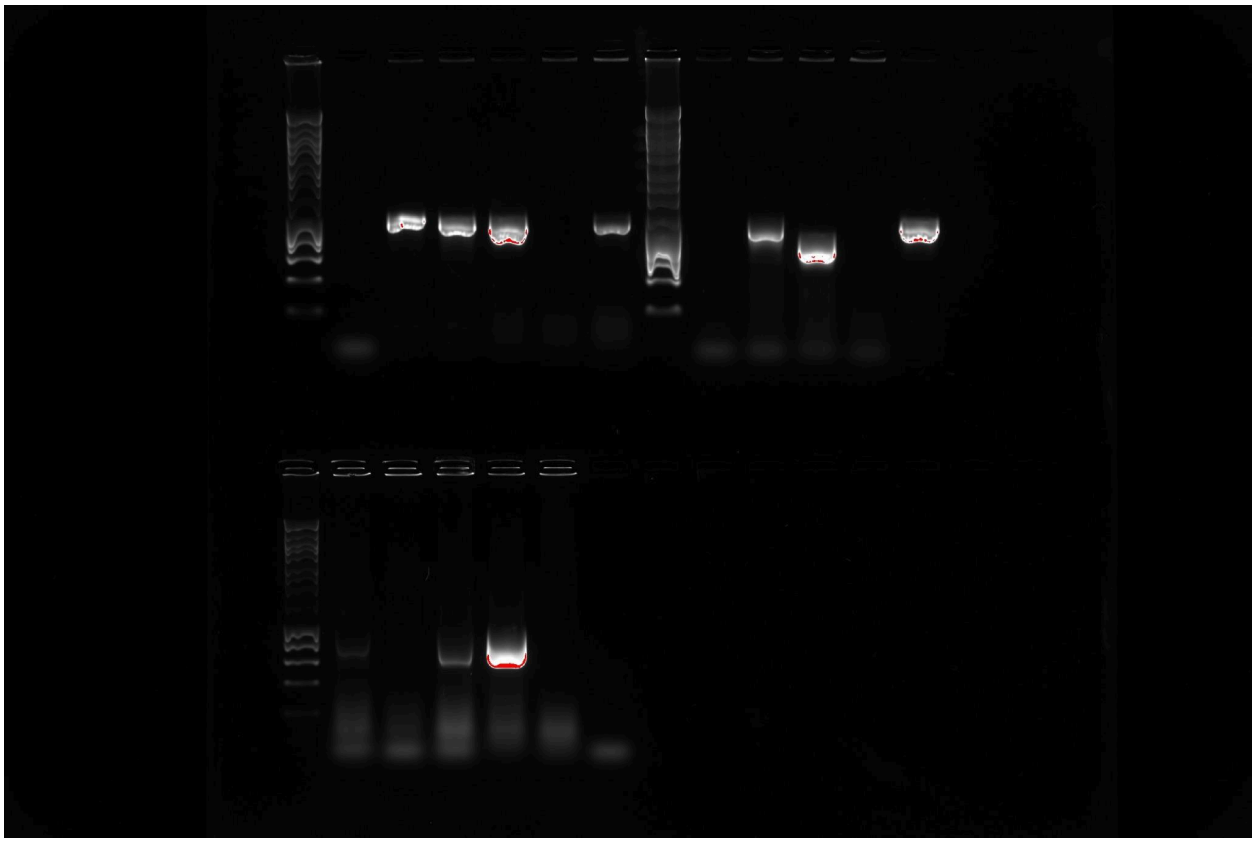
- Tryptone - 13.33 g
- Yeast Extract - 26.67 g
- Glycerol - 4.44mL

For solution B:

- KH_2PO_4 - 13.932g
- K_2HPO_4 - 2.566g

Colony PCR of AKR1D1-His transformations

Colonies transformed yesterday were analysed using colony PCR, using the same protocols as before. PCR reactions were put analysed on agarose gel, where a couple of them had correct inserts. Positive colonies were inoculated in 5 mL LB+Amp tubes for plasmid miniprep and sequencing.



Day 28: [July 24, 2026], Friday

Weekly responsible: Patrik, Eleonora, Giulia
Lab participants: Hema, Hamsini, Cecilia

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Plasmid miniprep of ci-MBP overnight culture	E.Z.N.A protocol	
Restriction Digestion of ci-MBP	Simple Restriction Di…	ci-MBP plasmid is cut to use the pET_blank vector for ligation with AKK1D1 and ci2350 inserts
Agarose gel of digested plasmid		
Gel extraction of digested plasmid		
Plasmid miniprep of AKR1D1 overnight culture	E.Z.N.A protocol	
Sequencing of extracted AKR1D1		24 samples with 3 different primers were prepared - T7 pro fw, T7 ter and AKR1D1 rev
Buffer Preparation for protein purification		Buffers recommended for Cytiva His Columns were made
Purification of ci2350+His and ci2350+MBP		

On Monday, the agar should be remelted and should be plated. Prepare 5 agar plates without ampicillin and then do the rest of the plates with ampicillin. Measure how much of the media is used up for the non-ampicillin plates so that you can accurately calculate the ampicillin amount to be added.

Results and interpretation

Plasmid Miniprep of ci2350-MBP

It was done with EZNA kit, and in total 10 samples were miniprepped. Elution was done 2x25 uL, preheated elution buffer to 60C. Washing of the column was done twice, as advised in the protocol.

Sample	**Concentration (ng/µL)**	**Abs 260/280**	**Abs 260/230**
1	38.8	2.167	2.786
2	52.2	2.143	1.117
3	35.3	2.219	1.690
4	29.8	2.222	3.000
5	18.9	2.714	3.167
6	42.7	2.205	2.529
7	25.3	2.429	3.000
8	24.9	2.381	3.333
9	38.3	1.925	1.974
10	30.3	2.033	2.652
Pooled	38.8	2.000	1.418

Restriction digestion of ci2350-MBP vector

Based on a concentration of 38.8 ng/µL and a volume of 478µL of undigested plasmid the volume of undigested plasmid was set to 16µL per sample leading to 620 ng of DNA per sample.

Reagent	Nde1 control (µL)	BamH1 control (µL)	Sample (µl)	Master Mix for 20x (µL)
FD Green Buffer	2	2	2	40

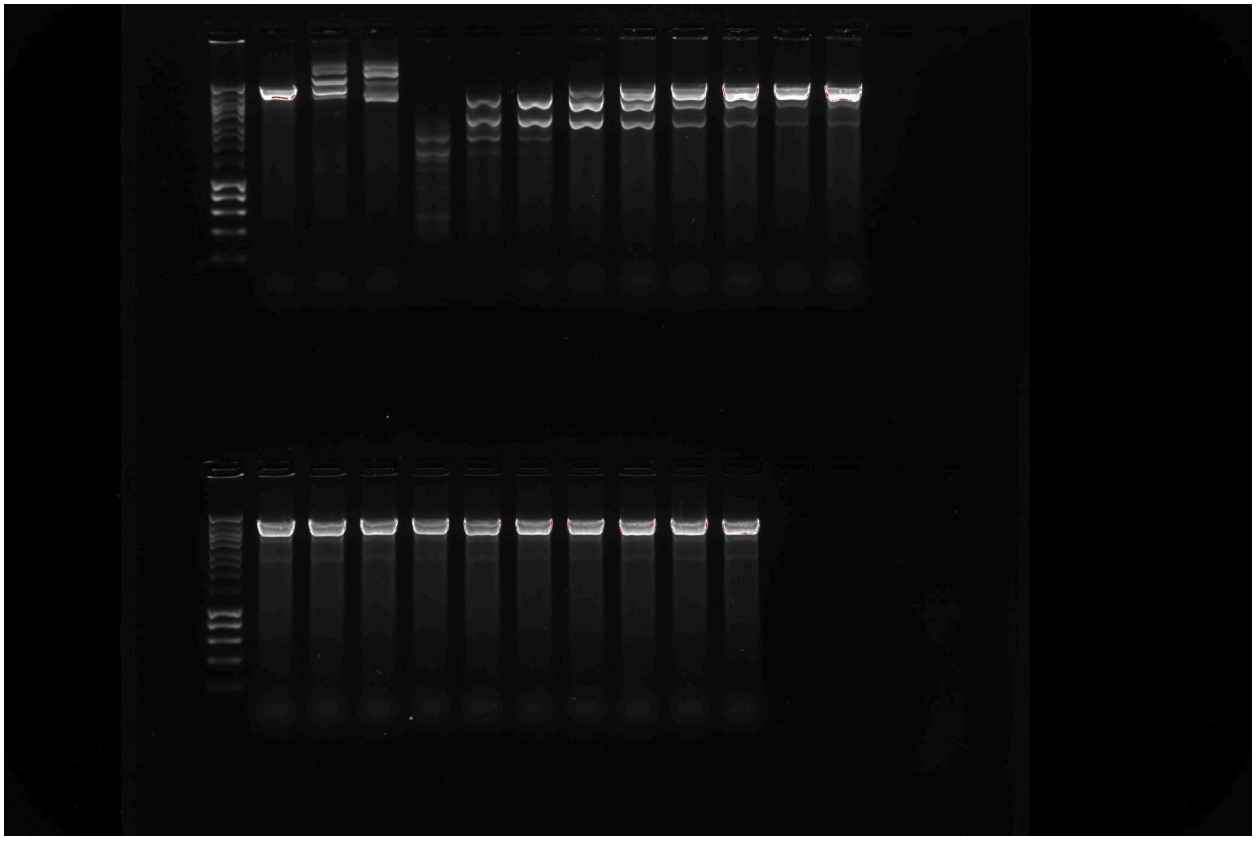
NdeI	1	-	1	20
BamH1	-	1	1	20
DNA	16	16	16	320
MilliQ H2O	1	1	-	-
Total Volume	20	20	20	400

Agarose gel electrophoresis

Following the digestion, samples were analysed on 1% agarose gel. Two times 15 wells were chosen. Gel was visualized after 15min at 60V, then ~__min at 90V.
Gel loading order is as followed:

Upper / Lower Gel	Lane	Sample
Upper Gel	1	Marker
	2	BamH1 control
	3	Nde1 control
	4	Undigested control
	5-13	Sample 1-9
Lower Gel	1	Marker
	2-11	Sample 10-19

Samples 2, 3 ,4 and 5 were cut from the gel and extracted. The samples were cut and the weight of the sample was measured.



Sample	Empty Tube (g)	Sample (g)	Weight of plasmid (g)	Volume of buffer (uL)
2	1.0215	1.2072	0.1857	185
3	1.0215	1.2380	0.2165	216
4	1.0215	1.1387	0.1172	117
5	1.0215	1.1886	0.1671	167

The nanodrop readings after elution will be taken on Monday (27/07/2026)

Plasmid Miniprep of AKR1D1 for sequencing

It was done with EZNA kit, and in total 8 samples were miniprepped. Elution was done 2x25 uL, preheated elution buffer to 60C. Washing of the column was done twice, as advised in the protocol and then they were used for sequencing..

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
A	176	1.839	2.052
B	89	1.884	2.266
C	188	1.844	2.112
E	87.5	1.872	1.978
G	138	1.853	1.891
H	128	1.843	2.115
J	136	1.845	1.936
N	117	1.844	2.185

Preparing samples for sequencing

The samples for sequencing were different colonies from the same culture plate and were thus named as A, B, C, E, G, H, J, N. The samples are AKR1D1 with His tags and after nanodrop, all samples except B and E were diluted 2X with MilliQ water to get the concentration range required for sequencing.
- For samples with high concentration, the added volume was 2.5 uL of Plasmids, 2.5 u: of MilliQ water for 2x dilution and 5 uL of Primers for a final volume of 10uL.
- For samples with concentration in expected range, the added volume for Plasmids was 5 uL of plasmids and 5 uL of Plasmids for a final volume of 10uL.
The primers used were T7 Pro Fw, T7 Ter and AKR1D1 Rev.

Identification codes:

With T7 pro fw:
- EF73935137 (A)
- EF73935161 (B)
- EF73935140 (C)
- EF73935164 (E)
- EF73935143 (G)
- EF73935146 (H)
- EF73935149 (J)
- EF73935152 (N)

With T7 ter:
- EF73935138 (A)
- EF73935162 (B)

- EF73935141 (C)
- EF73935165 (E)
- EF73935144 (G)
- EF73935147 (H)
- EF73935150 (J)
- EF73935153 (N)

With AKR1D1 Rev:
- EF73935139 (A)
- EF73935163 (B)
- EF73935142 (C)
- EF73935166 (E)
- EF73935145 (G)
- EF73935148 (H)
- EF73935151 (J)
- EF73935154 (N)

What and how much are we adding them:

→ Add 5uL of plasmid and 5uL of water for the codes:

- EF73935137 (A)
- EF73935140 (C)
- EF73935143 (G)
- EF73935146 (H)
- EF73935149 (J)
- EF73935152 (N)
- EF73935138 (A)
- EF73935141 (C)
- EF73935144 (G)
- EF73935147 (H)
- EF73935150 (J)
- EF73935153 (N)
- EF73935139 (A)
- EF73935142 (C)
- EF73935145 (G)
- EF73935148 (H)
- EF73935151 (J)
- EF73935154 (N)

→ Add 10uL of plasmids to samples B and E for the codes:

- EF73935161 (B)
- EF73935164 (E)

- EF73935162 (B)
- EF73935165 (E)
- EF73935163 (B)
- EF73935166 (E)

Buffer preparation for protein purification

Different buffers were made using already prepared stock solutions (3M NaCL, 1M Sodium Phosphate buffer and 1M Imidazol).

Note: The sodium phosphate buffer was not adjusted to pH 7.4, so when using it to prepare other solutions, the should be adjusted to pH 7.4 (by not adding all buffers, then 80% MQ water, the pH adjustments, following by topping up to the full buffer volume).

Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 20 to 40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein dependent; 20 to 40 mM is suitable for many proteins.)
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4 (The imidazole concentration required for elution is protein dependent).

500 mL of cell washing and binding buffers were made, and 250 mL of elution buffer. The cell washing buffer is the same composition as the binding buffer, just without imidazol.

Purification of ci2350+His and ci2350+MBP

Samples of cell pellets were taken from the -80C freezer (insert which samples exactly). They were washed in 10 mL of Cell washing buffer, by resuspending then centrifuging at 4000g for 10 minutes. Supernatant was discarded, then they were resuspended in the Binding buffer (20 mM sodium phosphate, 0.5 M NaCl, pH 7.4) (for cell lysis, we are using the same buffer).

Note: Lysis should be done either more times than just 3 cycles, or amplitude should be increased. The Internet suggests that the samples are fully lysed after the solutions become translucent, as non-lysed cells make the solution cloudy.

Before loading onto the column, samples were syringe filtered using a 0.4 µm filter.

They were loaded onto a purification column, and Cytiva protocol was followed for their purification. Samples were eluted in an elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4).

For all three samples, several fractions from the column were collected (waste, washing and elution fractions). Elution was done in 5 column volumes (5 mL total), and all samples were stored in the -20C freezer over the weekend.

Note: protein concentrations need to be measured on Monday