Week 29 - Overview

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Weekly summary:

Week 29 was dedicated to running colony PCRs to check multiple rounds of transformations, along with troubleshooting restriction digestion with NdeI and BamHI. We began protein expression of ci2350-His and ci2350-MBP, followed by cell lysis and SDS-PAGE analysis. We amplified our inserts with PCR to avoid running out of them.

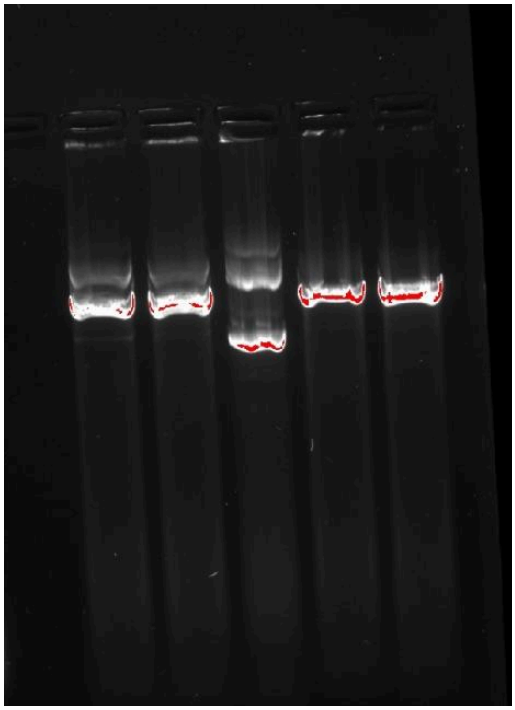
Main results of the week:

Agarose gel electrophoresis of colony PCR products of the July 10th transformants showed that all colonies, covering every construct transformed on Friday, were negative for inserts. Considering this was not the first time colony PCR had failed, it raised concerns about the integrity of our restriction digestion. It's possible the colonies were transformed with a religated pET-11a vector.

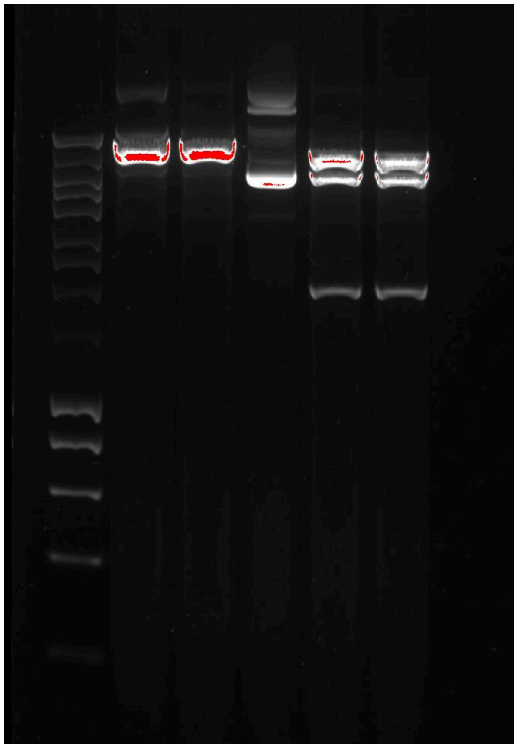
Agarose gel electrophoresis of restriction digestion performed on the same day confirmed our concerns. The NdeI control was almost identical to the undigested plasmid control. The double digested samples look identical to single digested ones, since the excised fragment is very small (~40bp), making it impossible to distinguish single and double digested pET-11a on the gel. This is likely why most of our transformations have been unsuccessful. We decided to test the NdeI and BamHI aliquots we had in the freezer, in order to identify which ones would digest correctly.



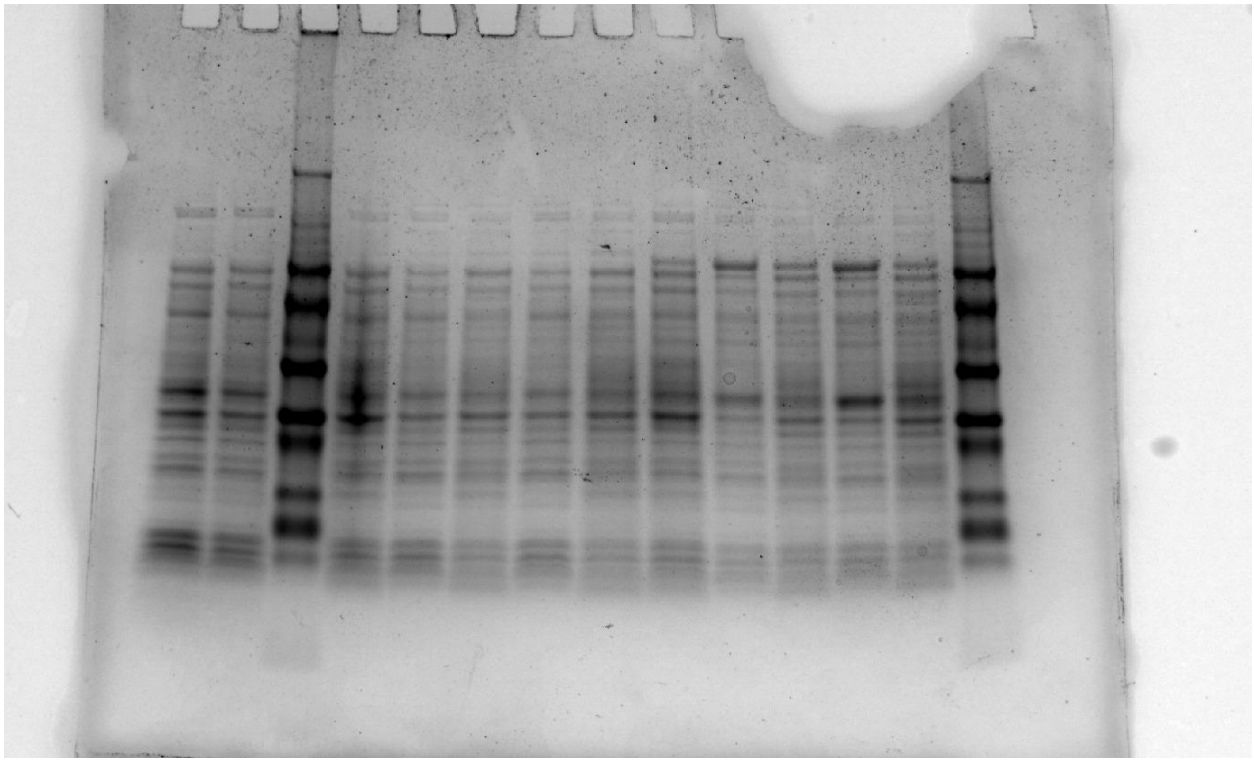
Testing enzyme integrity gave different results than the previous gel. Both NdeI aliquots cut the plasmid, though the digestion was still incomplete. BamHI doesn't seem to be a problem, as it fully digested the plasmid. To avoid any further mistakes, we have decided to place an order for a new NdeI enzyme, and use it for further digestion.



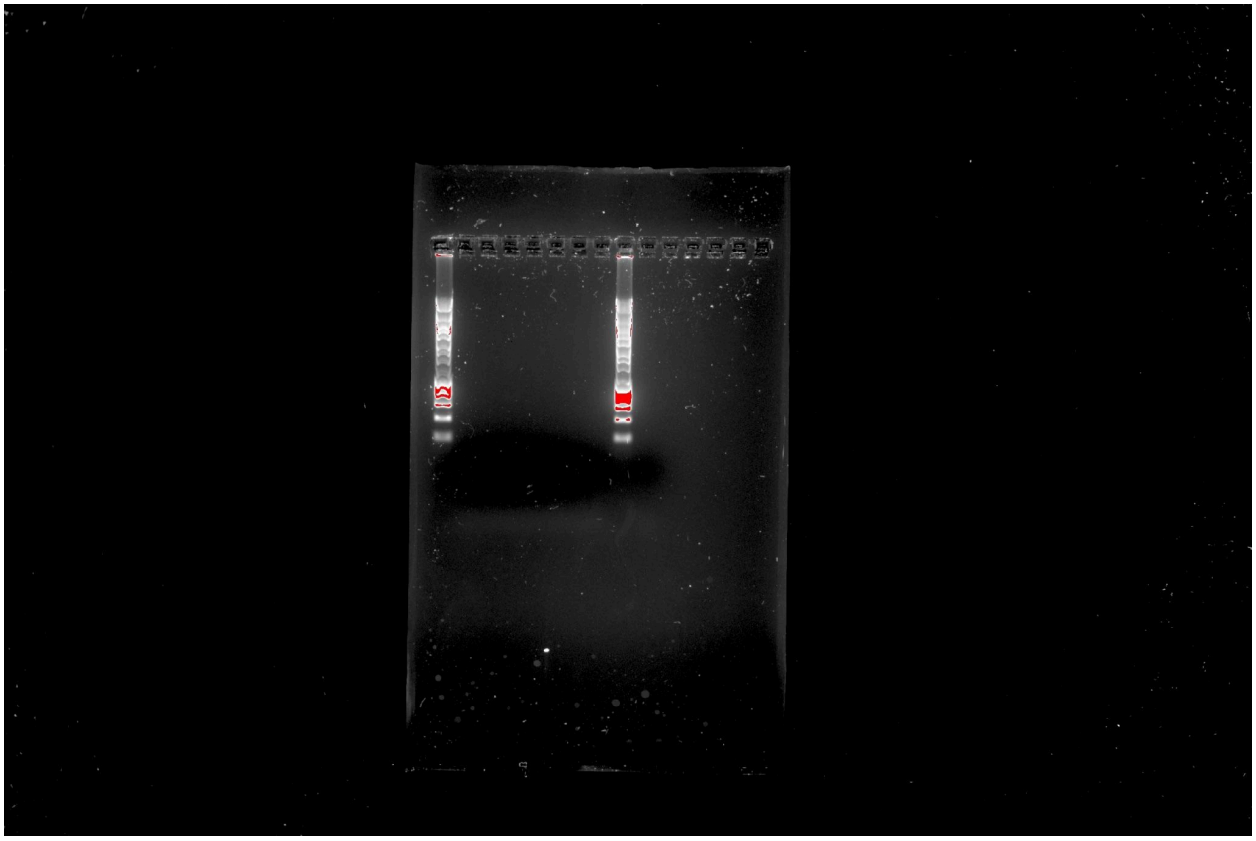
Because the pET-11a cutout was very small after digestion, we decided to approach restriction digestion from a different angle. We have previously ordered ci2350-MBP synthesized in the pET-BLANK plasmid, which has NdeI and BamHI sites flanking the insert. Since the pET-BLANK cutout corresponds to the full ci2350-MBP insert (~3000 bp), significantly larger than the pET-11a cutout, double digesting this plasmid would let us distinguish single from double-digested plasmid much more easily, allowing gel extraction of only the double-digested plasmid. Although this approach would not yield digested pET-11a, it would allow cloning directly into the pET-BLANK vector, which we had ordered with a T7 promoter. We adopted this method for further cloning, as it proved more reliable than digesting pET-11a directly.



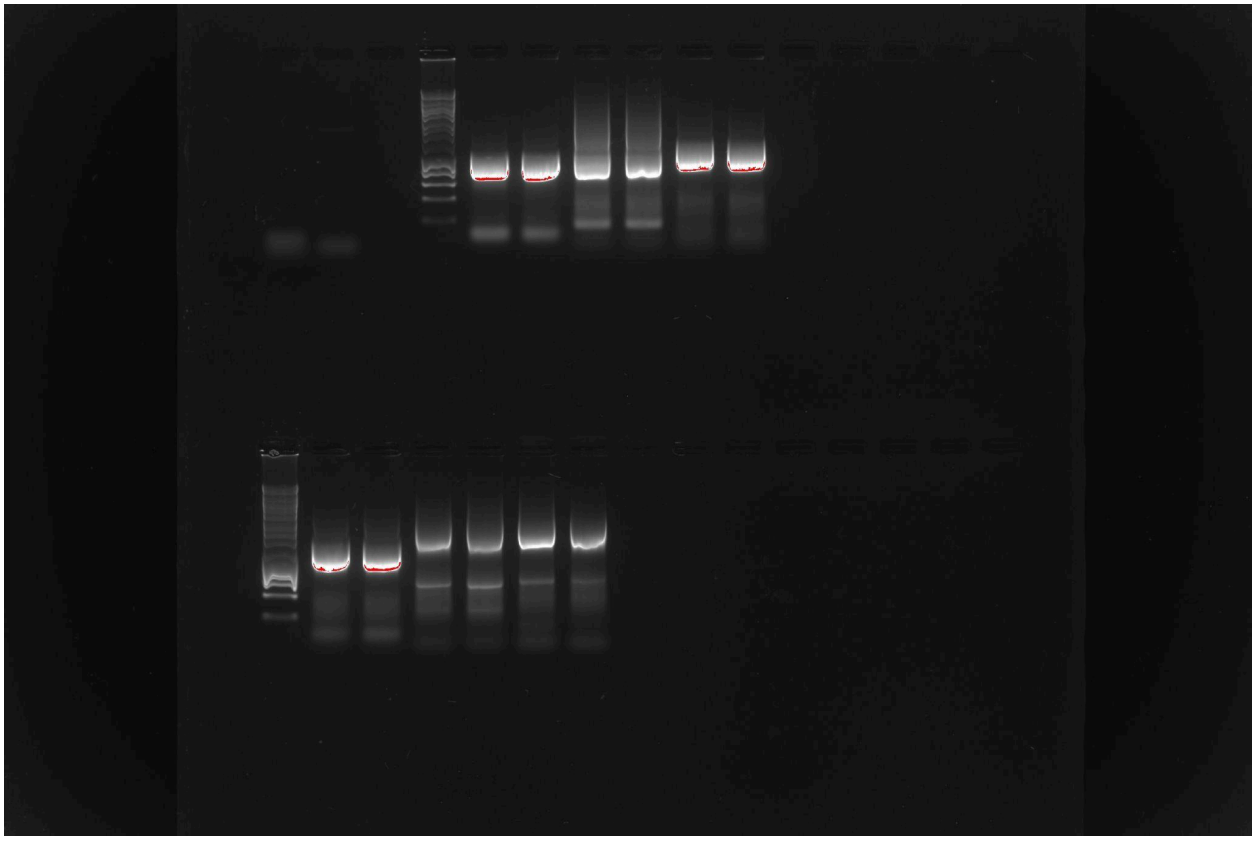
After visualising the SDS-PAGE of soluble fractions from ci2350-His and ci2350-MBP expression cultures, we found no visible overexpression of ci2350-His at 70kDa mark, while ci2350-MBP expression was visible at 102 kDa mark. Ci2350-His might have not been expressed due to 37ºC cultivation temperature, as ci2350 was cultivated at 25ºC in our reference paper, Arp et al (2025). For further cultivation, we decided to test lowering the expression temperature to 17ºC, to minimize inclusion body formation and allow for proper folding.



Agarose gel visualisation of PCR amplification products of our gBlocks showed the amplification was unsuccessful, as there were no bands present on the gel except the ladder. After consulting with our supervisor Camille, we realized that the reaction mix was incorrect, as too little primer had been added to the reaction.



We decided to repeat the gBlock amplification, adding more primer to the reaction mixture to bring the final concentration to 0.5 μM. This time the PCR worked, and we successfully amplified our insert gBlocks, as shown in the agarose gel picture below.



Day 20: [July 13, 2026], Monday

Weekly responsible: Eleonora, Rytis
Lab participants: Patrik, Giulia

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Colony PCR to confirm transformants	Colony PCR.docx	
Agarose Gel Electrophoresis	Gel electrophoresis.d…	To visualise Colony PCR and to visualise pET-11a digestion
Preparation of IPTG Stock Solution	Media preparation.docx	
Inoculation of starter cultures for protein expression	Enzyme expression	Ci2350+His and ci2350+MBP colonies
Restriction digestion of pET-11a	Simple Restriction Di…	With Fast Alkaline Phosphatase

Results and interpretation

Colony PCR of July 10th transformations

To test the presence of inserts in transformations, colony PCR was performed. Due to a very large number of formed colonies, it was decided to test a large number of colonies. In total 56

PCR reactions were done, with 28 testing for ci2350 inserts, and 28 testing for AKR1D1 inserts. Jena Bioscience Red Load Taq MasterMix kit was used.

Component	Volume per 1 reaction (µL)	Volume for 28 reactions (µL)
Red Load Taq Master (5x)	4	112
T7 promoter forward primer (10 µM)	0.8	22.4
Reverse primer (ci_rev/AKR_rev) (10 µM)	0.8	22.4
PCR Grade Water	14.4	403
Total	20	560

The thermocycler was set according to the table below:

Step	**Duration**	**Temperature**	**Cycles**
Cell Lysis	5 min	94°C	1
Denaturation	30 s	94°C	30
Annealing	30 s	56°C	
Elongation	3 min 15 sec	72°C	
Final Elongation	5 min	72°C	1
Hold	∞	4°C	

PCR products were analysed on 1% agarose gel below.

Well Number	Gel 1	Well Number	Gel 2
1	Ladder	1	Ladder
2-10	ci2350-TrxA	2	Negative control colony
11-14	ci2350-SUMO	3	Negative control colony

15-19	ci2350-TrxA	4-5	AKR1D1-His
20	Negative control colony	6-14	AKR1D1-MBP
21	Negative control colony	15-25	AKR1D1-TrxA
22-29	AKR1D1-His	26	Ladder
30	Ladder		

Gel PICTURE!!!!

Restriction Digestion of pET-11a

Simple Digestion Protocol was used, with increased size of reaction to 30 µL due to addition of Fast AP. One big MasterMix was made, then aliquoted into 20 reactions of 30 µL. Controls NdeI and BamHI were made, with their reaction volume of 30 µL. Incubated for 15 minutes at 37C, then 5 minutes at 80C.

Reagent	NdeI Control	BamHI Control	One reaction	MasterMix
FD Green Buffer	3	3	3	60
NdeI	1	0	1	30
BamHI	0	1	1	30
Fast AP	1	1	1	30
DNA	7.08	7.08	7.08	141.6
MilliQ H2O	16.92	16.92	16.92	338.4
Total Volume	30	30	30	600

Note: in the controls MilliQ H2O was added the same amount as in reaction, by mistake. It should have been added 1 µL more, as one enzyme is not added.

Agarose Gel Electrophoresis of Digested Plasmid

Insert experiment discussion and interpretation
NdeI visibly did not work. It was noted that an abnormal band was present in the last gel as well (**see gel picture of Thursday 9th July, NdeI control band**). The enzyme may have partially degraded in the previous reaction already, so that only part of the plasmid was digested,

resulting in the abnormal colonies found in the plates from the transformation performed over the weekend. The degradation of the enzyme may be linked to repeated thawing and freezing, and too extensive time spent on ice for the enzyme.

IMPORTANT: avoid thawing and freezing repeatedly the enzyme, do aliquots instead if required. Also avoid keeping the enzyme on ice for too long than necessary: it should be kept at -20 °C 90% of the time to avoid degradation. While PCR enzymes are thermostable and do not suffer thawing as much, restriction enzymes are VERY sensitive.



Day 21: [July 14, 2026], Tuesday

Weekly responsible: Eleonora, Rytis
Lab participants: Giulia, Patirk

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Measuring OD of overnight cultures for the inoculation of main culture		
Inoculation of Main culture for protein expression, followed by IPTG Induction and sampling	Enzyme expression Protein expression lo…	
Preparation of Buffer stock solutions for future use		1L - 3M NaCl 1L - 1M Sodium phosphate, pH 6.5
Restriction digestion of pET-11a	Simple Restriction Di…	Testing BamHI and NdeI enzymes to see if they work
Agarose gel electrophoresis		To visualize digestion
Inoculating E. coli TG1 pET-11a for overnight culturing		For plasmid miniprep, 10 cultures were inoculated

Results and interpretation

Measuring OD of overnight cultures

Overnight cultures of ci2350+His and ci2350+MBP were taken and each construct was mixed together (3 overnight tubes into 1), for easier calculation of main culture inoculation. LB Medium was taken as a blank for the spectrophotometer, and for dilution of the samples.

OD600 of ci2350+His was 0.170 after 10x dilution, meaning OD600 of culture was 1.7.

OD600 of ci2350+MBP was 0.164 after 10x dilution, meaning OD600 of culture was 1.64.

Preparation of Main culture inoculation

For the protein expression, ci2350+His and ci2350+MBP constructs were used. Multiple cultures were made, with different IPTG induction concentrations (0.4 and 0.25 mM) and different expression times (3h and overnight). One control culture was made, which will not be induced and will be cultured for the same time as 3h samples.

Construct	IPTG Concentration (mM)	Production time
ci2350+His	0	3h
	0.25	3h
	0.25	Overnight
	0.4	3h
	0.4	Overnight
ci2350+MBP	0	3h
	0.25	3h
	0.25	Overnight
	0.4	3h
	0.4	Overnight

Regarding the Terrific Broth (TB), 600mL were used in total (540 mL solution A and 60 mL solution B). They were mixed together, and ampicillin was added (600 µL), followed by dividing into 10 Erlenmeyer flasks for cultivation, with 50 mL medium each. A bit of excess medium was made, to account for pipetting mistakes.

After preparing the medium, cultures were inoculated, with 0.441 mL for ci2350+His and 0.457 ci2350+MBP (volumes were calculated from Johans instruction, where 0.5 mL of OD600 = 3

overnight culture was needed to inoculate 100 mL main culture). After inoculation, cultures were placed on a shaking incubator at 37C and 150 rpm at 10:50, where they will be sampled every ~30 minutes, starting from 1h after inoculation. (First sampling, 30 minutes after inoculation was skipped, due to too low OD600).

After cultivation finished, 6 flasks were taken out, 500 uL was taken from all of them, centrifuged at max speed for 1 minute. Supernatant was poured out and the pellets were resuspended in enough MilliQ H2O for the OD600 to be 20. They were put into the freezer overnight.

Sample prep after cultivation

After the cultivation and protein expression finished, flasks were poured out into preweighed 50 mL Falcon tubes, centrifuged at ???. Before centrifuge, the weight was adjusted by addition of TB, so they are balanced. Supernatant was poured out, followed by resuspension?

Buffer stock preparations

Buffers were made: 1L of 1M Sodium Phosphate Buffer (pH 6.5) and 1L of 3M Sodium Chloride stock solution. To prepare the solutions, ~800 mL of MilliQ H2O were poured into the flask, followed by addition of appropriate salts. After dissolving, volume was adjusted to 1L.

Calculations were done using this website: https://www.novoprolabs.com/tools/buffer-preparations-and-recipes/sodium-phosphate-buffer

Sodium Phosphate Buffer (1M, pH 6.5)	1L
NaH2PO4 H2O	94.013 g
Na2HPO4	45.241 g
Sodium Chloride Stock (3M)	1L
NaCl	175,32 g

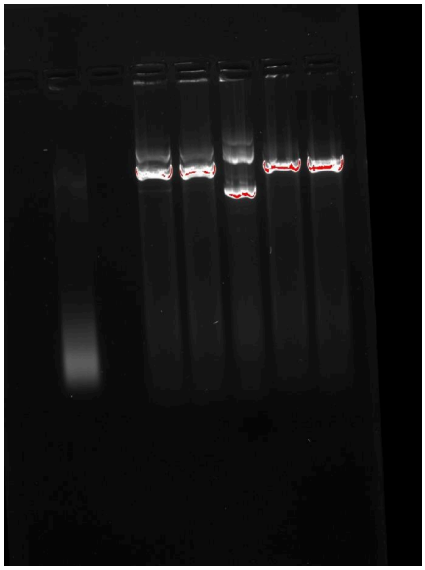
Restriction digestion of pET-11a

Test restriction digestion was done, to evaluate if enzymes work, due to their suspicious activity yesterday. They were incubated for 15 minutes at 37C, followed by inactivation at 80C.

Reagent	NdeI Control	BamHI Control
FD Green Buffer	2	2
NdeI	1	0
BamHI	0	1
DNA (0.5 µg)	7.08	7.08
MilliQ H2O	9.92	9.92
Total Volume	20	20

After digestion, samples were loaded onto the gel at 60V for 10 minutes, then 90V for 50 minutes.

Well Number	Sample
1	Camille's Sample
2	Empty
3	NdeI control
4	NdeI control
5	Undigested Plasmid
6	BamHI control
7	BamHI control



The gel picture showed that the NdeI enzyme has some suspicious activity, because a band is visible above the linearized plasmid band, indicating that the enzyme did not fully digest the plasmid. Even though it is better than yesterday, the digestion is still not complete. We should place a new order on the NdeI enzyme, or Camille will borrow it from someone, and we should test it tomorrow.

Day 22: [July 15, 2026], Wednesday

Weekly responsible: Eleonora, Rytis
Lab participants: Giulia, Patrik

Daily overview of experiments

Plasmid Miniprep was performed for overnight cultures. NdeI test with enzyme from other lab to compare with our enzymes. SDS page with samples from time zero, 3 hours incubation + overnight culture. Autoclaving of flasks for future experiments.

Activity	***Protocol File***	***Comments***
Plasmid Miniprep of overnight E. coli TG1 pET-11a cultures	Copy of QMF28.002… PP-204.0002.pdf	EZNA Kit was followed for 6 tubes, and Jenna Kit was followed for 4 tubes
Restriction digestion of extracted plasmid		
Agarose gel electrophoresis	Gel electrophoresis.d…	
Sampling of overnight cultures for SDS and OD measurement. The cell pellet was weighed and put in -80.		
SDS gel of samples from Tuesday expression	SDS-PAGE	

Results and interpretation

Plasmid pET-11a miniprep using E.Z.N.A. kit

In total 10 samples were miniprepped (EZNA Kit was followed for 6 tubes, and Jenna Kit was followed for 4 tubes).Plasmid was extracted using standard protocol, with 50 µL elution volumes.

Sample	**Concentration (ng/µL)**	**Abs 260/280**	**Abs 260/230**
1	63.6	1.829	2.133
2	44.2	1.854	2.225
3	39.3	1.837	2.394
4	61.1	1.836	2.365
5	35.8	1.846	2.483
6	45.2	1.857	2.022
7	126	1.992	1.318
8	119	1.943	1.975
9	114	1.974	1.202
10	127	1.855	2.1.407
Pooled Plasmid EZNA (1-6)	**49.2**	**1.800**	**2.062**
Pooled Plasmid JENA (7-10)	**117**	**1.934**	**1.774**

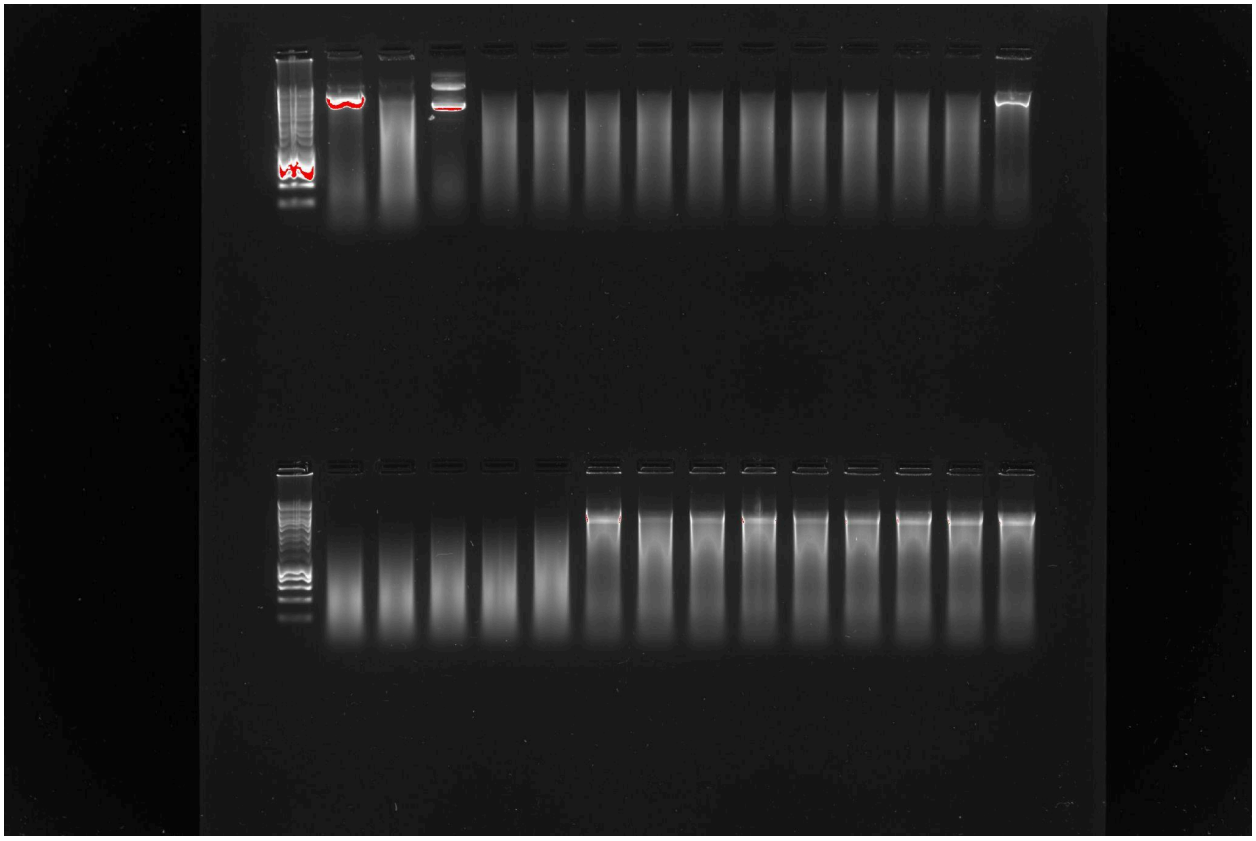
Restriction digestion of extracted plasmid

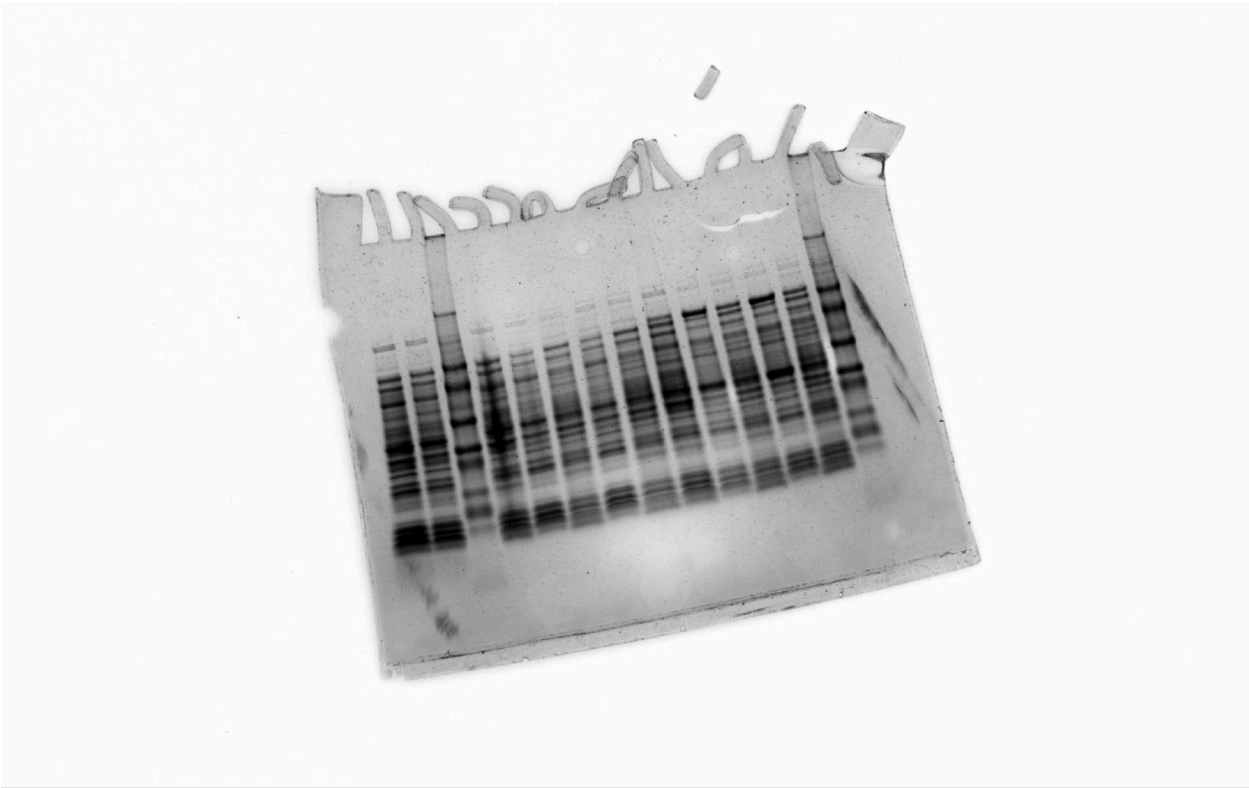
Reagent	MasterMix	1x reaction	NdeI Control	BamHI Control
FD Green Buffer	60	2	2	2
NdeI	-	1	1	0

BamHI	-	1	0	1
DNA (0.5 µg)	321	10.7	10.7	10.7
MilliQ H2O	159	5.3	6.3	6.3
Total Volume	540	20	20	20

Well Number	Upper gel	Lower gel
1	Ampliqon High Range DNA Ladder (1.5 µL)	Ampliqon High Range DNA Ladder (1.5 µL)
2	BamHI control	Sample
3	NdeI control	Sample
4	Undigested plasmid	Sample
5	Sample	Sample
6	Sample	Sample
7	Sample	Sample
8	Sample	Sample
9	Sample	Sample
10	Sample	Sample
11	Sample	Sample
12	Sample	Sample
13	Sample	Sample
14	Sample	Sample
15	Sample	Sample

*sample corresponds to one aliquot from the mastermix mixture





ci2350-MBP is visible above the 102 mark in the ladder, while no visible overexpression is visible at around 70 kDa mark for ci2350. It may be due to 37°C of incubation, as ci was cultivated at 25 °C in the reference paper.

Gel order is written on the following day.

Day 23: [16 July, 2026], Thursday

Weekly responsible: Eleonora, Rytis
Lab participants: Patrik, Giulia

Daily overview of experiments

Visualization of SDS gel left overnight, loading and visualization of new restriction attempt (Camille), attempt at restriction of pET-11a continuing ci2350 with insert to visualize the cut insert and be sure of double cut and functioning NdeI. Cell lysis and analysis of two pellets from Tuesday's expression.

Activity	***Protocol File***	***Comments***
Visualization of SDS gel left overnight		Clearer vision of the bands. See picture. Anomalous band visible at lower kDa than expected in one of the ci-tag samples.
Cell lysis and sonication of two pellets from Tuesday's expression experiment.		
SDS-PAGE of fractions from lysed cells.		
Plasmid miniprep of 3 samples of E. coli TG1 ci2350+His		
Restriction digestion of pET-11a containing ci2350+His		Testing NdeI digestion, as ci2350+His plasmid has a bigger cutout than regular pET-11a, making it easier to see the double digest, which is not the case with normal pET-11a
Agarose gel electrophoresis		Visualising restriction digestion
Inoculating *E. coli* TG1		10 tubes were inoculated

Primer resuspension	Following IDT protocol	100 uM stock solution was made in the IDT tube, and 2 aliquots of 10 uM solutions were made of each primer
Insert PCR amplification	PCR amplification g-…	Done due to having little inserts left Reaction done overnight

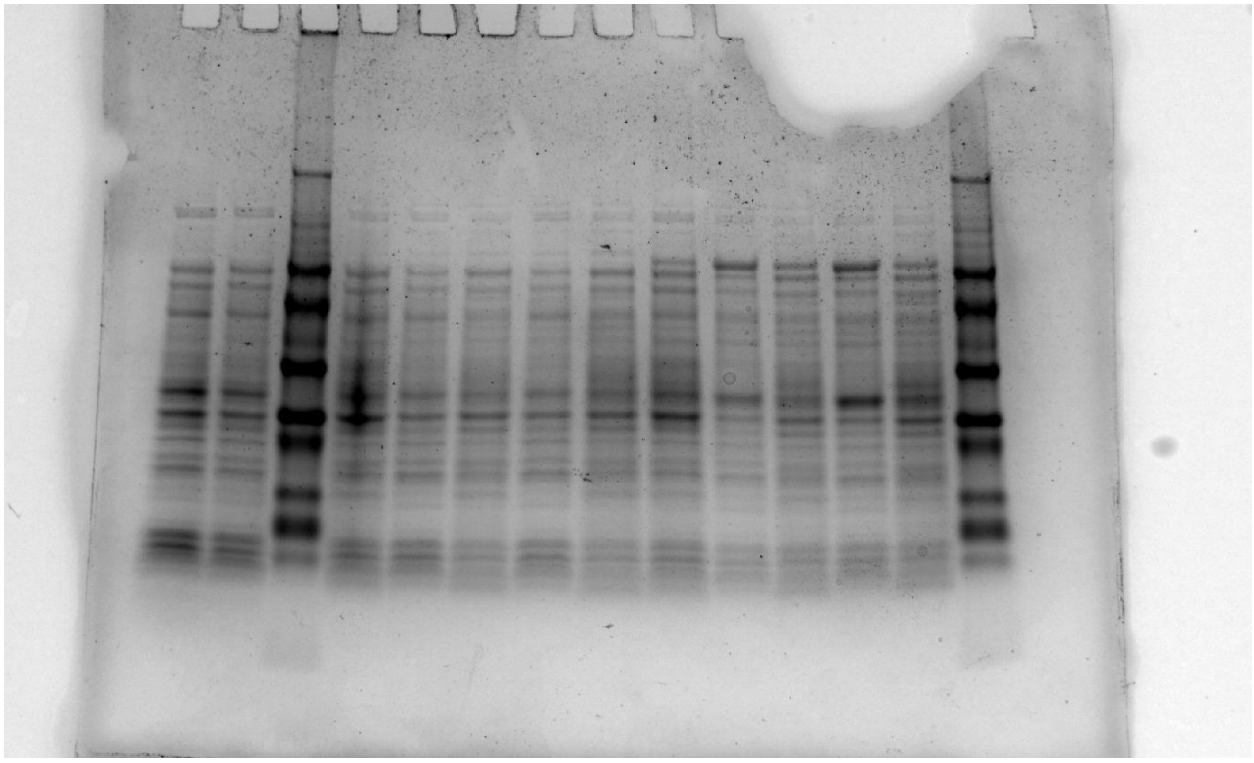
Results and interpretation

Visualization of SDS gel overnight

ci2350-MBP is visible above the 102 mark in the ladder, while no visible overexpression is visible at around 70 kDa mark for ci2350. It may be due to 37°C of incubation, as ci was cultivated at 25 °C in the reference paper.

The gel order is as follows:

ciHis 0.4 IPTG pre-ind, ciMBP 0.4 IPTG pre induction, Ladder, ciHis no induction, ciHis 0.25 IPTG 3 hrs, ciHis 0.25 IPTG overnight, ciHis 0.4 IPTG 3 hours, ciHis 0.4 IPTG overnight, ciMBP no induction, ciMBP 0.25 IPTG 3 hrs, ciMBP 0.25 IPTG overnight, ciMBP 0.4 3 hrs, ciMBP 0.4 overnight, Ladder.



Cell lysis of cell pellet

Two of the pellets left in -80°C were analysed: ci2350, 0.4 IPTG, 3 hours and ci2350_tag, 0.4 IPTG, 3 hours.
E.coli Lysis by sonication protocol was followed. Some adjustments were made (see protocol). The lysis buffer used for resuspension was based on Cytiva guidelines for purification. Lysis buffer is therefore 20 mM NaPho and 500 mM NaCl + 5 mM Imidazole instead of 100 mM NaPho and 200 mM NaCl.

Plasmid Miniprep of E. coli TG1 containing ci2350+His

Three tubes of E. coli TG1 containing ci2350+His were MiniPrepped. Elution was done twice using 30+30 uL of elution buffer, preheated to 60C.

Note: long centrifugation to remove residual ethanol was forgotten, which could have an impact on downstream applications. Restriction digestion worked, indicating that this step is not as crucial as it might seem. However, it must not be forgotten in the future, as instructions on the kit state that this is a crucial step.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
1	60.1	1.833	2.161
2	84.5	1.868	2.099
3	78.5	1.881	2.194
Pooled Plasmid	**92.9**	**1.851**	**2.011**

Note: the pooled concentration seems to be higher. There might have been mistakes with the mixing of individual samples, resulting in lower concentration.

Restriction digestion

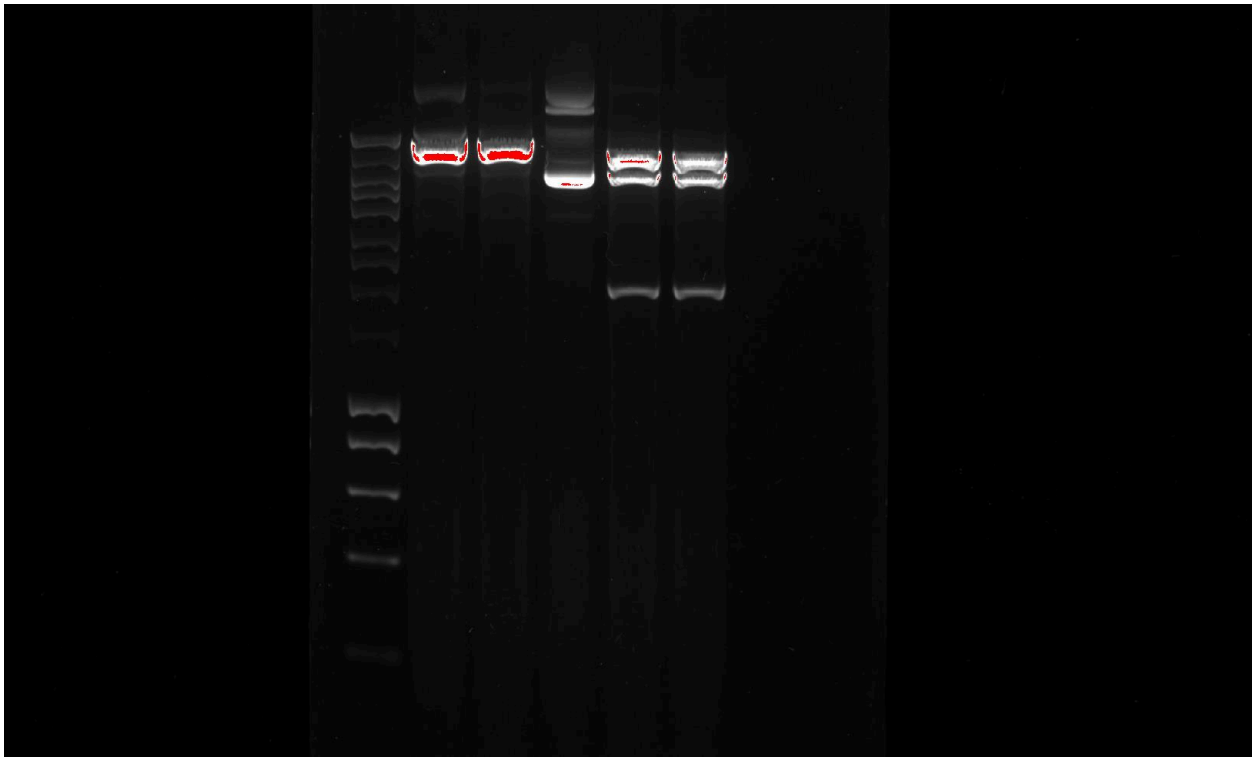
Restriction digestion was done, following the Simple Protocol. In total 2 samples were digested, along with NdeI control and BamHI control.

Note: by accident, 2 uL of NdeI were put into the NdeI control.

After the digestion, samples were loaded onto the 1% agarose gel and run for 10 minutes at 60V, followed by 90V for ~80 minutes. 1 uL of the Ampliqon High Range DNA Ladder was put onto the gel, and the bands seem good, meaning we can use this amount in the future, not 1.5 uL, which often gives smeared and uneven bands. Orange Loading Buffer was used for preparation of undigested plasmid.

Well Number	Sample
1	Ampliqon High Range DNA Ladder (1 uL)

2	NdeI control
3	BamHI control
4	Undigested sample
5	Sample 1
6	Sample 2



NdeI control shows the linearized vector (the red highlighted part) and a faint undigested vector. BamHI control shows fully linearized plasmid. On the both samples, it is visible that they were both linearized but also double digested, due to the showing of lower bands, which account to ci2350+His cutout.

This method could be used for reliable plasmid double digestion, as its clear which bands show single and which show double digestion. However, it cant be used for insert digestion, as they don't have a big difference in size between single and double digest.

Amplification primers resuspension

Primers received from IDT were resuspended in MilliQ Water, following the volumes shown in the table. Before resuspension, tubes were spinned down, to allow all of the primers to go down the tube. After resuspension, primers were vortexed, then spun down. All of the stock concentrations in the IDT tubes are 100 µM. Two aliquots of 100mL of each primer were made, with 10 µM concentration.

Primer Name	Nanomols received	Volume of MilliQ added (µL)
His fw	24.8	248
AKR1D1 rv	40.1	401
Ci2350 rv	20.8	208

Amplification PCR of Inserts

PfuTurbo Hotstart PCR Master Mix (2x) was used for amplification. Forward primer is the same for all constructs. Reverse primer is construct dependent. One reaction was made for all inserts that were amplified (ci2350+SUMO, ci2350+TrxA, AKR1D1, AKR1D1+SUMO, AKR1D1+TrxA, AKR1D1+MBP). One control was made for each set of primers (2 controls in total, one for ci2350 set, one for AKR1D1 set).

Component	Amount per reaction (µL)
Pfu Hotstart Master Mix (2x)	25
Primer Forward (0.5 µM)	1
Primer Reverse (0.5 µM)	1
DNA Template (1 ng/µL)	1
MilliQ H2O	22
Total volume	50

Note: This is a completely wrong reaction mix. While looking at the Agilent Technologies instructions for the PCR, concentration values were interpreted wrongly, leading to a mistake of not the amplification failing.

Temperature (C)	Duration	Cycles
95	2 minutes	1
95	30 seconds	30
57	30 seconds	
72	3 minutes	
72	10 minutes	1
4	infinite	-

Day 24: [July 17, 2026], Friday

Weekly responsible: Eleonora, Rytis
Lab participants: Giulia, Patrik

Daily overview of experiments

Activity	***Protocol File***	***Comments***
Plasmid miniprep of overnight E. coli TG1 pET-11a cultures	Copy of QMF28.002…	EZNA Kit, 10 minipreps were done, samples were pooled together afterwards for a uniform plasmid concentration
Restriction digestion	Simple Restriction Di…	Done ~27 digestions, and controls
Agarose gel electrophoresis	Gel electrophoresis.d…	For digestion visualization and PCR visualization from previous day
Amplification PCR of inserts	PCR amplification G-…	For amplification of all inserts (two 50 uL reactions per insert)
Bradford assay for protein quantification	Protein Quantificatio…	

Results and interpretation

Plasmid miniprep of E. coli TG1 pET-11a

EZNA Kit was followed, 10 samples were miniprepped. Elution was done 2 times of 30 µL. The elution buffer was not preheated.

Sample	Concentration (ng/μL)	Abs 260/280	Abs 260/230
1	59.1	1.889	2.052
2	61.1	1.864	2.016
3	42.7	1.870	1.830
4	45.2	1.896	1.820
5	58.6	1.844	1.356
6	33.3	1.914	1.914
7	64.1	1.897	2.115
8	54.2	1.912	1.879
9	37.3	1.923	1.8531
10	47.4	1.920	2.043
Pooled Plasmid	**46.7**	**1.843**	**2.089**

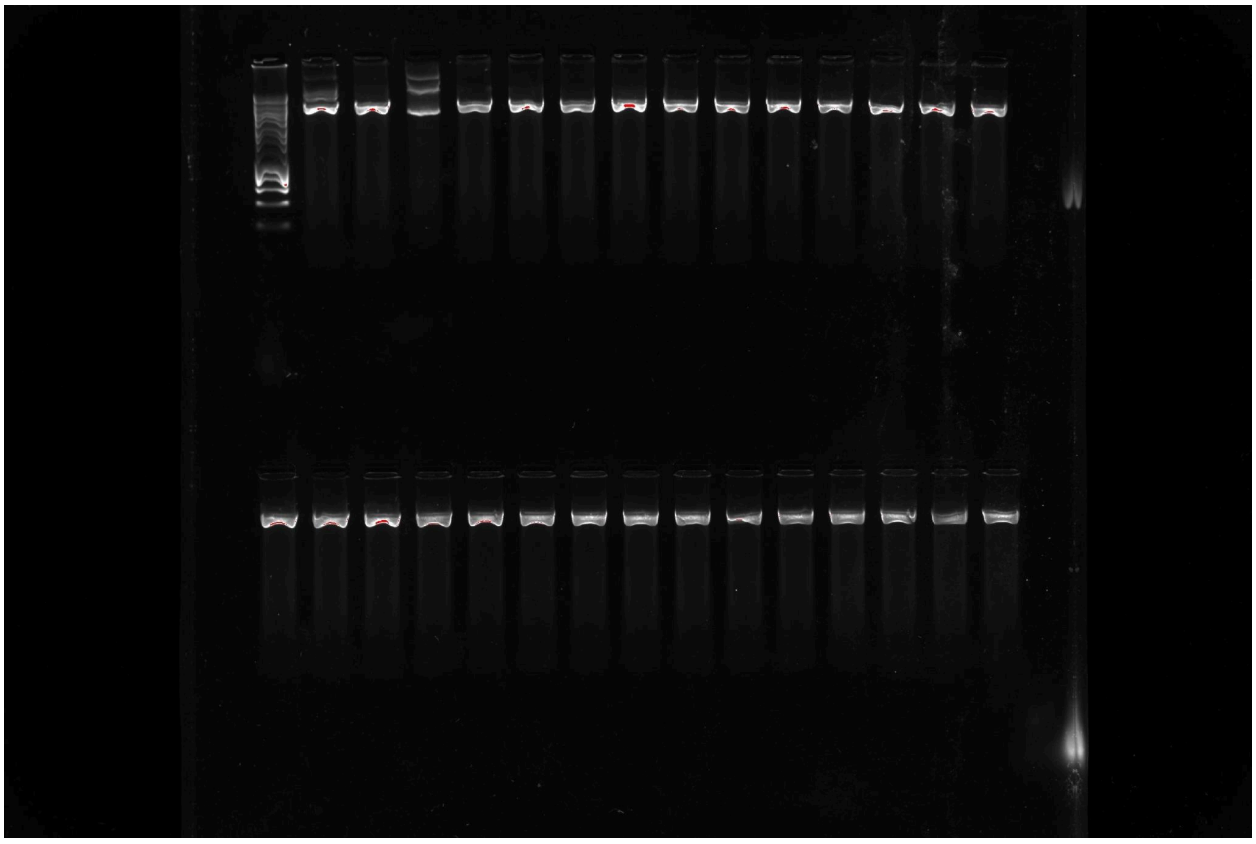
Restriction Digestion of pET-11a

For the digestion, one big mastermix was made without enzymes, then aliquoted into both controls. Enzymes were added to the leftover mastermix, then it was aliquoted into 20 μL reactions. Incubation was done at 37C for 20 minutes, followed by 5 min inactivation.

Reagent	MasterMix	1x reaction	NdeI Control	BamHI Control
FD Green Buffer	60	2	2	2
NdeI	-	1	1	0
BamHI	-	1	0	1
DNA (0.5 μg)	321	10.7	10.7	10.7
MilliQ H2O	159	5.3	6.3	6.3
Total Volume	540	20	20	20

Digestion was visualized on 1% agarose gel, 10 mins at 60V, followed by ~50 mins at 90V.

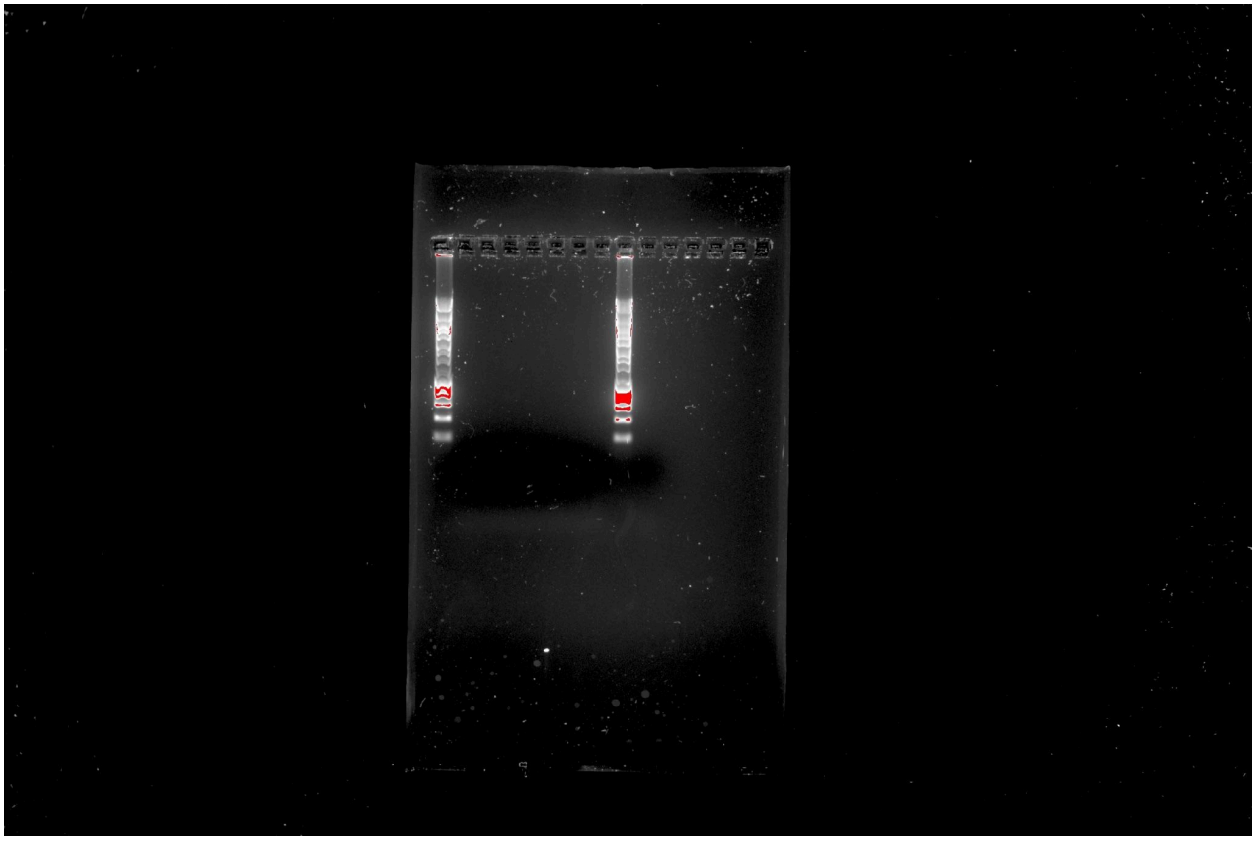
Well Number	Sample
1	Ladder
2	NdeI Control
3	BamHI Control
4	Undigested plasmid
All the rest	Samples



PCR Visualization of previous day

Samples were loaded onto 1% agarose gel, incubated for 8 minutes at 60V, followed by 30 minutes at 80V. 1 µL of each reaction was loaded onto the gel.

Well Number	Sample
1	Ladder
2	AKR1D1 Control
3	AKR1D1-His
4	AKR1D1-Trx
5	AKR1D1-SUMO
6	Ci2350 Control
7	Ci2350-Trx
8	ci2350-SUMO
9	Ladder
10	AKR1D1-MBP



The gel showed PCR was unsuccessful. After troubleshooting, it was found that the concentration of the added primers were too low, and that more of the PCR reaction needed to be loaded onto the gel (not only 1 µL).

Amplification PCR of inserts

PfuTurbo Hotstart PCR Master Mix (2x) was used for amplification. Forward primer is the same for all constructs. Reverse primer is construct dependent. One reaction was made for all inserts that were amplified (ci2350+SUMO, ci2350+TrxA, AKR1D1, AKR1D1+SUMO, AKR1D1+TrxA, AKR1D1+MBP). One control was made for each set of primers (2 controls in total, one for ci2350 set, one for AKR1D1 set). 2 reactions were made for each insert, with a total of 12 reactions + 2 controls. Reaction concentrations were corrected and a master mix prepared with water, Pfu Hotstart Master Mix and forward primer, which are in common for all samples.

For future PCR experiments: prepare master mix (count 2 reactions more due to volume lost pipetting) and add the different components afterwards (different primer, insert etc…) to avoid errors.

Component	Amount per reaction (µL)	Final concentration
Pfu Hotstart Master Mix (2x)	25	1x
Primer Forward (10 µM)	2.5	0.5 µM
Primer Reverse (10 µM)	2.5	0.5 µM
DNA Template (20 ng/µL)	0.5	10 ng/µL
MilliQ H2O	22	
Total volume	50	

Reaction parameters are based on Agilent Pfu guidelines, annealing temperature is based on primer melting temperature of 62°C - 5°C, 3 minutes are chosen in the elongation part based on the guideline of 1 min per kilobase considering the inserts vary from 1.3 kb to 2.3 kb.

Temperature (C)	Duration	Cycles
95	2 minutes	1
95	30 seconds	30
57	30 seconds	
72	3 minutes	
72	10 minutes	1
4	infinite	-

The gel was visualized the next week, so the gel picture is in next week's notes.

Bradford Assay for Protein Quantification

The BSA standard curve was created following the protocol attached. Samples (B - F) were duplicates and values reported in the table below. Sample A was used as a blank and therefore not included. The buffer used was the same one used for protein purification (mM NaPHO, mM NaCl).

Sample	Absorbance - S1	Absorbance - S2	Absorbance - Average
B	0,62	0,606	0,613
C	0,926	0,928	0,927
D	1,202	1,215	1,2085
E	1,311	1,337	1,324
F	1,354	1,328	1,341

Ci2350 - His Tag (WT)

Sample	Absorbance (y)	[Protein] µg/mL	Washing Buffer
E.A1	1,433	4,890447922	20mM [Imidazole]
E.A2	0,919	2,116567728	
E.B1	1,408	4,75553157	50mM [Imidazole]
E.B2	0,772	1,323259579	
E.C1	1,289	4,113329736	100mM [Imidazole]
E.C2	0,6	0,395035078	

Elution 1 - [Imidazole] 200 mM
Elution 2 - [Imidazole] 500 mM

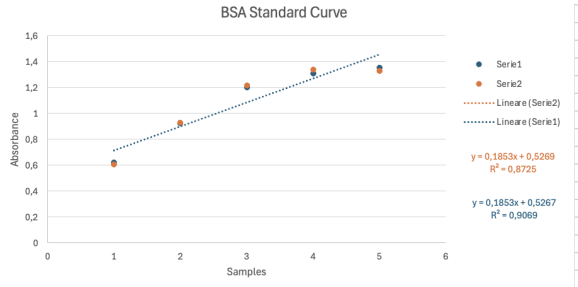
Ci2350 -MPB (TAG)

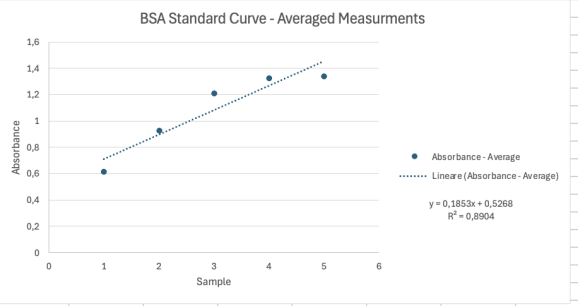
Sample	Absorbance (y)	[Protein] µg/mL	Washing Buffer
E.A1	1,367	4,534268753	20mM [Imidazole]
E.A2	1,299	4,167296276	
E.B1	1,401	4,717754992	50mM [Imidazole]
E.B2	1,096	3,071775499	
E.C1	1,427	4,858067998	100mM [Imidazole]
E.C2	1,19	3,579060982	

Elution 1 - [Imidazole] 200 mM
Elution 2 - [Imidazole] 500 mM

Equation	x= (y-0,5268)/0,1853

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





Protein concentration was determined from the equation given by the average absorbance of the duplicates. After the obtained results we decided to continue with condition A and B for washing buffer (20 and 50 mM imidazole concentration) and a single elution with 500mM Imidazole.