Week 28 - Overview

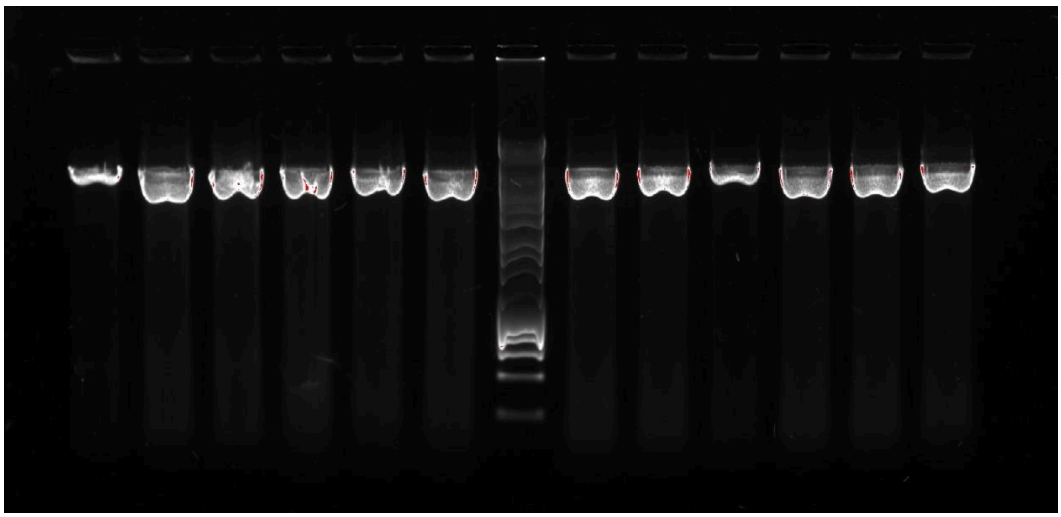
Week 28 - Overview..... 1
Day 15: [July 6, 2026], Monday.....5
Day 16: [July 7, 2026], Tuesday..... 11
Day 17: [July 8, 2026], Wednesday..... 17
Day 18: [July 9, 2026], Thursday..... 21
Day 19: [July 10, 2026], Friday.....29

Weekly summary:

Week 28 focused on troubleshooting restriction digestion of pET-11a with NdeI and BamHI enzymes, and on redoing transformations of *E. coli* TG1 with our inserts. To reduce loss of digested plasmid during gel electrophoresis and extraction, we tested a PCR cleanup kit. Based on our lab supervisor Camille, we developed a new digestion protocol aimed at shortening the reaction time. Sequencing of positive transformations was carried out to check insert integrity, but unfortunately it revealed mutations. We received a safety introduction for handling testosterone, as it is a CMR reagent requiring special safety precautions.

Main results of the week:

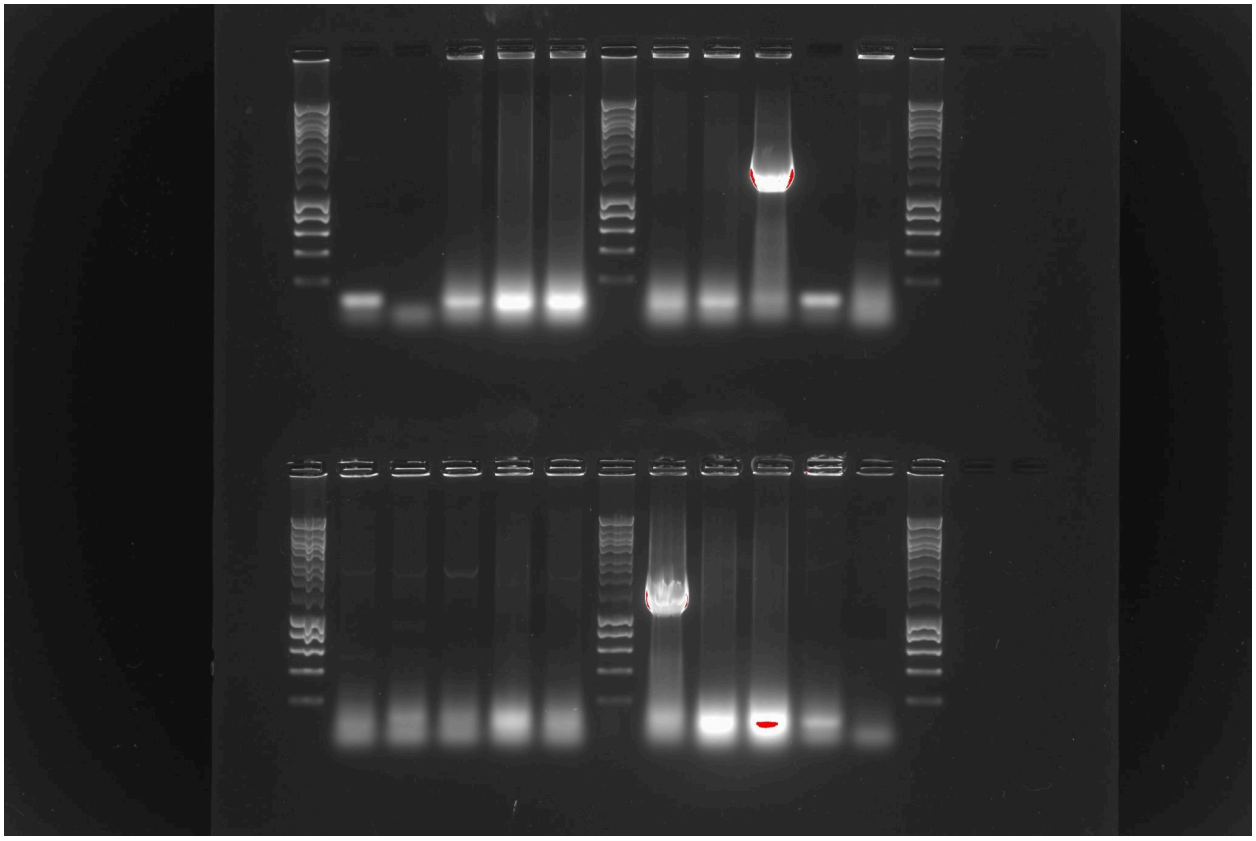
After visualising restriction digestion of pET-11a on Monday, we found that the NdeI control was invalid, due to adding BamHI by mistake. The gel was discarded, since we were unsure whether to proceed, due to compromised control. Digestion was redone the next day, where the controls were set up correctly.



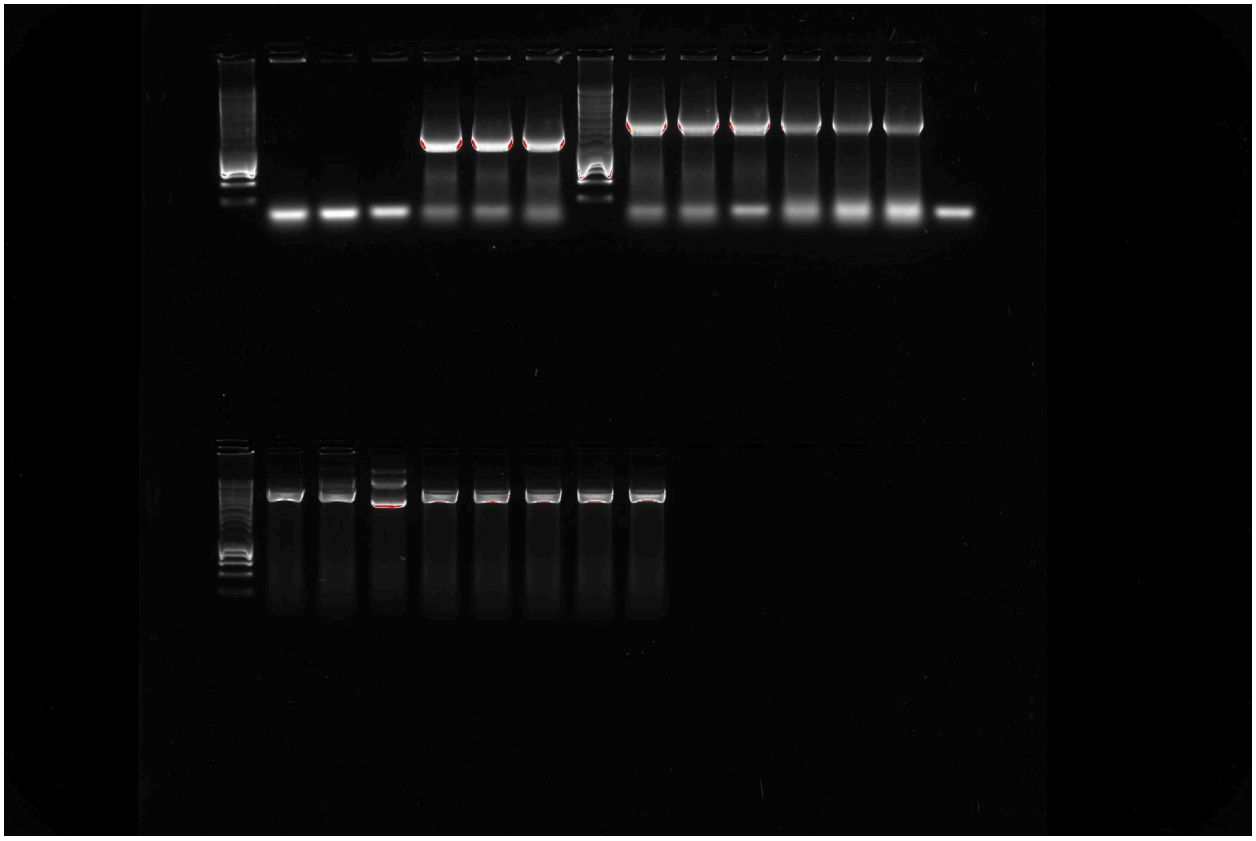
Restriction digestion of pET-11a was also carried out on Tuesday, followed by gel extraction and NanoDrop readings. The absorbance ratios were interesting to say the least. Nucleic acid concentrations were really low, and A260/280 were unusually high. The samples were discarded, and the digestion was repeated in the following days, using a PCR cleanup kit instead of gel extraction. Camille suggested this, as the yields are usually higher than gel extraction.

Sample	**Concentration (ng/µL)**	**Abs 260/280**	**Abs 260/230**
AP	0.994	-	0.133
Undigested plasmid	2.485 / 1.491	2.5 / 3.0	0.161 / 0.111
pET 2A	2.485	5.000	0.076
pET 2B	1.988	4.000	0.049
pET 2C	6.461	1.857	0.333
pET 4A	4.970	2.500	0.213
pET 4B	5.964	2.400	0.194
pET 4C	7.952	1.778	0.112
pET 4D	0 / 0.994	0 / 1.000	0 / 0.154
pET 4E	6.958	2.000	0.389
pET 4F	10.4	2.333	0.420

Colony PCR was performed on transformations from last week, retesting the colonies from July 1st, and adding AKR1D1-Trx and ci2350-Trx colonies from July 3rd transformation. The gel showed different results from last week, as new colonies were positive for the inserts, while the colony positive last week was now negative. Sequencing the positive colonies revealed mutations in all samples, rendering them unsuitable for further use.



E. coli TG1 and BL21 were successfully transformed with ci2350-MBP (colony PCR results shown on gel below). The insert was ordered in a plasmid from Twist Bioscience, as the insert was too large to be synthesized as a gBlock. Additionally, *E. coli* BL21 was transformed with empty pET-11a, which will serve as a comparison on SDS-PAGE during protein expression, making it easier to identify our overexpressed protein.



Digestion reactions of pET-11a were set up in larger volumes (100 µL) to avoid wasting tubes and reduce the amount of pipetting needed. Digested pET-11a was cleaned up using a PCR cleanup kit, instead of gel extraction. Gel electrophoresis was still performed to confirm digestion, but using a smaller aliquot of the reaction (20 µL).

Digested pET-11a	Concentration (ng/µL)	Abs 260/280	Abs 260/230
1	17.4	1.842	0.077
2	14.9	1.765	0.400
3	14.4	1.813	0.592
4	12.4	2.083	0.134
5	12.9	1.857	0.325
6	13.9	1.867	0.384
Pooled Plasmid	**16.9**	**1.789**	**0.206**

Day 15: [July 6, 2026], Monday

Weekly responsible: Patrik
Lab participants: Hema, Cecilia and Ruiqi

Daily overview of experiments

Digestion of the samples from Friday (July 3, 2026) was performed. pET-11a samples from Friday numbered as 1, 3, 5 and 6 were completely used.

Activity	***Protocol File***	***Comments***
Restriction digestion of plasmid pET-11a with NdeI and BamHI	Johan's Restriction D…	Samples for pET-11a: 1, 3, 5, 6. DNA amount increased to 1µg per sample. Control with BamHI; no control for NdeI
Agarose gel electrophoresis of digested pET-11a plasmid	Gel electrophoresis.d…	0.8% agarose. Samples - 12 and a ladder
Colony PCR	Colony PCR.docx	Checking transformants from July 3rd, along with ci2350+Trx and AKR1D1+Trx
Agarose gel electrophoresis of colony pcr	Gel electrophoresis.d…	To visualize Colony PCR, 1% agarose

Results and interpretation

Restriction digestion of plasmid pET-11a with NdeI and BamHI

Reaction volumes for different samples for a total volume of 21 µL after the addition of and BamHI and Dephosphorylation solution (Fast AP). A larger amount of DNA was added as the amount of plasmid was set as 1 µg instead of 0.25 µg as mentioned in the protocol.

Note: some of the samples (pET 1 normal and pET 5C) were done incorrectly, with **less total volume of the reaction**, because there was no DNA left for it. MilliQ-H2O should have been added, to compensate for the lack of volume of plasmid. Not adding this resulted in the buffer and enzymes to be almost double the concentration compared due to less volume.

Another Note: To the **NdeI control, BamHI was added by mistake**. This resulted in this sample not being a control anymore, as it was the same as other samples.

Sample	**DNA [µL]**	**FD Buffer (µL)**	**Water (µL)**	**NdeI (µL)**	**BamHI (µL)**
pET 1 normal	11.36	2	4.64	1	1
BamHI control	11.36	2	5.64	0	1
Nde1 control (not used)	11.36	2	4.64	1	1
pET 3A	14	2	2	1	1
pET 3B	14	2	2	1	1
pET 3C	14	2	2	1	1
pET 5A	11.64	2	4.37	1	1
pET 5B	11.64	2	4.37	1	1
pET 5C	11.64	2	4.37	1	1
pET 6A	12.28	2	3.73	1	1
pET 6B	12.28	2	3.73	1	1
pET 6C	12.28	2	3.73	1	1

Gel Electrophoresis of the digested plasmid

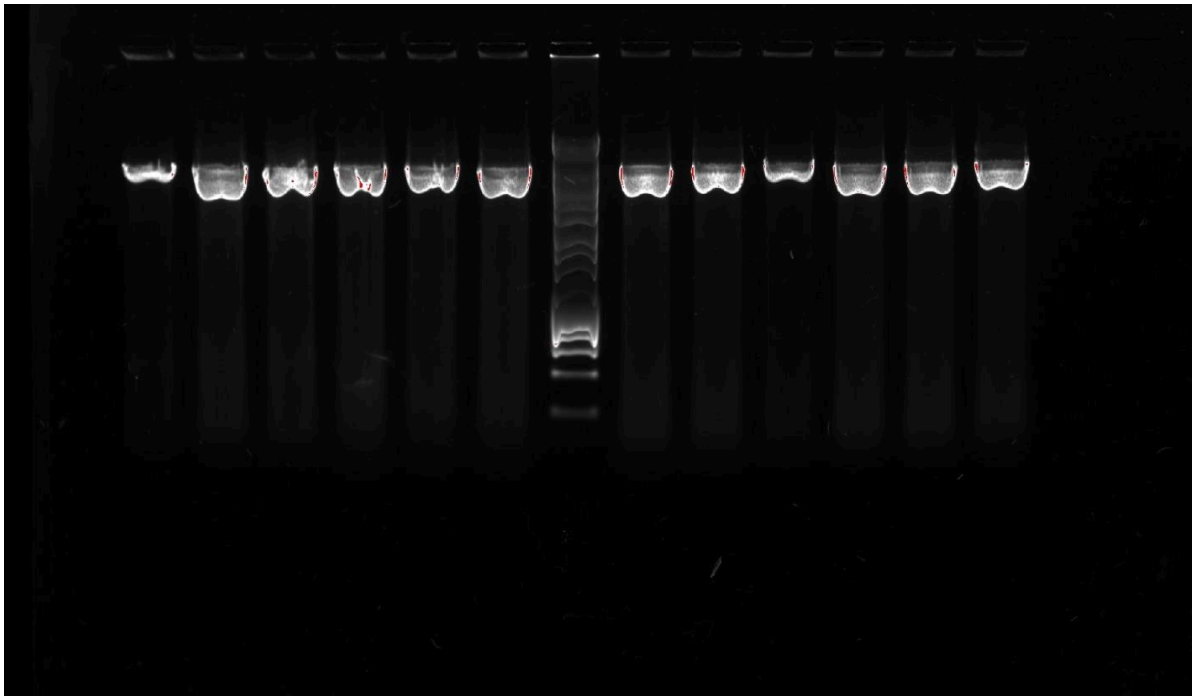
The agarose gel used had 1% of agar. So, 150 mL of gel had 1.5 g of agar. Undigested plasmid was forgotten to put onto the gel, and we could not compare it with our digested samples. We have to redo the experiment tomorrow.

Note: the TAE buffer has been reused a couple of times, so it would be better to change it tomorrow.

Plasmid Sample pET 1 was divided into three reactions: 2 controls and 1 normal digestion. Other samples pET 3, pET 5 and pET 6 were divided into 3 normal digestion samples.

Gel lane Order:

Lane	**Sample**
1	pET 1A
2	BamHI control
3	NdeI control (not an actual control)
4	pET 3A
5	pET 3B
6	pET 3C
7	Marker (1.5 µL of Ampliqon High Range)
8	pET 5A
9	pET 5B
10	pET 5C
11	pET 6A
12	pET 6B



Colony PCR

Colonies transformed yesterday were tested, along with ci2350+Trx and AKR1D1+Trx colonies made on July 3rd.

Same primers were used for all ci2350 genes, and the same primers were used for all AKR1D1 genes. An identical PCR reaction was made on July 2nd, with different primers. Jena Bioscience Red Load Taq MasterMix was used.

Gene	Primer Fw	Primer Rv
ci2350	T7 pro fw	Ci2350 rv
AKR1D1	T7 pro fw	AKR1D1 rv

Reagent	Volume
Red Load Taq Master	4 µL
Fw Primer (10 µM)	0.8 µL

Rv Primer (10 µM)	0.8 µL
MQ H2O	14.4 µL

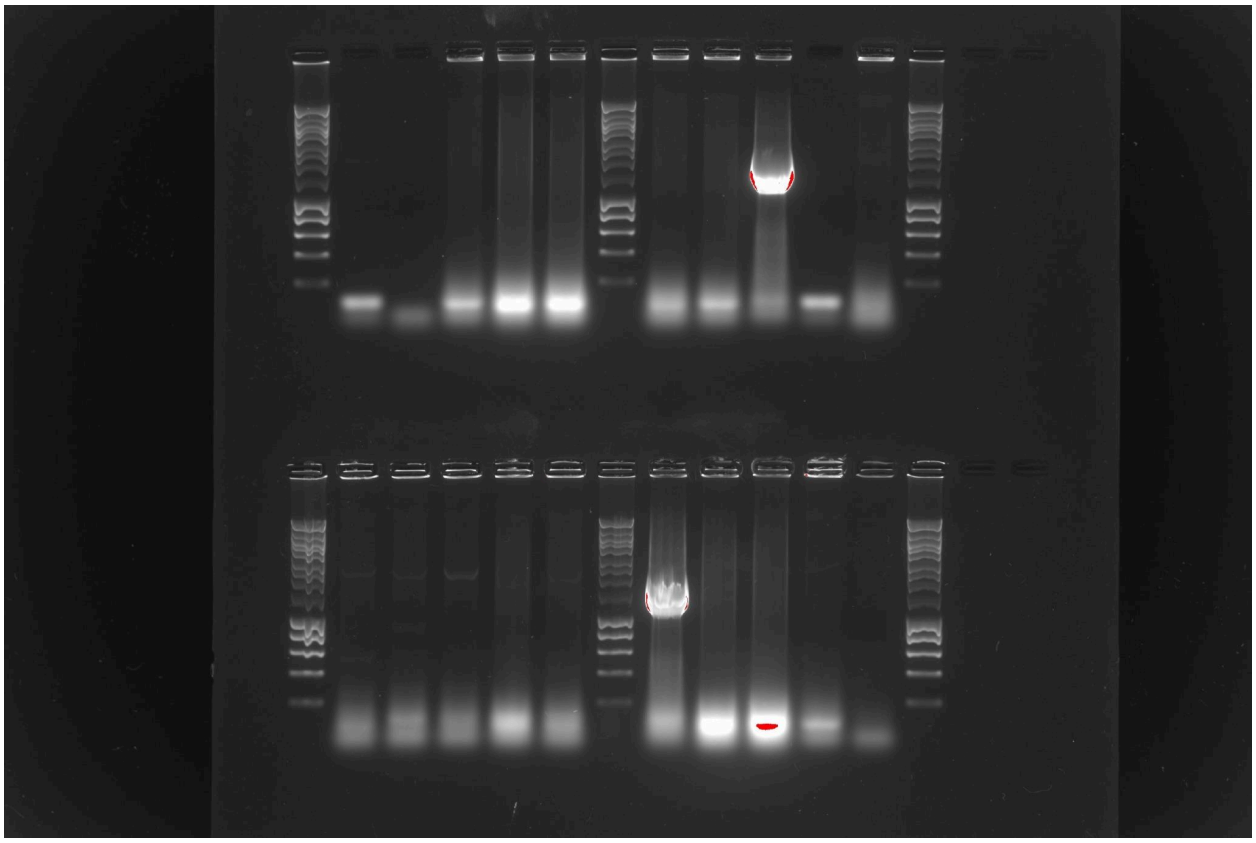
Number	Sample	Number	Sample
1	ci2350 PCR control	11	AKR1D1
2	AKR1D1 PCR control	12	AKR1D1
3	ci2350 Neg. control	13	AKR1D1 SUMO
4	AKR1D1 Neg. control	14	AKR1D1 SUMO
5	ci2350 Pos. control	15	AKR1D1 SUMO
6	AKR1D1 Pos. control	16	AKR1D1 MBP
7	ci2350 SUMO	17	AKR1D1 MBP
8	ci2350 SUMO	18	AKR1D1 MBP
9	ci2350 SUMO	19	ci2350 Trx
10	AKR1D1	20	AKR1D1 Trx

Samples 19 and 20 were taken from Friday's July 3rd transformation, and the rest were retested from July 2nd.

Number	Sample	Number	Sample
1		14	
2	ci2350 PCR control	15	AKR1D1
3	AKR1D1 PCR control	16	AKR1D1
4	ci2350 Neg. control	17	AKR1D1 SUMO
5	AKR1D1 Neg. control	18	AKR1D1 SUMO
6	ci2350 Pos. control	19	AKR1D1 SUMO
7		20	
8	AKR1D1 Pos. control	21	AKR1D1 MBP

9	ci2350 SUMO	22	AKR1D1 MBP
10	ci2350 SUMO	23	AKR1D1 MBP
11	ci2350 SUMO	24	ci2350 Trx
12	AKR1D1	25	AKR1D1 Trx
13		26	

After PCR, the samples were loaded onto the gel (big gel, 1% agarose), in the same order. Ladders were added (Ampliqon High Range DNA Ladder, 1.5 µL), as seen on the picture.



After visualising the gel, it was confirmed that samples 8 (ci2350 SUMO) and 16 (AKR1D1 MBP) were positive! They were inoculated overnight for sequencing prep the next day.

Note: it was realised that the Colony PCR results did not match the results from July 2nd, and the same colonies were tested (plus 2 extra today). After sequencing, the colonies found positive today indeed did have an insert, which could make us doubt if other colonies did have inserts which did not show on Colony PCR.

Day 16: [July 7, 2026], Tuesday

Weekly responsible: Patrik
Lab participants: Ruiqi, Hamsi, Cecilia

Daily overview of experiments

We received a testosterone handling introduction by our PI Johan. Repeating restriction digestion using a fast protocol. Sending ci2350-SUMO and AKR1D1-MBP for sequencing.

Note: try another faster protocol for digestion, by putting all enzymes at once
Note: change the TAE buffer for the gel, reusing it might have caused the wavy bands

Activity	***Protocol File***	***Comments***
Testosterone introduction	Testosterone Handlin…	
pET-11a restriction digestion	Simple Restriction Di…	Fast AP was added.
1x TAE buffer preparation	Gel electrophoresis.d…	40mL of 50x TAE with 1.96 L of distilled H2O; pH =8.4
Agarose Gel Electrophoresis	Gel electrophoresis.d…	TAE buffer was changed
Gel Extraction	Gel Extraction from A…	Elution: 2x 25µL of EB (heated at 60°C)
Plasmid miniprep of transformants	QMF28.0029.D6942 …	
3M NaOH Preparation	File	Diluted from 5M NaOH, combining 60 mL 5M NaOH and 40 mL DI H2O
Sequencing Prep	Colony PCR.docx	Sent out AKR1D1-MBP and ci2350-SUMO for sequencing

Results and interpretation

Restriction digestion of plasmid pET-11a with NdeI and BamHI

Testing a new protocol approach (developed by Patrik and Camille based on Thermo fisher protocol). Compared to the former protocol, the adjusted version adds NdeI and BamHI simultaneously with new incubation settings of 10min incubation on 37°C following a 5min inactivation step on 80°C. The total amount of plasmid in one reaction was 0.5 µg.

The pET vectors used were made by mini-prep on 3rd of July. The samples used were pET-11a duplicate 2 and 4 with a total volume of ~48µL (initial amount = 50µL, 2µL were used for nanodrop).

Plasmid concentrations:

- pET-11a, duplicate 2; concentration = 85 ng/µL
- pET-11a, duplicate 4; concentration = 74.6 ng/µL

Reagent	AP Control	NdeI Control	BamHI Control	pET11a 2	pET11a 4
FD Buffer Green (µL)	3	3	3	3	3
Plasmid (µL)	5.88	5.88	5.88	5.88	6.70
NdeI (µL)	1	1	0	1	1
BamHI (µL)	1	0	1	1	1
FAST AP (µL)	0	1	1	1	1
MiliQ H2O (µL)	9.12	19.12	19.12	18.12	17.3
Total volume (µL)	20	30	30	30	30

Agarose gel electrophoresis

Big gel (~150 mL) was made, with 1% agarose and 7.5 µL of GelRed. 2x15 wells were used. 1.5 µL of Ampliqon High Range Ladder was used. Gel settings were set to: 60V for 10 min, 90V for 45min.

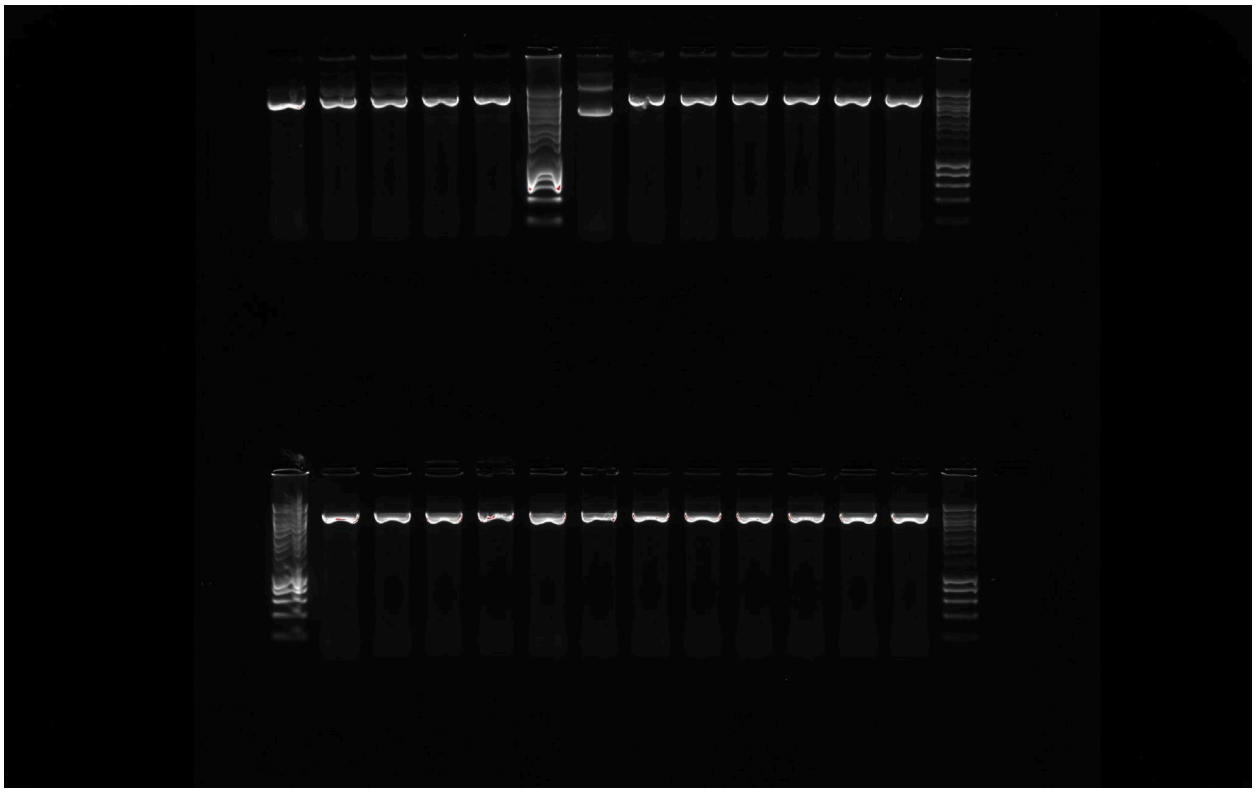
Samples were 30µL - split into 15µL/well leading to two duplicates (dup1; dup.2), as 30 µL was too much to load into one well. An exception is the AP control which is 20µL of which 15µL was put into the well.

For the undigested plasmid, 0.25 µg solution was made, and diluted in 5x DNA Loading Buffer Orange from Ampliqon. (2.94 µL of pET11-2 with 9.06 µL of MiliQ H2O and 3 µL of Loading Orange Buffer).

Wells order:

Well number	Lane 1	Lane 2
1	AP control	Ladder
2	NdeI control	pET-4 A
3	NdeI control	pET-4 A
4	BamHI control	pET-4 B
5	BamHI control	pET-4 B
6	Ladder	pET-4 C
7	Undigested pET-2	pET-4 C
8	pET-2 A	pET-4 D
9	pET-2 A	pET-4 D
10	pET-2 B	pET-4 E
11	pET-2 B	pET-4 E
12	pET-2 C	pET-4 F
13	pET-2 C	pET-4 F
14	Ladder	Ladder
15	-	-

Comment: while applying samples pET-4 A, B, C to the wells, the first duplicate had more volume, the second less than 15 µL due to incorrect pipetting. The difference should be small.



Gel Extraction

Digested plasmid was cut out from the gel, and extraction was done with the E.Z.N.A. kit. Two duplicates from the gel that belonged to the same reaction aliquot were collected into the same extraction tube, this was done for all duplicates. Two elution steps were done, with 25 µL each. Elution buffer was preheated to 60C.

NanoDrop readings are presented the following day.

Johan Testosterone introduction

Testosterone is a CMR and doping agent. Johan ordered 250 mg of testosterone in powder form. If in higher concentrations, testosterone can be a CMR substance, that's why our powder form is ‘restricted’, if it were dissolved in a smaller concentration, it would not have been a CMR agent.

Every time someone takes some of the dry testosterone, it needs to be documented and written down, so anyone can take a look at how much testosterone is left. There is no need to write down when we use it from the stock solution.

Camille, Johan and Eleonora are allowed to handle powder testosterone. Camille will make a testosterone solution each week for the current week, so we need to plan in advance how much she needs to make. Dry powder will be locked in, only Camille has access to it.

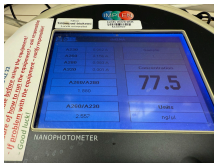
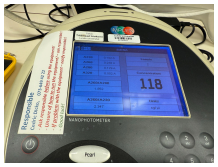
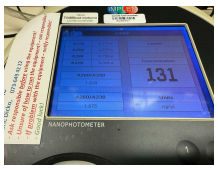
Furthermore, if testosterone is out of 'the locker', it needs to be under supervision at all times. However, after it is dissolved, it is not a viable doping agent (due to low concentration and the organic solvents used to dissolve (methanol/acetonitrile) because you would probably die due to the solvent toxicity. Diluted testosterone is considered flammable material (due to methanol), so it needs to be in the 'flammable chemicals storage cabinet'. Tips used to pipette testosterone stock solutions will be put into separate tip bags and in the chemical storage?? (or the bacterial cultures, not sure)

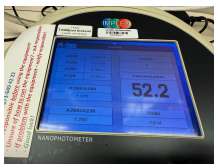
All of us are allowed to handle testosterone solutions at lower concentrations (1mg/mL and below), as in diluted form it is not a CMR agent anymore.

Testosterone is a chemical hazard, so we need to make a special chemical waste bin for it, in our fume hood.

Plasmid MiniPrep from overnight transformed cultures

Plasmid MiniPrep was made from ci2350-SUMO and AKR1D1-MBP overnight cultures, according to E.Z.N.A. protocol. 4.5 mL of culture was taken from each tube. Elution was done 2 times of 25 µL, with a heated elution buffer to 60ºC. NanoDrop measurements were taken:

Sample	Concentration (ng/µL)	A260/280	A260/230	**Pictures**
ci2350-SUMO 1	77.5	1.880	2.557	
ci2350-SUMO 2	118	1.852	2.347	
AKR1D1-MBP 1	131	1.839	1.675	

AKR1D1-MBP 2	52.2	1.875	1.780	

Preparation for Mix2Seq

After evaluation of results, samples were prepared for Mix2Seq. Sequencing reactions were made as following:

Note: AKR1D1-MBP had to be diluted to fall into the range of 50-100 ng/µL, that's why it was taken 2.5 µL DNA and 2.5 µL water. ci2350-SUMO was already in the range, and 5 µL was taken directly.

	ci2350-SUMO	AKR1D1-MBP
MiliQ H2O	2.5	5
DNA	5	2.5
Primer (10 µM)	2.5	2.5
Total volume	10	10

All of the codes begin with EF735913**, the stars are written in the table below, corresponding to the primer added to the mixture.

	ci2350-SUMO			AKR1D1-MBP		
Primer	T7 fw	T7 rv	ci2350 fw	T7 fw	T7 rv	AKR1D1 fw
Code	36	26	25	33	34	35

Day 17: [July 8, 2026], Wednesday

Weekly responsible: Patrik
Lab participants: Hema, Ruiqi and Cecilia

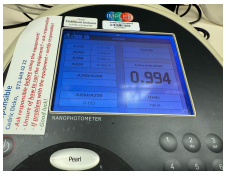
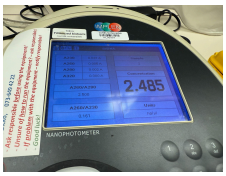
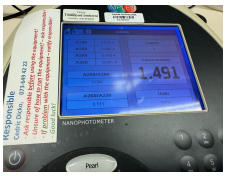
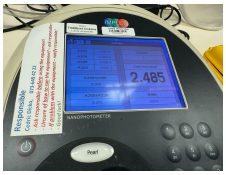
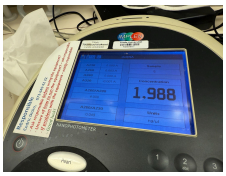
Daily overview of experiments

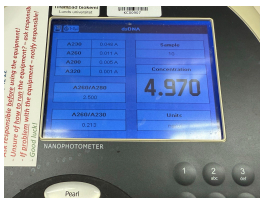
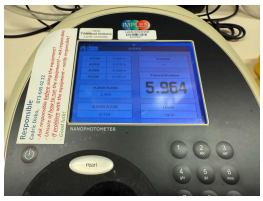
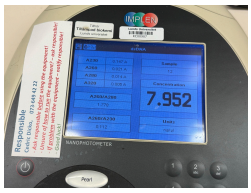
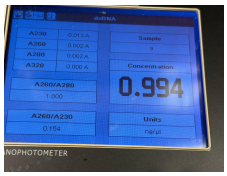
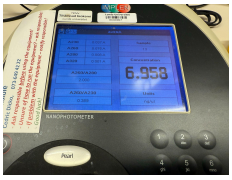
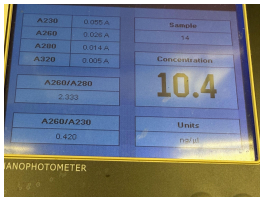
Autoclaving was done and ci2350-MBP plasmid was resuspended and transformation of *E. coli* TG1 and BL21(DE3) with ci2350 + MBP was performed. We have to wait till 1 p.m in the afternoon to get the delivery for us and hope that it has ligase so that we can do ligation in the afternoon. It was not ligase afterall, but some salts.

Activity	***Protocol File***	***Comments***
Autoclaving	Autoclaving.docx	We autoclaved pipette tips (3 boxes each of 10 and 200 µl and 2 boxes of 1 mL tips), 5 tiny bottles of MQ water and aliquoted MQ water in 0.5 mL tubes and empty glass bottles just in case we would need it at a later time.
Resuspension of ci2350+MBP plasmid	DOC_4045_DNA_R…	1 aliquot was made of 5 µL, labeled 'MBP ci2350 plasmid', and put into the yellow box in the freezer
Transformation of E. coli TG1 and BL21 with ci2350+MBP	E. coli Transformatio…	Adjusted protocol
Transformation of E. coli BL21 with empty pET11 and ci2350+His	E. coli Transformatio…	Adjusted protocol
Inoculation of E. coli TG1 pET-11a for overnight incubation		Inoculated 10 LB Medium tubes for plasmid miniprep and digestion tomorrow.
Streaking a E. coli TG1 backup plate		Due to the oldness of the plates Johan streaked.

Results and interpretation

Nanodrop measurements of digested samples from the previous day. The blank was 2 µL of the elution buffer.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Pictures
AP	0.994	-	0.133	
UD (Undigested plasmid)	2.485 / 1.491	2.5 / 3.0	0.161 / 0.111	 
pET 2A	2.485	5.000	0.076	
pET 2B	1.988	4.000	0.049	
pET 2C	6.461	1.857	0.333	

pET 4A	4.970	2.500	0.213	
pET 4B	5.964	2.400	0.194	
pET 4C	7.952	1.778	0.112	
pET 4D	0 / 0.994	0 / 1.000	0 / 0.154	
pET 4E	6.958	2.000	0.389	
pET 4F	10.4	2.333	0.420	

Note: these concentrations are really low, and most of them have really high 260/280 ratios, so another round of digestion will be done, which will be purified by PCR-cleanup. Camille suggested this, as the yields are usually higher than gel extraction.

Resuspension of ci2350+MBP Plasmid from Twist Bioscience

Plasmid has been resuspended in 20 µL of MiliQ Water according to Twist Bioscience protocol. The plasmid was not heated, as that was an optional step. Final concentration of diluted plasmid

is: 43.45 ng/µL. 2 µL were taken from the transformation, and 5 µL aliquot (‘MBP ci2350 plasmid’, and put into the yellow box in the freezer) was made, leaving 11 µL in the stock solution.

Transformation of *E. coli* TG1 and BL21 with ci2350+MBP plasmid

The plasmid ci2350+MBP plasmid was transformed into competent *E. coli* TG1 and *E. coli BL21*. Protocol steps were followed, with slight adjustments. 50 µL of competents cells (for both strains) were used instead of 200 µL, as Johan advised to lower the amount. Negative control was added, with just 1 µL of MiliQ Water. Normal samples were transformed with 1 µL of resuspended ci2350+MBP plasmid, meaning 43,45 ng of plasmid was used for transformation in one sample. Cells were incubated for 50 minutes during the initial ice incubation step, and then 50 minutes during the LB Medium shaking step.

In total 4 samples were transformed:

- Negative control for *E. coli* TG1
- *E. coli* TG1 ci2350+MBP sample
- Negative control for *E. coli BL21*
- *E. coli BL21* ci2350+MBP sample

Transformation of *E. coli* BL21 with ci2350+His plasmid

After the ci2350+His plasmid was confirmed by sequencing on June 30th, E. coli BL21 was transformed with it. Previously minipreped ci2350+His plasmid that was sequenced was used for transformation. 0.5 µL of the plasmid was used (134 ng/µL x 0.5 µL = 67 ng) for transformation. The empty pET-11a vector was also transformed into BL21, which could serve as a comparison during the SDS-PAGE gel, or protein expression. Negative control was added (only MilliQ H2O).

Day 18: [July 9, 2026], Thursday

Weekly responsible: Patrik
Lab participants: Ruiqi, Cecilia, Hema, Hamsi

Daily overview of experiments

Colony PCR was done on yesterday's transformants to double check if transformation was successful. Plasmid MiniPrep was done

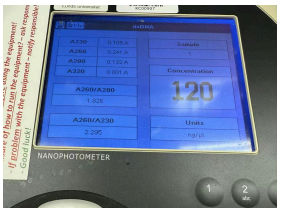
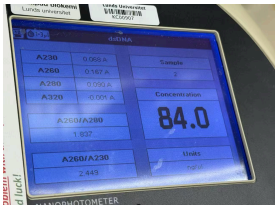
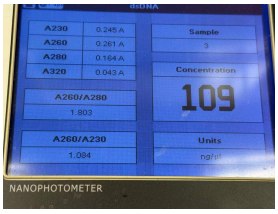
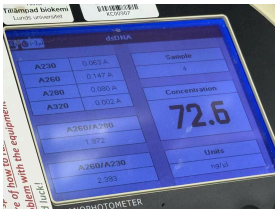
Activity	***Protocol File***	***Comments***
Colony PCR	Colony PCR.docx	To confirm yesterday's transformants.
3M NaOH Preparation	Self-calculated	Diluted from 5M NaOH
Plasmid pET-11a MiniPrep of overnight cultures	QMF28.0029.D6942 …	Done with EZNA Kit.
Nanodrop	DNA Concentration …	Samples pooled; final concentration = 72.15 ng/µL
Restriction Digestion of pET-11a	Simple Restriction Di…	
Agarose Gel Electrophoresis	Gel electrophoresis.d…	• Check Colony PCR • Check Restriction Digestion
PCR Cleanup Kit	NucleoSpin Gel and …	Testing if its a better option than Gel Extraction, it should be faster and with higher yield.

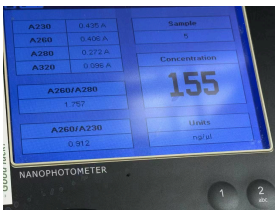
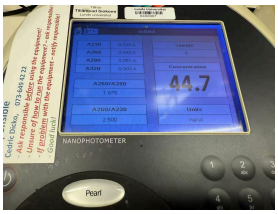
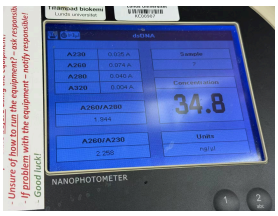
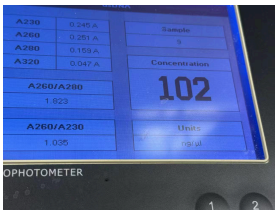
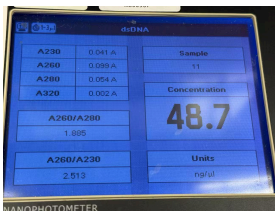
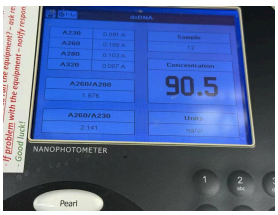
Results and interpretation

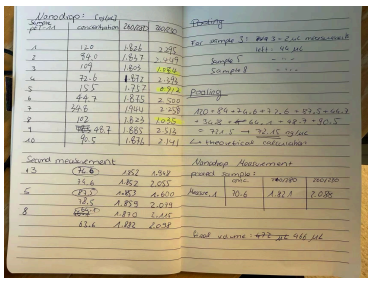
Plasmid MiniPrep pET-11a from overnight cultures

10 tubes with 5 mL LB Amp Medium were cultured overnight, followed by EZNA Plasmid MiniPrep. An additional washing step was done (Step 9). Elution was done in 2 stages (25 uL each, with the elution buffer preheated to 60C).

NanoDrop readings:

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Pictures
1	120	1.826	2.295	
2	84.0	1.837	2.443	
3	109	1.803	1.084	
4	72.6	1.872	2.393	

5	155	1.757	0.912	
6	44.7	1.875	2.500	
7	34.8	1.944	2.258	
8	102	1.823	1.035	
9	48.7	1.885	2.513	
10	90.5	1.876	2.141	

S3 re1	74.6	1.852	1.948	
S3 re2	74.6	1.852	2.055	
S5 re1	87.5	1.853	1.600	
S5 re2	78.5	1.859	2.079	
S8 re1	64.1	1.870	2.115	
S8 re2	63.6	1.882	2.098	
Pooled	**70.6**	**1.821**	**2.088**	

After NanoDrop was done, all ten samples were pooled together, and aliquots were made. Pooled mixture was also tested on NanoDrop with a concentration of 72.15 ng/µL. The pooled sample was aliquoted into 5x 100µL samples.

Restriction digestion of pET-11a, and inserts

After plasmid miniprep, restriction digestion of plasmid was done, by instruction from the ‘simple’ protocol, with each reaction having 0.5 µg of plasmid. One big reaction mix was made, with a total of 30 reactions (600 µL), which was then aliquoted into 6 tubes, with each tube containing 100 µL (totaling to 5 reactions per tube). Incubation was done at 37C for 15 minutes, followed by 80C inactivation for 5 minutes.

Digestion on inserts was also done, with each digestion having 200 ng of insert. The reaction consisted of 10 µL insert, 1 µL NdeI, 1 µL BamHI, 2 µL Fast Digest Buffer Clear and 6 µL MQ H2O.

Reagent	Volume for 1 Reaction (µL)	Volume for 30 Reactions (µL)	Control NdeI	Control BamHI
Fast Digest Buffer Green	2	60	2	2
NdeI	1	30	1	0
BamHI	1	30	0	1
Pooled pET-11a	7.08	212.4	7.08	7.08
MQ H2O	8.92	267.6	9.92	9.92
Total volume	20	600	20	20

Colony PCR to confirm yesterday's transformation

For colony PCR, Jena Bioscience Red Load Taq Master Kit was used. In total 14 reactions were made, with each reaction consisting of 20 µL. One big PCR mix was made containing 280 µL, enough for 14 reactions (including one extra reaction to account for pipetting errors).

Component	Volume per 1 reaction (µL)	Volume for 14 reactions (µL)
Red Load Taq Master (5x)	4	56
T7 promoter forward primer (10 µM)	0.8	11.2
Ci2350 reverse primer (10 µM)	0.8	11.2
PCR Grade Water	14.4	201.6
Total	20	280

In total 14 reactions were done:

- Tubes 1 - 3 → pET 11 E.coli BL21
- Tubes 4 - 6 → ci2350 His E.coli BL21
- Tubes 7 - 9 → ci2350 MBP E.coli BL21 positive
- Tubes 10 - 12 → ci2350 MBP E.coli TG1
- Tube 13 → PCR Negative control (no colonies added)
- Tube 14 → Autoclaved MilliQ Water (just water, no pcr mixture)

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	

Agarose gel electrophoresis for confirming successful Colony PCR and Plasmid Digestion

Big gel (~150 mL) was made, with 1% agarose and 7.5 µL of GelRed. 2x15 wells were used. 1.5 µL of Ampliqon High Range Ladder was used. Gel settings were set to: 60V for 15 min, 90V for 45min.

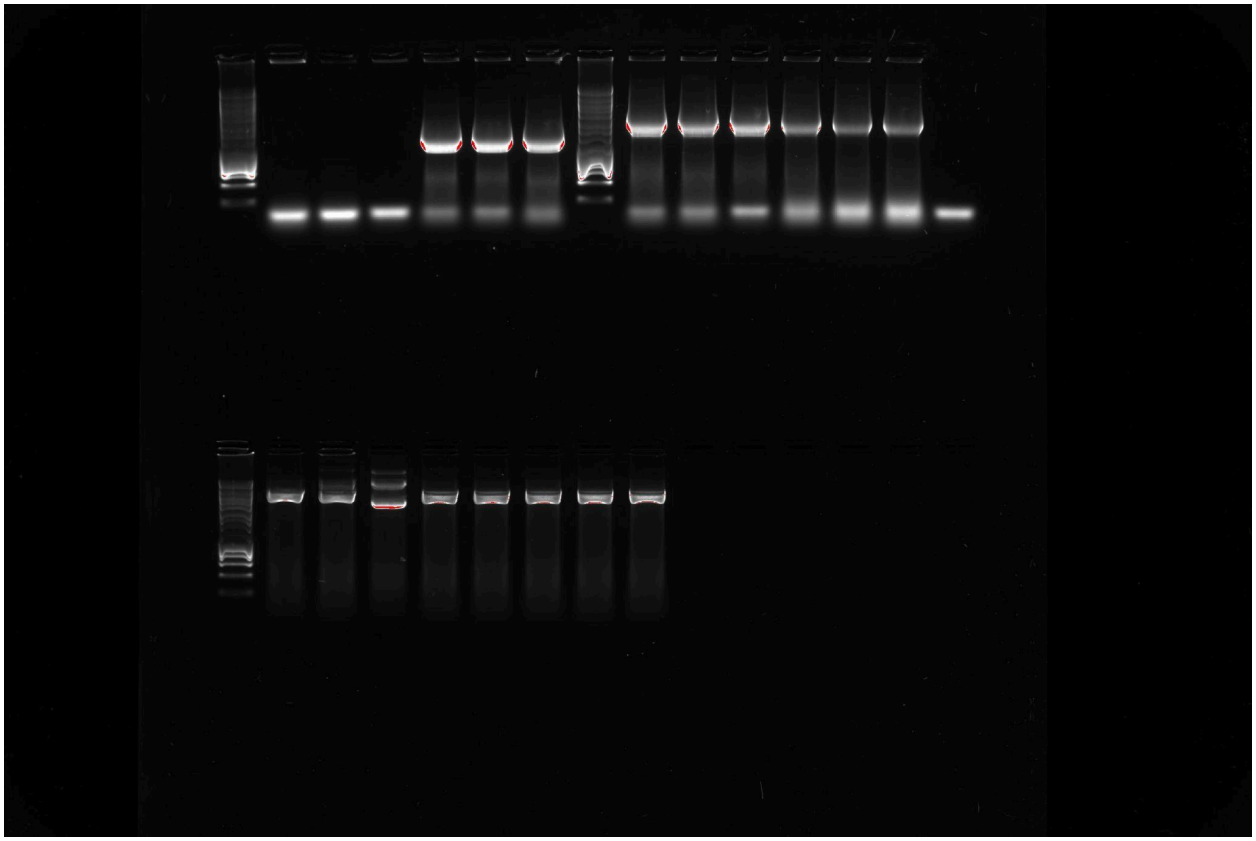
The upper lane contained the samples from colony PCR (volume loaded = 15µL/lame); lower lane from plasmid digestion (20µL/lane).

Wells order:

Well number	Upper	Well Number	Lower
15	PCR Control (no colonies)	30	-
14	E.coli TG1 ci2350 MBP	29	-
13	E.coli TG1 ci2350 MBP	28	-
12	E.coli TG1 ci2350 MBP	27	-
11	E.coli BL21 ci2350 MBP	26	-
10	E.coli BL21 ci2350 MBP	25	-
9	E.coli BL21 ci2350 MBP	24	Digested sample 5
8	Ladder	23	Digested sample 4
7	E.coli BL21 ci2350 His	22	Digested sample 3
6	E.coli BL21 ci2350 His	21	Digested sample 2
5	E.coli BL21 ci2350 His	20	Digested sample 1
4	E.coli BL21 PET 11	19	Undigested control

3	E.coli BL21 PET 11	18	Ndel control
2	E.coli BL21 PET 11	17	BamHI control
1	Ladder	16	Ladder

Note: Sample 6 from restriction digestion was forgotten to load on the gel, as the eppendorf tube was marked with ‘digestion mastermix’. Camille said there is no need to load all of the samples on the gel, as they are the same, and are aliquots from the mastermix. By loading more samples on the gel we are losing the sample volume for PCR Cleanup, and potentially decreasing our final purified concentration of digested sample.



PCR clean-up with Cytiva kit

After the gel confirmed successful plasmid restriction digestion, the rest of the samples (80 µL x 5 samples, + one sample of 100 µL which was forgotten to put onto the gel) were purified with PCR cleanup by Cytiva Kit, from last year. Elution was done with Elution Buffer 4 (grey buffer), with 2 x 25 µL. The Elution buffer was not prewarmed. Elution of digested inserts was done with 2 x 10 µL. NanoDrop measurements were taken.

After taking NanoDrop readings, the plasmids were pooled, to make it easier to do ligation, as only one concentration needed to be used, instead of 6 different ones.

NanoDrop Readings

Digested pET-11a	**Concentration (ng/μL)**	**Abs 260/280**	**Abs 260/230**
1	17.4	1.842	0.077
2	14.9	1.765	0.400
3	14.4	1.813	0.592
4	12.4	2.083	0.134
5	12.9	1.857	0.325
6	13.9	1.867	0.384
Pooled Plasmid	**16.9**	**1.789**	**0.206**

Digested insert	**Concentration (ng/μL)**	**Abs 260/280**	**Abs 260/230**
AKR1D1	8.449	1.889	0.121
AKR1D1+SUMO	12.9	2.187	0.019
AKR1D1+Trx	12.4	2.083	0.052
AKR1D1+MBP	8.449	1.889	0.148
ci2350+SUMO	5.964	2.000	0.096
ci2350+Trx	6.958	1.556	0.059

Day 19: [July 10, 2026], Friday

Weekly responsible: Patrik
Lab participants: Hema, Cecilia, Ruiqi, Hamsi

Daily overview of experiments

Testing the efficiency of different vector:insert ligation ratios, followed by transformation into *E. coli* TG1. Preparation of terrific broth and LB Agar, for future cultivations.

Activity	***Protocol File***	***Comments***
Ligation with different vector:insert ratios (1:1 & 1:3)	Ligation with pET-11…	ci2350+Trx ci2350+SUMO AKR1D1 AKR1D1+Trx AKR1D1+SUMO AKR1D1+MBP
Transformation of *E. coli TG1* with pET11-a containing inserts	E. coli Transformatio…	All inserts from ligation were transformed into E. coli TG1
Autoclaving	Autoclaving.docx	Autoclaving waste Autoclaving medium and tips
Medium Preparation	Media preparation.docx	LB Agar Preparation and plating. Around 40 Amp plates were made
Terrific Broth Preparation	TB-medium.doc	900mL of Solution A and 100 mL of Solution B was made

Results and interpretation

Ligation of pET-11a with our inserts

For ligation reactions, plasmid extracted on July 9 was used (Sample S5), with 70 ng of plasmid in each ligation sample for 1:1 ratios and 40 ng of sample for 1:3 ratios. Inserts are ci2350+Trx, ci2350+Sumo, AKR1D1, AKR1D1+Trx, AKR1D1+Sumo, AKR1D1+MBP, and 1 control without insert.

Ligation Reaction Calculations
Insert (ng)= (Insert size/vector size) *Vector amount(ng)* 3

Ligation reaction 1

	Size	Concentration	Insert (ng)	Volume (µL)
AKR1D1	1032	8.449	21.81	2.589
AKR1D1 +SUMO	1335	12.9	28.22	2.18
AKR1D1 +Trx	1368	12.4	28.92	2.33
AKR1D1+MBP	2142	8.449	45.28	5.36
ci2350+SUMO	2283	5.964	48.26	8.09
Ci2350 +Trx	2316	6.958	48.96	7.04

Vector length = 5677 bp
Pooled Concentration of pET 11 = 16.9 ng/µL
40 ng of vector
Vector : insert molar ratio = 1:3

Volume of vector to be added = 2.3 µL

Ligation Reaction mixtures for 40 ng (1:3)

	T4 Buffer (µL)	Volume of vector (µL)	Volume of insert (µL)	Ligase (µL)	Water (µL)
AKR1D1	2	2.3	2.59	2	11.11

AKR1D1 +SUMO	2	2.3	2.18	2	11.52
AKR1D1 +Trx	2	2.3	2.33	2	11.37
AKR1D1+MBP	2	2.3	5.36	2	8.34
ci2350+SUMO	2	2.3	8.09	2	5.61
Ci2350 +Trx	2	2.3	7.04	2	6.66
Negative Control	2	2.3	0	2	13.7

Note: 2 µL of T4 DNA Ligase have been added. Old protocol was followed instead of a new one.

Ligation reaction 2

Vector length = 5677 bp
Pooled Concentration of pET 11 = 16.9 ng/µL
70 ng of vector
Vector : insert molar ratio = 1:1
Digested vector amount =0.02 pmol
Volume of vector to be added = 4.4 µL

	Size	Concentration	Insert (ng)	Volume (µL)
AKR1D1	1032	8.449	12.73	1.50
AKR1D1 +SUMO	1335	12.9	16.46	1.28
AKR1D1 +Trx	1368	12.4	16.87	1.36
AKR1D1+MBP	2142	8.449	16.41	3.13
ci2350+SUMO	2283	5.964	28.15	4.72
Ci2350 +Trx	2316	6.958	28.56	4.1

Ligation Reaction mixtures for 70 ng (1:1)
Volume of vector to be added = 4.4 µL

	T4 Buffer (µL)	Volume of vector (µL)	Volume of insert (µL)	Ligase (µL)	Water (µL)
AKR1D1	2	4.4	1.50	0.2	12.16

AKR1D1 +SUMO	2	4.4	1.28	0.2	12.38
AKR1D1 +Trx	2	4.4	1.36	0.2	12.3
AKR1D1+MBP	2	4.4	3.13	0.2	10.53
ci2350+SUMO	2	4.4	4.72	0.2	8.94
Ci2350 +Trx	2	4.4	4.1	0.2	9.56
Negative Control	2	4.4	0	0.2	13.66

After making the 20 µL reaction mixture, let it react at room temperature for two hours.

Transformation of *E. coli* with pET11-a containing inserts

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 16 reactions were made; 2 transformation controls, 2 ligation controls and 12 transformations with inserts (7 of 1:1 ratio, and 7 of 1:3 ratio).

After transformation, plates were left at room temperature over the weekend.