Week 26 - Overview

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Weekly summary:

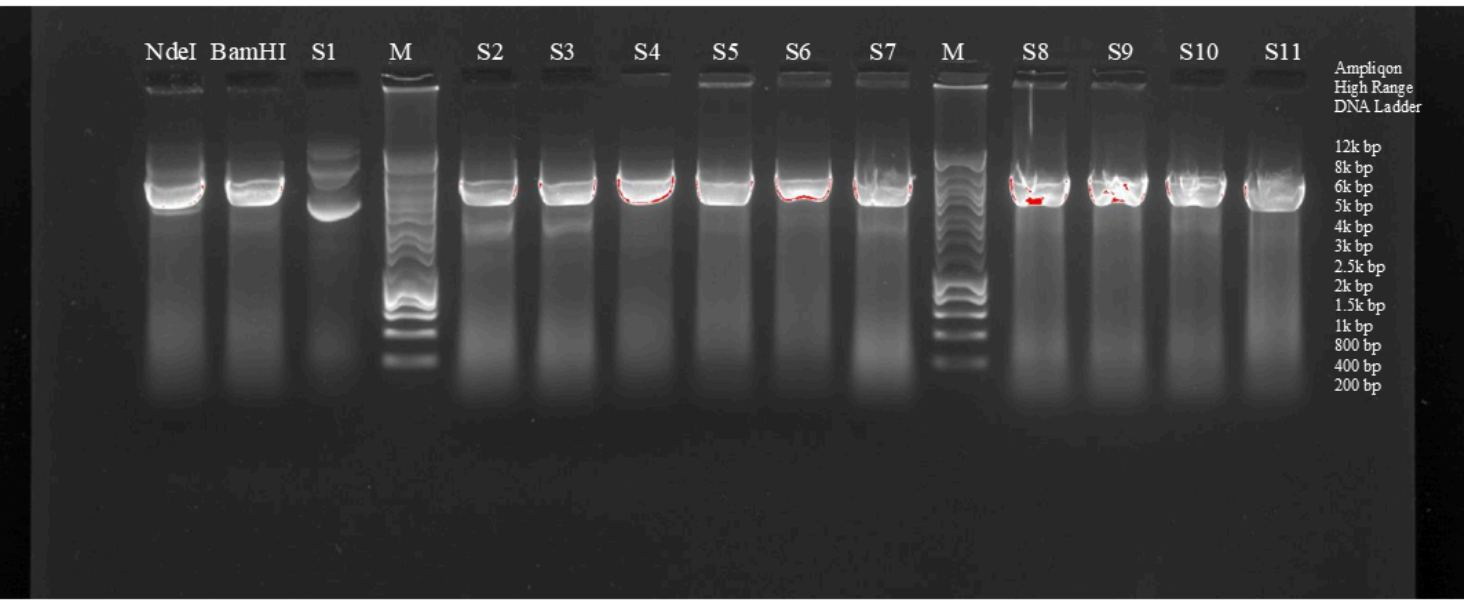
The second week of the lab was dedicated to repeating and troubleshooting restriction digestion of pET-11a with BamHI and NdeI. Different protocols were tested, with different incubations times. Additionally, another plasmid miniprep kit was tested, which resulted in purer samples. Digestion of ci2350-His was done, followed by ligation and successful transformation into *E. coli* TG1. Competent *E. coli* BL21 cells were prepared.

Main results of the week:

E.Z.N.A. plasmid miniprep kit was used as an alternative to Jena Bioscience kit, resulting in less concentration with higher purity. After consulting with our PI Johan, we concluded that samples prepared with Jena Bioscience Kit were contaminated with RNA, due to absorbance ratio 260/280 being ~2.0, and the concentration of nucleic acids being a lot higher than our PI usually gets. For future experiments, E.Z.N.A. plasmid miniprep kit was used.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
S1	73.1	1.838	2.410
S2	92.4	1.824	1.979
S3	75.5	1.831	1.810
S4 (column equilibrated)	93.9	1.853	2.305
S5 (column equilibrated)	95.9	1.804	2.474
S6 (column equilibrated)	112	1.852	2.306

Restriction digestion with BamHI and NdeI of plasmid extracted with E.Z.N.A. plasmid miniprep kit showed noticeably stronger bands on agarose gel electrophoresis. This supports our assumption that earlier plasmid samples were significantly RNA contaminated. When we thought we were quantifying plasmid concentration, it was mostly RNA, leaving only a small fraction of DNA for digestion. This would explain both the faint bands in earlier digestions and the low plasmid yields after gel extraction.



Quantification of digested plasmid after gel extraction showed better results than previously. We continued using E.Z.N.A. gel extraction kit, as it showed better results.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
S1 (positive control-undigested plasmid)	8.449	1.889	0.195
S2 **clear FD buffer, no phosphatase**	19.9	1.905	0.506
S3 **clear FD buffer, no phosphatase**	13.9	2.000	0.538
S4 **clear FD buffer, no**	15.9	2.133	0.232

phosphatase			
S5 **clear FD buffer, with phosphatase**	16.4	2.082	0.083
S6 **clear FD buffer, with phosphatase**	11.9	2.000	0.304
S7 **clear FD buffer, with phosphatase**	19.4	2.053	0.402
S8 **green FD buffer, with phosphatase**	19.4	2.053	0.722
S9 **green FD buffer, with phosphatase**	13.9	2.000	0.483
S10 **green FD buffer, no phosphatase**	15.9	1.882	0.542
S11 **green FD buffer, no phosphatase**	17.4	2.059	0.347

A test transformation of competent *E. coli* TG1 with empty pET-11a was done, which resulted in many colonies, indicating that our competent cells are viable for transformations.

Transformation of *E. coli* TG1 with pET-11a containing ci2350-His resulted in colonies, which will be tested next week with colony PCR, to confirm the presence of ci2350-His insert, followed by sending for sequencing. Sample 2 with higher amount of ligation mixture resulted in more colonies than Sample 1 with less ligation mixture. If future transformations give too few colonies, increasing ligation mixture volume is a good variable to try to gain more transformed colonies.

Day 5: [June 22, 2026] - Monday

Weekly responsible: Patrik, Rytis
Lab participants: Giulia, Shun, Ruiqi

Daily overview of experiments

Today we redid the restriction digestion of pET-11a with NdeI and BamHI, and did agarose gel electrophoresis, and gel extraction with two different kits; EZNA and Jena Bioscience. Mockup transformation of E. coli TG1 with a pET-11a plasmid was done, to see if our cells were alive and competent. Finally, streaking of E. coli TG1 on the LB Agar plate without ampicillin was done, to check if cells were alive from glycerol stock.

Activity	*Protocol File*	*Comments*
Restriction digestion of pET-11a	Restriction Digestion …	Previously we got very low concentrations of digested plasmids after gel extraction, so we are redoing it in hope of getting a better result.
Agarose Gel Electrophoresis	Gel electrophoresis.d…	Large gel (150 mL) was made, and ladders from previous year were used to assess them.
Gel Extraction	Gel Extraction from A… Make a protocol for Jena Bioscience Kit	Redoing the Gel Extraction with EZNA Kit and Jena Bioscience Kit, to see if there are differences between them.
NanoDrop Measurements of gel-extracted samples	DNA Concentration …	
E. coli transformation with pET-11a	E. coli Transformatio…	Trial transformation, to check if our cells are chemically competent, so we do not waste resources later.

Plating competent *E. coli TG1* from glycerol stock	-	Plating it on LB Agar without ampicillin, to see if cells are viable, incubated overnight at 37C.
Autoclaving Waste	Autoclaving.docx	1 box of 1.5 mL tubes, 1 box of 2 mL tubes, 2 boxes of small, medium, big pipettes respectively; 6 conical flasks with 100 mL LB and ~10 tubes LB medium (5 mL)

Results and interpretation

Restriction digestion

Reaction:

- Water: 14 µL
- FD Green buffer: 2 µL
- FD NdeI: 1 µL
- pET11a (1 µg of DNA): 2.97 µL

Total volume: 20 µL

+ BamHI: 1 µL

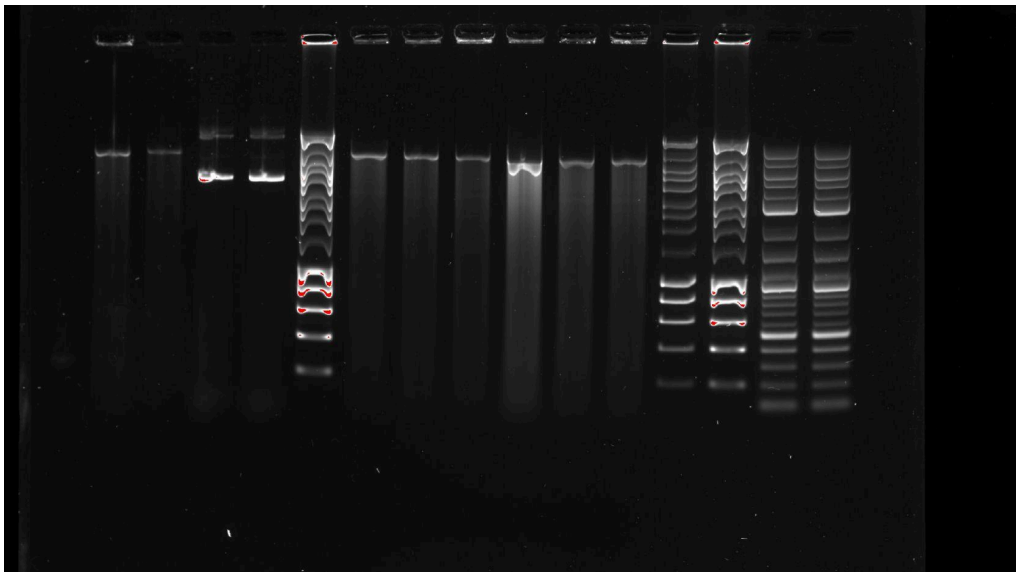
Digestion time was extended: 60 min for NdeI and 15 min for BamHI, at 37C.
Afterwards, the protocol was followed (alkaline phosphatase, heat inactivation…)

Keep in mind that in this reaction, extra 1 µL of water was added by accident, do not redo it the next time.

Agarose Gel Electrophoresis of Digested Plasmid

Well Number	Description
1	NdeI Control
2	BamHI Control
3	Undigested pET-11a

4	Undigested pET-11a
5	Ampliqon high range ladder (OLD) (2 µL)
6-11	pET11a (BamHI + NdeI)
12	Ampliqon high range DNA ladder new (1 µL)
13	Ampliqon high range DNA ladder old (2 µL)
14	NEB Ladder 1 (2 µL)
15	NEB Ladder 2 (2 µL)



Gel excision

Samples from well 3, 4, 6-11 were cut, and labeled 3, 4 and 5-10.
Gel fragments were combined: 5-6; 9-10 (new labels, not the wells), in hopes of higher DNA concentration.

For the Jenna Bioscience Gel Extraction Kit, 400 mg of gel was too large to extract in one Eppendorf tube, so the combined bands (9-10) had to be dissolved first and aliquoted divided in 2 tubes, so effectively the fusion of two bands did not work.

Note: for one Jena Bioscience gel extraction of plasmid, 250 mg of gel is maximum, otherwise it won't fit into the 1.5 mL Eppendorf tube

Nanodrop Measurements of Digested and Gel Extracted Plasmids

For blanking, Elution Buffer was used from each of the kits. 2 µL of volume was used for both blanking and the samples. The concentration values were very low, most of the time an error message was shown ‘concentration too low’. The 260/280 values are good in some samples, but some samples such as P5, P9.1 and P9.2 deviate, with P9.2 deviating too much. Aside from this, all samples have a very low 260/230 value, indicating the presence of some impurities.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Kit Used
P3	0.994	2.00	0.087	Jenna
P4	0.45	Not written down due to low conc.	Not written down due to low conc.	EZNA
P5 (5+6)	2.982	1.5	0.231	EZNA
P7	0.994	2.00	0.800	EZNA
P8	2.982	2.00	0.182	Jenna
P9.1	2.485	5.00	0.294	Jenna
P9.2	2.982	1.5	0.078	Jenna

Day 6: [June 23, 2026], Tuesday

Weekly responsible: Patrik, Rytis
Lab participants: Giulia, Shun, Ruiqi

Daily overview of experiments

Redid restriction digestion - followed Johan's protocol exactly, not the one from last year, as it does not work. A big restriction digestion reaction was done, with more plasmid (5 µg) and bigger volume (50 µL). Loading the samples on gels, following with gel extraction. Checked if yesterday's mockup transformation was a success. Inoculating E. coli TG1 with pET-11a on overnight incubation for plasmid miniprep the next day.

Activity	*Protocol File*	*Comments*
Restriction digestion	Johan's Restriction D…	Normal reaction (20 µL reaction with 0.25 µg pDNA) and a bigger reaction was done (50 µL containing 5 µg plasmid)
Gel electrophoresis	Gel electrophoresis.d…	Small gel (50 mL) and a big gel (150 mL) were made, each for their restriction reaction.
Gel extraction	Gel Extraction from A…	Big gel was extracted with EZNA protocol, and smaller one with Jena Bioscience one.
Checking the transformation from previous day	-	Success! The transformants with pET11a showed colonies. Streaking of just competent cells resulted in bacterial lawn (almost mistaken for no cells, would have been bad)
Inoculating E. coli TG1 with pET-11a for overnight incubation	-	37C, with 150 rpm, for plasmid miniprep.

Results and interpretation

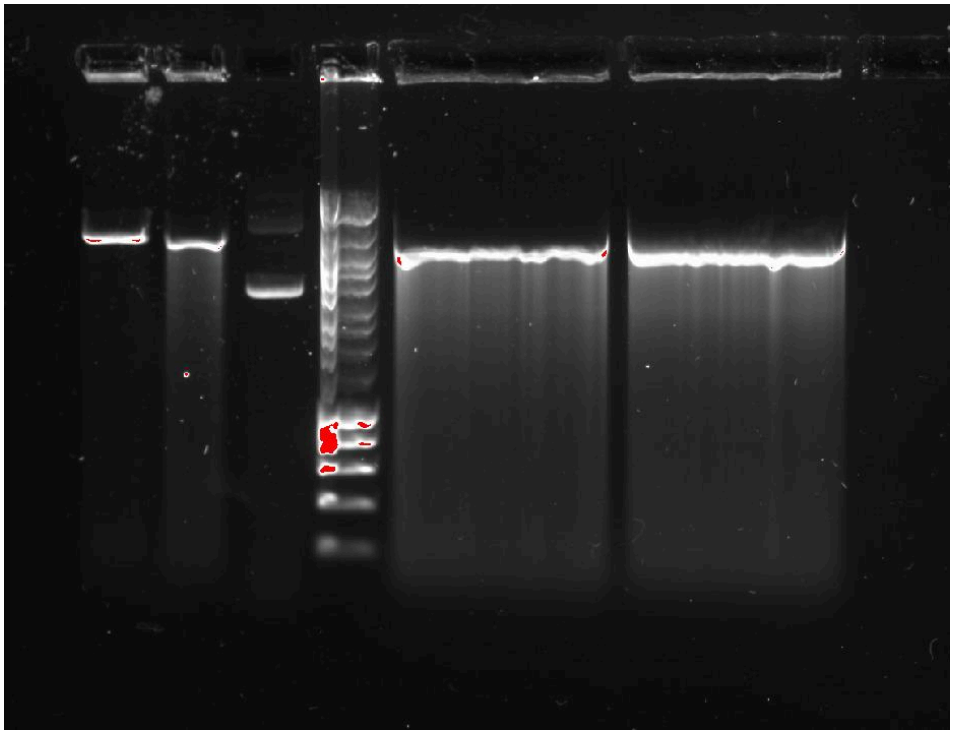
Digestion restriction (strictly adhering to Johan's protocol)

3 replicates of pET11a were digested for 45 min at 37C with NdeI, then additionally 10 min with BamHI., controls (pET11a cut with BamHI; pET11a cut with NdeI; undigested pET11a) were used. Concentration of miniprepped pET-11a was 337 ng/µL.

Reaction 1:

- Water: 20.16 µL
- FD Green buffer: 5 µL
- FD NdeI: 5 µL
- pET11a (~5 µg of DNA): 14.84 µL
- + BamHI: 5 µL
- + Alkaline phosphatase: 1 µL

Well Number	Description
1	NdeI Control
2	BamHI Control
3	Undigested Control
4	Ladder
5-7	Sample 1
8-10	Sample 2



Reaction 2:

- Water: 14 µL
- FD Green buffer: 2 µL
- FD NdeI: 1 µL
- pET11a (0.25 µg of DNA): 0.74 µL

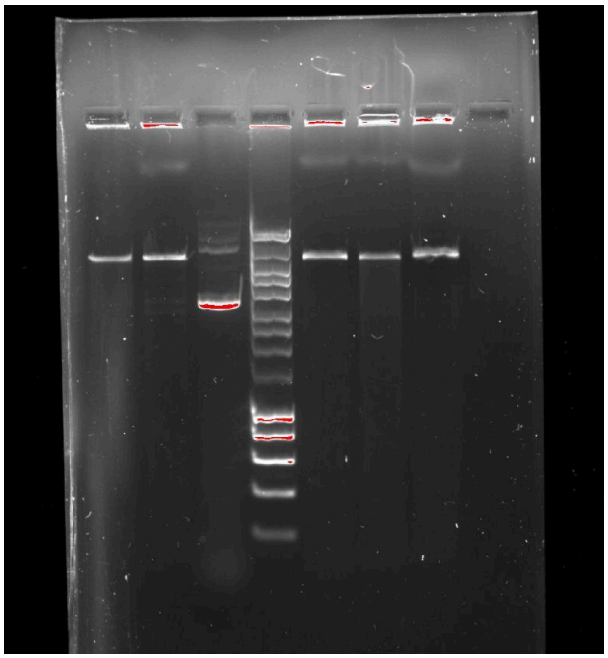
Total volume:

+ BamHI: 1 µL

Digestion time: 45 min for NdeI and 15 min for BamHI, at 37C.
Johans protocol was followed (alkaline phosphatase, heat inactivation…)

Well Number	Description
1	NdeI Control
2	BamHI Control
3	Undigested Control
4	Ladder

5	Sample 1
6	Sample 2
7	Sample 3



Day 7: [June 24, 2026], Wednesday

Weekly responsible: Rytis
Lab participants: Giulia, Shun, Ruiqi

Daily overview of experiments

We repeated pET-11a extraction, followed by restriction digestion and gel extraction. We also resuspended AKR1D1-His and ci2350-His gene fragments we received from IDT. Restriction digestion was done on ci2350-His.

Activity	*Protocol File*	*Comments*
pET11a extraction	EZNA and Jena kit	Overnight cells were incubated at 21C, 150 RPM. The temperature was increased to 37C, and cultivation continued for another 5 hours. 2 kits were used: Jena and EZNA. For EZNA, two 4 mL cell suspensions were combined into one, which might've overloaded the column. The concentrations after extraction were much less than previously gotten.
pET11a restriction digestion	Johan's Restriction D…	3x50 µL reactions (5 µg pDNA) were set up. One followed Johan's protocol exactly. Two had no steps 5-8. One of the reactions with no phosphatase (steps 5-8) was incubated for a longer time (1 h first enzyme and 15 min the second).
IDT gene (AKR1D1-His & ci2350-His) resuspension	https://eu.idtdna.com/page/support-and-education/dec	Followed the IDT protocol to get 50 µL of 20 ng/µL of

	oded-plus/how-to-resuspend-store-and-improve-the-longevity-of-your-idt-gene-fragments	ci2350-His and AKR1D1-His. Resuspended in autoclaved MQ-water (opened today). Made 2 aliquots of 10 µL for each gene, leaving 30 µL inside AKR1D1-His IDT stock, and 20 µL in ci2350-His IDT stock.
Ci2350 restriction digestion	Johan's Restriction D… Digestion of PCR products	Used 10 µL (200 ng) of DNA for restriction digestion following Johan's protocol (Digestion of PCR products).
Gel electrophoresis	Gel electrophoresis.d…	Big gel (150 ml) was prepared with three big wells for the plasmid and regular for ci2350 insert. Both plasmid and insert were run in 0.8% agarose gel.
Gel extraction	Gel Extraction from A…	EZNA kit was used as it yielded the best results the previous day.

Results and interpretation

pET11a & ci2350-His restriction digestion

3 replicates of pET11a were digested, controls (pET11a cut with BamHI; pET11a cut with NdeI; undigested pET11a) were used. A big reaction was set up for each 50 µL sample (5 mg pDNA plasmid), following Johan's protocol:

MQ water: 20.16 µL
10x FD Green: 5 µL
pET 11: 14.85 → 15 µL
NdeI: 5 µL
BamHI: 5 µL
Tot: 50 µL

Digestion time was 45 min for NdeI and 10 min for BamHI, at 37C, one sample was set to have a longer incubation time (1 hour and 15 minutes). Steps 5-8 of Johan's protocol were skipped in the same sample. One sample was meant to have no phosphatase as well but normal incubation time. However the final volume of such sample was not 50 mL but 45, therefore it was hypothesized that one enzyme, likely NdeI, was not added to the reaction mixture. This sample was discarded after gel electrophoresis due to uncertainty of its double cut.

A small reaction was set up for controls NdeI and BamH1, following Johan's protocol:

MQ water: 15.22 µL
10x FD Green: 2 µL
pET11a: 0.74 µL
NdeI: 1 µL → changed to H2O for the BamH1 control
BamHI: 1 µL → changed to H2O for the NdeI control

A small reaction was set up for the ci2350-His gene, following Johan's protocol:

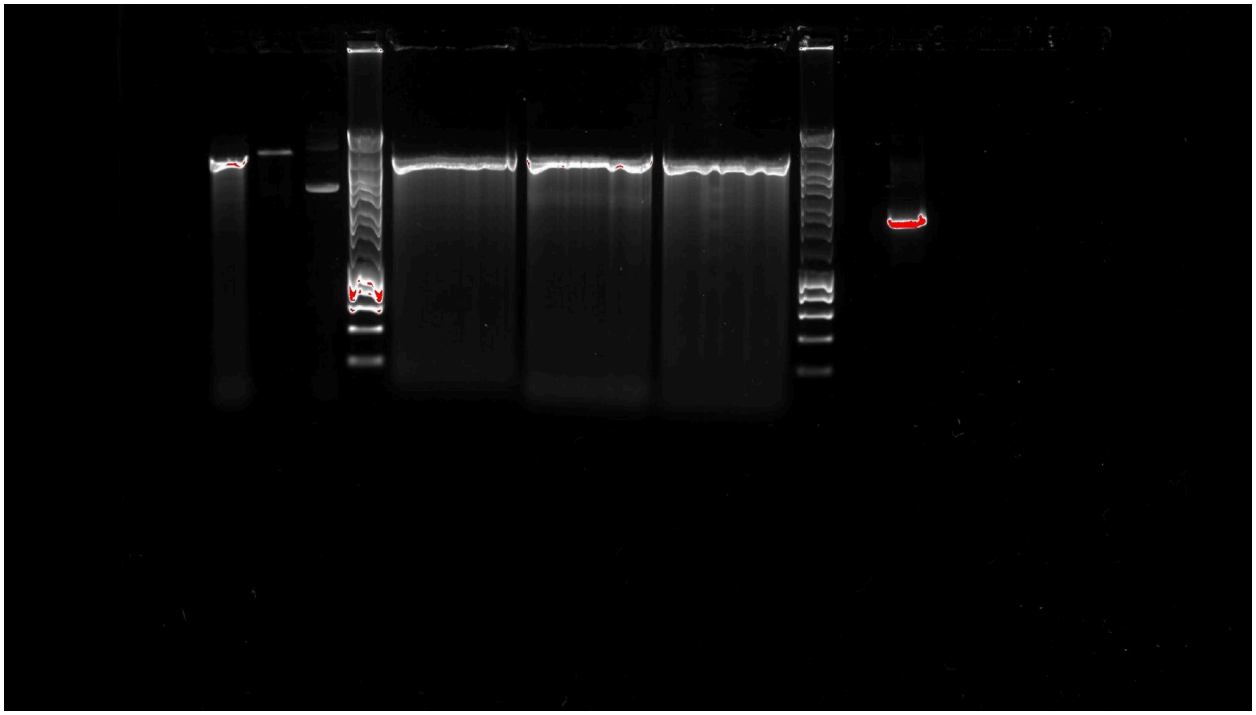
MQ water: 6 µL
10x FD Green: 2 µL
Gene: 10 µL
NdeI: 1 µL
BamHI: 1 µL

******genes were resuspended following IDT instructions as linked in the table entry. Followed the IDT protocol to get 50 µL of 20 ng/µL of ci2350-His and AKR1D1-His.

Samples were loaded in the following order:

Well Number	Description
1	NdeI Control
2	BamHI Control
3	Undigested Control
4	ladder 2 µL
5-7	pet11a (LACKING NdeI, thus DISCARDED)
8-10	pET (longer incubation, no phosphatase)
11-13	pet11a (normal conditions)
14	ladder 1µL

15	empty
16	Ci2350



The plasmid fragments are present in the right range. There is some smearing. NdeI looks more concentrated than BamHI. Two different batches of enzymes were likely used, even if the initial goal was to use the fully new enzymes due to mixing up. Next digestion attempts should be performed with the new enzymes to rule out influence in the results.

pET11a and ci2350-His extraction

Insert experiment discussion and interpretation

When performing gel extraction, feedback from Johan was implemented to make sure that no unmelted agarose was left in the tube to avoid clogging the membrane. Pre heating of the elution buffer at 70°C was also implemented to favour the elution step. These changes were applied to all samples.

Sample	Concentration (ng/μL)	Abs 260/280	Abs 260/230	Kit Used
Uncut	4.970	1.667	0.357	EZNA
pET11a longer incubation time and no phosphatase	6.461	1.625	1.000	EZNA
pETa normal	4.970	1.429	0.556	EZNA
Ci2350	3.479	1.400	0.412	EZNA

One sample with longer incubation time and no phosphatase had better concentration yield. These conditions could therefore be used in further experiments. Purity was lower than 1.8, however it is expected after gel extraction. The EZNA kit was used as it provided the best results the day before.

The gel and measurements were shown to Johan, who helped us with troubleshooting. In his opinion, it may be that the quantity of plasmid extracted with miniprep is actually lower than what reported by NanoDrop measurement. He reported that typical results using EZNA kit are around 80 ng/μL, while our results are much higher. Also regarding purity measurements, he said normal values are around 1.8, while results higher than 2 can be suspicious and indicative of contamination (RNA for example). He mentioned that many steps can cause this in mini prep and suggested repeating mini prep using EZNA kit instead of Jena, being mindful of sterility and all the possible causes of contamination in the steps of extraction. If the quantity of plasmid initially extracted is lower than what reported in the measurement, the amount finally recovered from the gel is lower than expected. This will be tested on Friday.

He also said to be mindful when adding ampicillin to culture, to make sure selective pressure is applied and sufficient plasmid can be extracted.

He mentioned he will check for old results of purification with the pet11a for us to compare our results.

It was noticed that the elutions were left overnight at room temperature. Check how that affects their stability. Preferably do not use the digested plasmids from this batch.

Day 8: [June 25, 2026], Thursday

Weekly responsible: Rytis
Lab participants: Gulia, Shun, Ruiqi

Daily overview of experiments

Checked if there was a problem with the electrophoresis buffer by checking its pH.
Did plasmid extraction using JENA kit. Discussed possible errors.

Activity	*Protocol File*	*Comments*
Check TAE buffer pH	N/A, universal pH strip	Universal pH strip showed pH similar to what is expected >7, <8
Plasmid extraction using Jena kit	W Plasmid miniprep - J…	6 x 5 mL cultures, spun down 5 mL in 6 1.5 mL tubes
Ligation of pET11a and insert	W E. coli Transformatio…	Set up ligation of pET11a (B2 sample) with ci2350 insert. Reaction done at +4C, overnight.
Discussion about troubleshooting		Since extracted pDNA showed A_{260}/A_{280} >2.0, it could be RNA contamination, and actual pDNA conc. is much lower. From Johan's words, they usually get 80 ng/µL
Autoclaving	W Autoclaving.docx	Pipette tips (2 boxes of grey and yellow, 1 box blue); 2 bottles of MQ H2O
Inoculating *E.coli TG1* pET11a for plasmid extraction.	🗋 File	Inoculate 6 tubes (5 mL each) of LB-Amp for TG1
Inoculating *E. coli BL21* for competent cell preparation	🗋 File	2 tubes of 5 mL were inoculated

Results and interpretation

pET11a extraction using Jena kit

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Kit Used
P1	252	2.000	2.352	Jena
P2	225	2.013	2.311	Jena
P3	300	2.000	2.204	Jena
P4	271	2.022	2.353	Jena
P5	331	2.059	2.293	Jena
P6	231	2.039	2.435	Jena

Concentration is very high, according to John, usually they expect ~80 ng/µL, due to pET11a being a low copy plasmid. However, the Jena kit is brand new, so a bit higher concentration is expected. Though the 260/280 for all the samples was >2, which could mean that while the DNA concentration seems high, due to some RNA contamination, our concentrations are much lower than shown.

We should re-do the extraction using another kit (EZNA), to see if it shows the same results.

Ligation of vector pET11a and insert (ci2350-His)

Insert length: ~1980 bp Insert amount: (1980 bp/5677 bp)×40×3 = 41.8 ng
Vector length: 5677 bp Vector amount: 40 ng

Ligation reaction (1:3 vector insert ratio):

T4 DNA ligation buffer:	2.5 µL
Vector (B2 (pET11a: 4.97 ng/µL):	8 µL
Insert (ci2350-His: 3.48 ng/µL):	12 µL
T4 ligase:	2.5 µL
Total volume:	25 µL

Day 9: [June 26, 2026], Friday

Weekly responsible: Patrik, Rytis
Lab participants: Giulia, Ruiqi, Shun

Daily overview of experiments

Insert day description

Activity	*Protocol File*	*Comments*
pET11a extraction using EZNA protocol		Used EZRA kit, got 70-90 ng/µL, similar to Johan's
E. coli BL21 competent cell preparation		~~Cultivation: ~13:00–16:00 (3 h)~~ ~~Prepared X aliquots of competent E. coli BL21~~
Transformation of *E. coli* TG1 competent cells using ligation mix	E. coli Transformatio…	Used 10 and 15 uL of ligation mix for XX µL of cells. Incubation started at 13:30, at 37C
Restriction digestion of pET-11a using new BamHI & NdeI	Johan's Restriction D…	
Agarose gel electrophoresis		Used big gel, ran at 90 V for 1 h.
Gel extraction		Used EZRA kit

Results and interpretation

Plasmid miniprep extraction with E.Z.N.A. kit

Plasmid extraction of six 5 mL LB medium tubes incubated with e.coli TG1 the day before. E.Z.NA. kit was used to test differences with Jena due to suspiciously high results in past extractions. First 3 samples are done with no column equilibration step, last 3 have the equilibration step included.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Kit Used
S1	73.1	1.838	2.410	E.Z.NA.
S2	92.4	1.824	1.979	E.Z.NA.
S3	75.5	1.831	1.810	E.Z.NA.
S4 (column equilibrated)	93.9	1.853	2.305	E.Z.NA.
S5 (column equilibrated)	95.9	1.804	2.474	E.Z.NA.
S6 (column equilibrated)	112	1.852	2.306	E.Z.NA.

Results from E.ZN.A kit extraction are more in line with usual results, both in terms of concentration and purity, suggesting the problem with the restriction digestion rose from the initial plasmid mini prep.

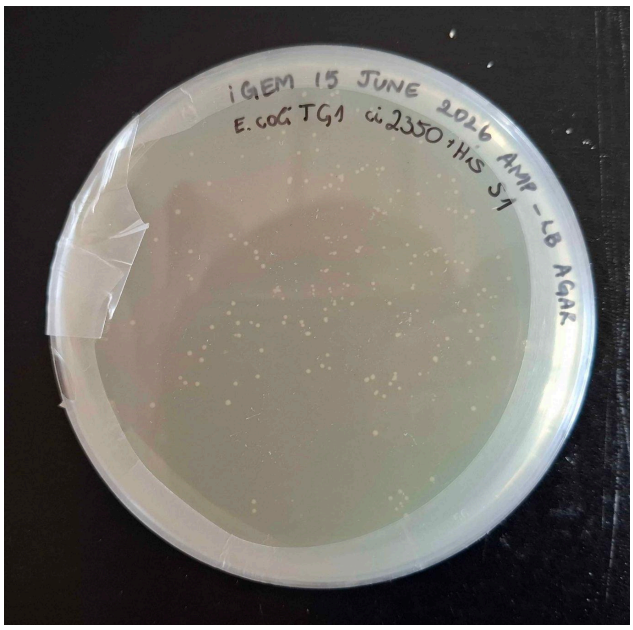
Regarding the difference in plasmid miniprep results between EZNA kit and Jena, a known issue was reported by Patrik: the RNAse in Jena kit was not stored properly in the fridge when the package arrived. While RNAse should be quite stable, it cannot be therefore ruled out that this influenced the concentration and purity results, given RNA contamination was suspected. Another RNAse is included in the package, therefore it could be tested in future extractions to check whether the one used is the cause of the anomalous results.

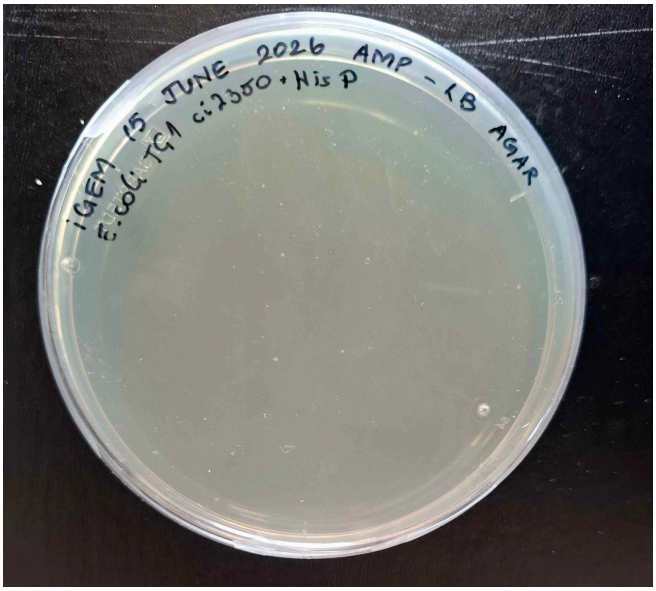
Transformation of E. coli TG1 with pET-11a ci2350 + His

Four different samples were transformed; positive control with empty pET11-a vector (added 1 uL); negative control with 10 uL ligation control reaction (no insert); transformant 1 (with 10 uL ligation reaction; transformant 2 (with 15 µL ligation reaction). Samples were transformed according to the protocol, and put in an incubator at 37C around 13:30h.

Note: previous day the ligation reaction was done in 25 uL

Colonies were observed the morning after incubation. Results will be confirmed on Monday with colony PCR. The positive and negative controls were likely swapped (See figures below).

	E.coli TG1, Transformation of ci2350-His cloned in pET11a **sample1 (S1) 10 uL of ligation reaction used.** Visible colonies
	E.coli TG1, Transformation of ci2350-His cloned in pET11a **sample2 (S2). 12.5 uL of ligation reaction used. (originally 15 uL was to be used, but in the eppendorf it was a smaller amount)** Visible colonies showing analogue growth pattern.

	E.coli TG1, Transformation of ci2350-His cloned in pET11a **Negative control.** **Likely swapped with positive when plating.**
	E.coli TG1, Transformation of ci2350-His cloned in pET11a **Positive control.** **Likely swapped with negative when plating.**

E. coli BL21 Competent Cell Preparation

Inoculation of 6 big flasks (1L flasks filled with 100 mL LB Medium) with baffles was done at 12:30 and put to incubation at 37C and 150 rpm. OD_{600} was checked periodically to ensure the growth did not exceed required OD_{600} of 0.30-0.45.

Sample	Time incubated	OD_{600}
S1	1h 35min	0.110
S3	1h 35min	0.133

Incorrect prediction of doubling time caused overshooting of OD for competent cells preparation. The cultures were therefore discarded. Johan mentioned he most probably still has competent BL21 cells from his master student work that we could use. Since not as much is required as for TG1 cells, this should suffice our needs. Check with him about this though!!

For future reference: doubling time of E. coli is ~30 mins, and the OD600 of 0.3-0.45 should be 'foreseen' by previous measurements and growth curve, so in the future if doing it, it would be good to take continuous measurements and construct this growth curve

Restriction digestion of pET11a extracted with E.Z.N.A. kit

The reaction scheme had the purpose of troubleshooting previous results and was based on the following conditions: all samples were incubated for a longer time than indicated in the general protocol, 1 hour first incubation, 15 minutes the second.
Digestion reactions were prepared as follows:

Single reaction
10x FD buffer: 2 uL
pET11a: 16 uL
NdeI: 1 uL
BamHI: 1 uL
Total: 20 uL

Control reaction
10x FD buffer: 2 uL
pET11a: 16 uL
NdeI/BamHI: 1 uL

H2O: 1 uL
Total: 20 uL

Gel extraction was performed integrating feedback from Johan: elution buffer was heated to 70°C for 3 minutes, and elution was repeated twice, with 15 uL each (15*2 = 30 uL).
The results from NanoDrop measurements for all conditions and replicates are reported below.

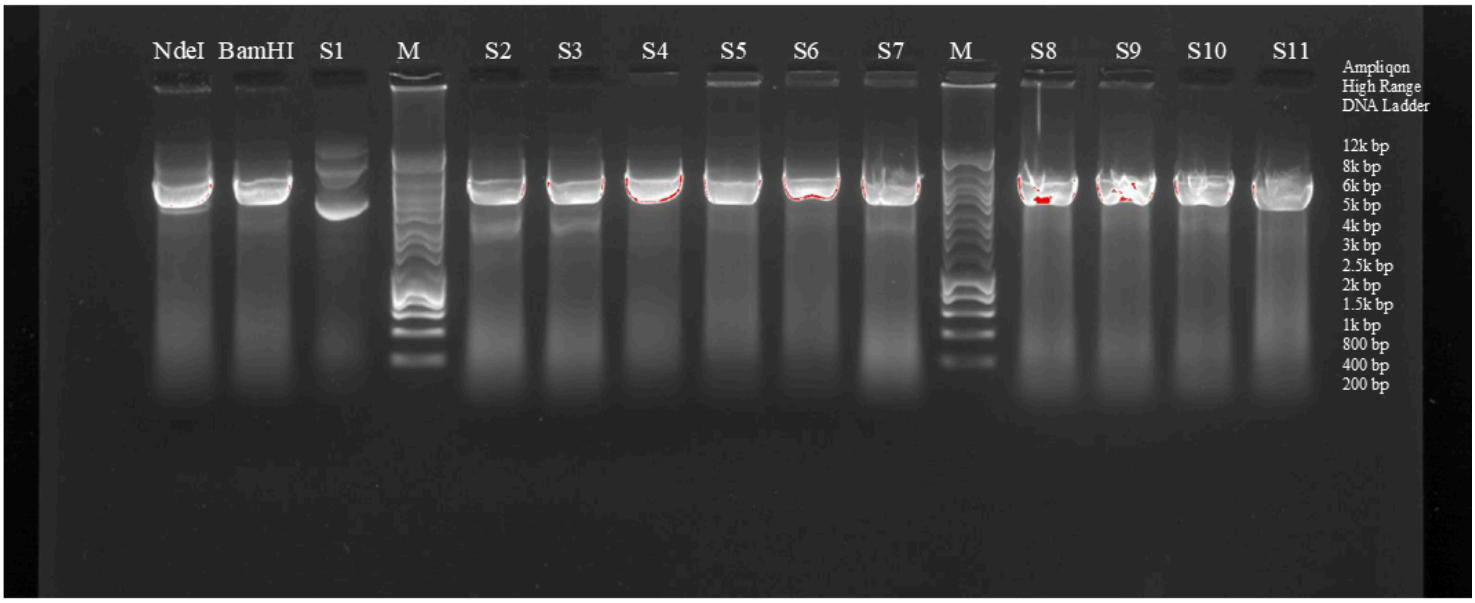
Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Kit Used
S1 (positive control-undigested plasmid)	8.449	1.889	0.195	E.Z.NA.
S2 **clear FD buffer, no phosphatase**	19.9	1.905	0.506	E.Z.NA.
S3 **clear FD buffer, no phosphatase**	13.9	2.000	0.538	E.Z.NA.
S4 **clear FD buffer, no phosphatase**	15.9	2.133	0.232	E.Z.NA.
S5 **clear FD buffer, with phosphatase**	16.4	2.082	0.083	E.Z.NA.
S6 **clear FD buffer, with phosphatase**	11.9	2.000	0.304	E.Z.NA.
S7 **clear FD buffer, with phosphatase**	19.4	2.053	0.402	E.Z.NA.
S8 **green FD buffer, with phosphatase**	19.4	2.053	0.722	E.Z.NA.
S9 **green FD buffer, with phosphatase**	13.9	2.000	0.483	E.Z.NA.
S10 **green FD buffer, no phosphatase**	15.9	1.882	0.542	E.Z.NA.

S11 **green FD buffer, no phosphatase**	17.4	2.059	0.347	E.Z.NA.

Best results were obtained in samples S2 and S10 in terms of purity; no clear pattern visible between conditions and replicates.

Agarose gel electrophoresis of plasmid restriction

Describe the order of the wells, and state how much of plasmid was used for the control



S1 is pET11a plasmid control, S2-24 digested with clear FD buffer and no phosphatase step, S5-S7 digested with clear FD buffer and phosphatase step included, S8-S9 digested with green FD buffer and phosphatase step included, S10-S11 with green FD buffer and no phosphatase step. Two marker ladders are included, the first 2 uL shows evident smearing, the second 1 uL has overall less smearing.