Week 25 - Overview

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Weekly Summary:

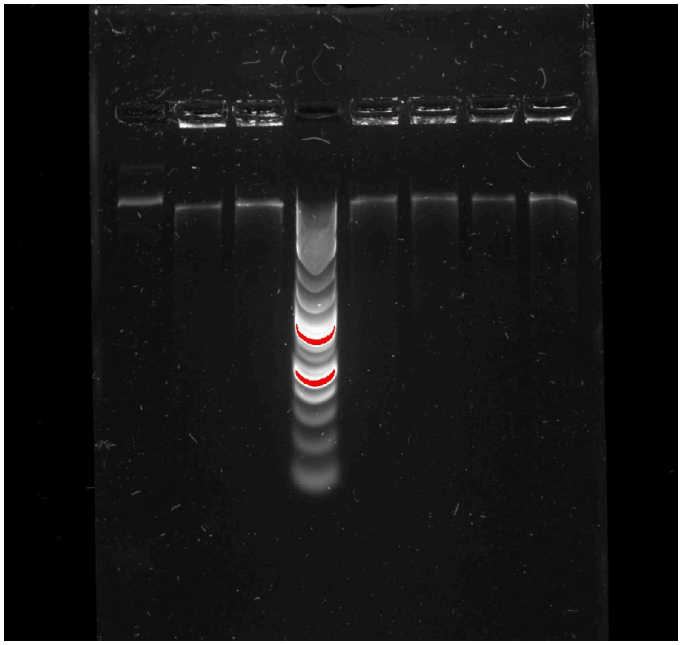
During the first week of the lab we received a lab equipment introduction from Johan, where we learned how to operate equipment properly and safely. Many consumables were made, including agar petri dishes, sterilized tips, Eppendorf tubes and MilliQ-H2O. Plasmid miniprep was done on *E. coli* TG1 cells containing pET-11a, followed by restriction digestion using BamHI and NdeI enzymes. Additionally, we prepared competent E. coli TG1 cells for future transformations.

Main results of the week:

Plasmid Miniprep of *E. coli* TG1 cells containing pET-11a. Jenna Bioscience Plasmid Miniprep Kit instructions state how the cells could be resuspended in Lysis buffer by pipetting or vortexing, and we decided to test if there was a difference. By looking at the results, non-vortexed samples had higher DNA concentration, and resuspension by pipetting was done in future experiments. Samples were pooled, allowing for more convenient downstream applications. A260/280 were slightly higher than expected, but we still decided to use these samples for restriction digestion.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
Non-vortexed sample 1	340	2.079	2.243
Non-vortexed sample 2	487	2.081	1.972
Non-vortexed sample 2 (second reading)	471	2.097	2.257
Vortexed sample 3	309	2.073	2.383
Vortexed sample 4	251	2.053	1.996
Pooled sample	347	2.068	2.164

Agarose gel visualisation of restriction digestion was done, without the holder for the gel. The ladder taken from last year's iGEM Team (New England Biolabs 1kb Plus Ladder DNA Ladder) was overloaded, and bands of digested pET-11a are slightly faint. In the future experiments, newly bought Ampliqon High Range DNA ladder can be used, along with keeping the gel in the holder.



Gel extracted plasmid concentrations were too low to be measured using NanoDrop, thus microcuvettes were used. Since the sample volume was 30 µL, they were diluted to 70 µL using MQ water. Their concentration was too low to be used for transformations, so restriction digestion will be repeated next week, with prolonged incubation time.

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230
P1	0.30	3.0	0.035
P2	0.15	3.0	0.023
P3	0.65	3.250	0.046
pET-11a (5 times diluted)	67.45	2.063	2.279

Competent E. coli TG1 cells were prepared, with a total of 125 tubes. They will be tested next week, to make sure they are competent for transformations.

Day 1: [June 15, 2026] - Monday

Weekly responsible: Patrik, Rytis
Lab participants: Hema, Hamsini, Shun

Daily overview of experiments

We will be given a lab equipment introduction from Johan, where we will be introduced to how to operate equipment properly and safely. If time allows us, we could prepare LB agar plates containing Ampicillin and organize the lab space. If not, shift these activities to the next day.

Activity	*Protocol File*	*Comments*
Equipment introduction		
Preparation of LB agar (+ AMP)	Media preparation.docx	46 plates were made with Amp, and 5 without.
Preparation of Ampicillin stock	Media preparation.docx	Made 1.5 mL, with concentration of 100 mg/mL
Autoclaving	Autoclaving.docx	LB Agar 1,5 L in 2L Schott flask 3 boxes of 1000 tips 2 small buckets of Eppendorf tubes of 1.5 mL 6 x ~80 mL of dH_2O in 100 mL Schott flasks

Day 2: [June 16, 2026] - Tuesday

Weekly responsible: Patrik, Rytis
Lab participants: Hema, Hamsini, Shun

Daily overview of experiments

Continuation of lab space organization, streaking of *E. coli* TG1 with pET-11a, inoculation of *E. coli* TG1 with pET-11a in LB media for overnight incubation. Autoclaving material.

Activity	*Protocol File*	*Comments*
Streaking of *E. coli* TG1 with plasmid pET-11a on a plate with Ampicillin to use for plasmid isolation	N/A	Johan streaked it. Incubated during the day (~11 -), for inoculation in the afternoon.
Day 1 of competent cell: Streaking of *E. coli* TG1 on a plate to use for competent cells.	E. coli competent cell…	Johan streaked it. Put on overnight incubation at -h.
Streaking of *E. coli* BL21 on a plate to use for competent cells.	N/A	Johan streaked it. Put on overnight incubation at -h.
Inoculation of *E. coli TG1 with pET-11a* on LB Medium with Amp for competent cells Autoclaving.docx	N/A	4 tubes containing 5 mL medium. Put on overnight incubation (8 p.m to next day 9 a.m) at 37°C with shaking 200 rpm.
Preparation of LB Media (+ AMP)	Media preparation.docx	6x100 mL of LB Media in 1L Erlenmeyer Flasks with baffles. 40 tubes (5 mL medium) were made with Amp, and 5 tubes without.
Preparation of CaCl2 stock solution (50 mM)	Media preparation.docx	3 x 100mL was prepared in 250 mL Schott flasks

Preparation of glycerol stock	N/A	Poured ~20 mL in 100 mL Schott bottle and autoclaved. Glycerol bottle stored near John's room, in base cabinet
Autoclave material		6 x 100 mL of LB Media in 6 x 1L Erlenmeyer Flasks 2 x 100 mL $CaCl_2$ stock 2 x ~80 mL MiliQ H_2O 1 x Glycerol flask of ~20 mL 45 tubes with 5 mL LB Medium 2 boxes of small tips 2 boxes of medium tips
Left to autoclave	Autoclaving.docx	4 x ~80 mL MiliQ H_2O 1 x 100 mL CaCl2 stock
Preparation of Plasmid Prep Kit: Washing buffer and Neutralization buffer	Plasmid miniprep - J…	Added 64 mL EtOH (99%) to the Washing Buffer, stored at RT. Added RNase (4 mg) to the Neutralization Buffer, stored in the fridge (+4C). NOTE: RNase powder was not stored at -20C, but in RT. RNase is supposedly very stable, so it might be fine but keep this in mind for later troubleshooting. However, it was only stored for a week or so, from the time the kit arrived.

Day 3: [June 17, 2026] - Wednesday

Weekly responsible: Patrik, Rytis
Lab participants: Hema, Shun, Hamsini

Daily overview of experiments

Performing restriction digestion for plasmids. We do not yet have the cis gene and we will only get them delivered on June 18th, before EOD.

Activity	*Protocol File*	*Comments*
Centrifugation		The overnight inoculated samples were centrifuged to prepare them for plasmid miniprep.
Plasmid mini prep using the Jena Bioscience Plasmid Miniprep Kit	Plasmid miniprep …	4 plasmid preps done of pET-11a
Measuring plasmid concentration	DNA Concentratio…	Nanodrop reading was performed with samples of 2 µL for each reading. Total readings - 6 ● Non-vortexed sample 1 ● Non-vortexed sample 2 ● Vortexed sample 3 ● Vortexed sample 4 ● Non-vortexed sample 2 (done again as the concentration seemed higher) ● Pooled together samples Elution was done differently, to compare if eluting twice yielded different amounts of DNA vs eluting once: → non-vortexed samples were eluted once with 50 µL →vortexed samples were

		eluted twice, with 25 µL each time (50 µL total)
Day 2 of competent cell preparation: Inoculate E. coli TG1 colonies into LB medium for overnight cultivation	E. coli competent …	2 tubes of 5 mL were inoculated, and put on 37C and 150 RPM.
Restriction digestion of plasmid pET-11a	Restriction clonin…	Samples - 6 1. Only BamHI (B) - control 2. Only NdeI (N) - control 3. Digested pET-11a four samples: P1, P2, P3, P4
Preparing the TAE buffer required for agarose gel	Gel electrophoresi…	2 L of TAE buffer was prepared from 50X stock (40 mL of stock and 1960 mL of distilled water)

Results and interpretation

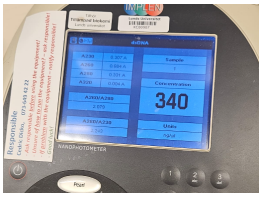
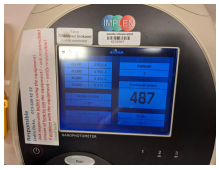
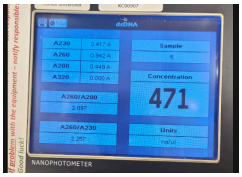
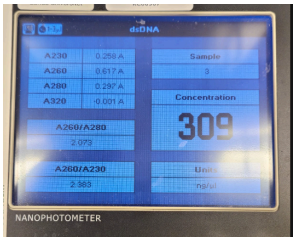
Centrifugation of overnight samples

The overnight inoculated samples of *E. coli* TG1 containing pET-11a were centrifuged, as the cultivation volume (5 mL) was too high for one eppendorf tube. The samples were centrifuged on a tabletop centrifuge for 3 rounds, with each round being 10,000 RPM / 5,585 g for 4 minutes, supernatants were discarded. Cell pellets were used for Jena Bioscience Plasmid MiniPrep Kits.

Johan told us that we could do this, because the plasmid miniprep kits usually have the capacity to isolate plasmids from more culture volume than they state so in the kit. This is the reason we used 4.5 mL of overnight culture in the end for each miniprepped sample, instead of 1-3 mL suggested in the kit.

Insert experiment discussion and interpretation, any comment regarding future uses of the kit, any things done differently from the instructions.

Plasmid Miniprep and NanoDrop Measurements

Sample	Concentration (ng/µL)	Abs 260/280	Abs 260/230	Pictures
Non-vortexed sample 1	340	2.079	2.243	
Non-vortexed sample 2	487	2.081	1.972	
Non-vortexed sample 2 (second reading)	471	2.097	2.257	
Vortexed sample 3	309	2.073	2.383	
Vortexed sample 4	251	2.053	1.996	
Pooled sample	347	2.068	2.164	

The absorbance ratio is a measurement of the purity of the DNA.

The ratio of absorbance A_{260}/A_{280} is used to assess the purity of DNA and RNa. The ratio ~1.8 is accepted as ‘pure’ for DNA, while ~2.0 as ‘pure’ RNA. Looking at our samples, they might be

contaminated with RNA, as this ratio is slightly higher than expected, but still follows in the accepted range. If we want to, we could try and extend the incubation time with RNase during plasmid miniprep.

The ratio of absorbance A_{260}/A_{230} is used as a secondary measure for purity. The value of 2.0-2.2 indicates pure nucleic acids, and values outside of this range may indicate presence of contaminants. Our samples have a good 260/230 ratio.

Johan suggested we pool all the plasmid samples together and make aliquots, to make it easier in the future as they will have the same concentration. We did this, and made **4 aliquots of 47 µL** with a concentration of **348 ng/µL**. One aliquot was spent on restriction digestion and as a negative control for agarose gel electrophoresis the next day.

Restriction Digestion

As the plasmid concentration was too high for the required 250 ng of DNA per digestion reaction, the plasmid was eluted 5 times in an elution buffer from the plasmid miniprep kit. (5 µL pET-11a from aliquot 1 + 20 µL elution buffer). This resulted in the final concentration of 69.6 ng/µL, and putting 3.6 µL of diluted plasmid into digestion reaction for a total of 250 ng DNA per reaction.

Initial digestion mix (total volume 19 µL):

- MiliQ Water - 12.4 µL (for P1, P2, P3 and P4 to make it up-to 19 µL) and 13.4 µL (for N and B)
- Plasmid DNA - 3.6 µL
- FastDigest Buffer (10X) - 2 µL
- NdeI - 1 µL
- BamHI - 1 µL

Day 4: [June 18, 2026] Thursday

Weekly responsible: Patrik, Rytis
Lab participants: Hema, Shun, Hamsini

Daily overview of experiments

Checking restriction digestion by agarose gel electrophoresis, followed by gel extraction. Preparing competent *E. coli* TG1 cells.

Activity	*Protocol File*	*Comments*
Agarose gel preparation	Gel electrophoresis.…	Small gel was made, 1% agarose
Gel electrophoresis	Gel electrophoresis.…	Gel was run at 60V initially (5 min), then it was changed to 80V (40 min)
Plasmid gel extraction	Make a protocol for Jena Bioscience Kit	Samples 5-8 were extracted from the gel. Next time extract also the uncut plasmid, to compare the efficiency
NanoDrop of extracted plasmids	DNA Concentration …	Concentration using NanoDrop was too small for proper measurement, and had to use microcuvettes - 30 µL of eluted DNA was diluted to reach 70 µL.
Autoclaving	Autoclaving.docx	1 box of big tips? 2 boxes of medium tips 2 boxes of small tips 2 boxes of 1.5 mL tubes 1 Schott flask of CaCl2 (200 mL) …

Competent cell preparation Day 3	E. coli competent cel…	*E. coli* TG1 competent cells were made, 125 aliquots in total.

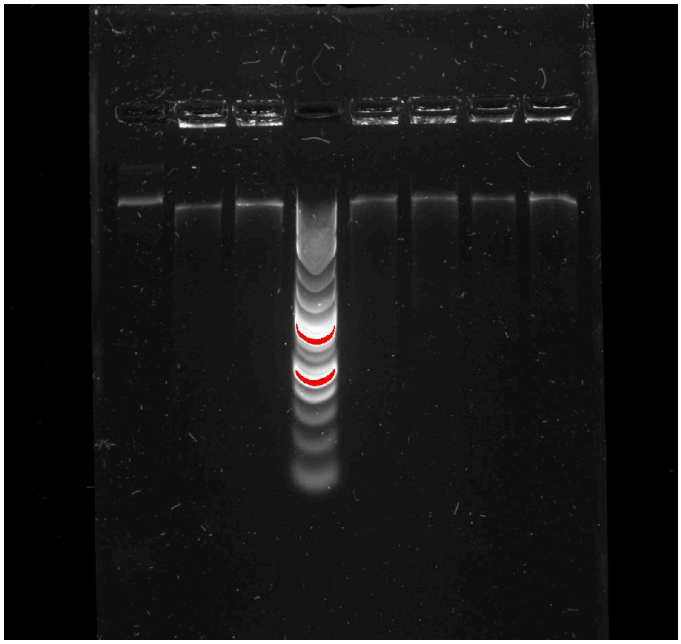
Results and interpretation

Gel electrophoresis

Well Number	Sample ID
1	pET11a (5x diluted)
2	pET11a digested with NdeI
3	pET11a digested with BamHI
4	NBE 1kb Plus Ladder
5	P1 (pET11a digested with NdeI and BamHI)
6	P2 (pET11a digested with NdeI and BamHI)
7	P3 (pET11a digested with NdeI and BamHI)
8	P4 (pET11a digested with NdeI and BamHI)

Gel electrophoresis was done without using a gel holder. Note: use it next time. A small gel was used, with a volume of 50 mL. For the first 5 min, 60 V was used, until DNA went inside the gel, then increased to 80 V for the remaining 40 min.

Try 0.8% agarose next time and test the new Amplicon ladder, the NBE 1kb Plus ladder was not so good.



Gel extraction

Insert experiment discussion and interpretation. Insert which samples were extracted from the gels, what was their mass and any deviations made in the experiment (like different elution or the dilution with MiliQ Water)

4 x 1.5 mL tubes were weighed beforehand using analytical scales. Samples 5-8 (cut pET11a with BamHI+NdeI) were cut, each placed into a separate tube and weighted again.

Sample	Mass
P1	0.1235 g → 124 mg
P2	0.1127 g → 113 mg
P3	0.1624 g→ 162 mg
P4	0.2137 g → 214 mg

Gel extraction was performed using the J**ena Bioscience Gel extraction kit**, in accordance with their instructions. Since our DNA is >5 kbp, isopropanol volume was 2:1 to gel, as advised in Jena's protocol. Secondary washing of the column was done, as advised in the kit for DNA larger than 5kb.

For the last step, pDNA was eluted using a 30 µL **Elution buffer**, to concentrate it as much as possible.

NanoDrop Measurement of Extracted Digested Plasmids

During the gel extraction, sample 4 was discarded due to accidentally mixing the sample with the washing buffer before the sample was placed on the activated filter.

Sample concentrations were too low to be measured using NanoDrop, thus microcuvettes were used. Since the sample volume was 30 µL, they were diluted to 70 µL using MQ water.

Sample	**Concentration (ng/µL)**	**Abs 260/280**	**Abs 260/230**
P1	0.30	3.0	0.035
P2	0.15	3.0	0.023
P3	0.65	3.250	0.046
~~P4 - discarded~~	n/a	n/a	n/a
pET11a (5 times diluted)	67.45	2.063	2.279

For the Blank, 30 µL elution buffer was mixed with 40 µL MQ water (70 µL total).

At the advice of John, the uncut plasmid pET11a concentration was measured again, this time using microcuvette for more accurate results. The undiluted pET11a had too high concentration, thus it was diluted 5 times: 14 µL of pET11a + 56 µL Elution buffer (Jena Plasmid extraction kit). The undiluted pET11 concentration was thus 337.25 ng/µL, slightly lower than the NanoDrop showed (347 µL).

Competent Cell Preparation Day 3

Insert experiment discussion and interpretation, what the deviations were, if the cells were incubated longer, difficulties with resuspension in cacl2, which force they were centrifuged, how many vials we prepared

Competent cell preparation Day 3 protocol was followed:

Initially, 3 x shaking flasks with 100 mL LB media were inoculated with 500 µL overnight cell culture (cultivation time ~ h: 09:50-12:XX). The OD_{600} was measured to be: ~0.4

After incubation, the flasks were placed on ice.

After reconsideration, more 3 x shaking flasks (100 mL LB) were inoculated (cultivation time ~2.5h: 11:20-14:00). The final OD_{600} of each was 0.492; 0.437; 0.538, accordingly (slightly more than protocols 0.3-0.45 guidelines). After incubation, the cells were placed on ice.

The following steps were the same for both: cell suspensions were aliquoted into 50 mL Falcon tubes, each of them weighed, and equilibrated for centrifugation at 3428 x g for 10 min.

A total of **125** vials of competent cells were prepared.