Nuclease

Week 9

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Transformaton of pSB1C3-T7-Nuc in Top10 and BL21(DE3) cells with Colony PCR analysis and inoculation in liquid media

Responsible

Ellinor Lindholm and Oscar He

Protocols used

Transformation
PCR Amplification (Taq)
Gel electrophoresis
Colony picking

Modifications and comments to protocols

PCR Amplification (Taq) primers:	VR (reverse)
	VF2 (forward)

Experimental Setup

Table 1: Volumes of liquid media for each colony inoculated.

Sample	Volume [μl]
Nuc 1.2 HQ BL21(DE3) 1	3
Nuc 1.2 HQ BL21(DE3) 2	3
Nuc 1.2 HQ Top10 1	3
Nuc 1.2 HQ Top10 2	3
Nuc 1.2 HQ Top10 3	3
Nuc 2.1.2 GS BL21(DE3) 1	3
Nuc 2.1.2 GS BL21(DE3) 2	3
Nuc 2.1.2 GS BL21(DE3) 3	3
Nuc 2.1.2 GS Top10 1	3
Nuc 2.1.2 GS Top10 2	3

Results and Conclusions

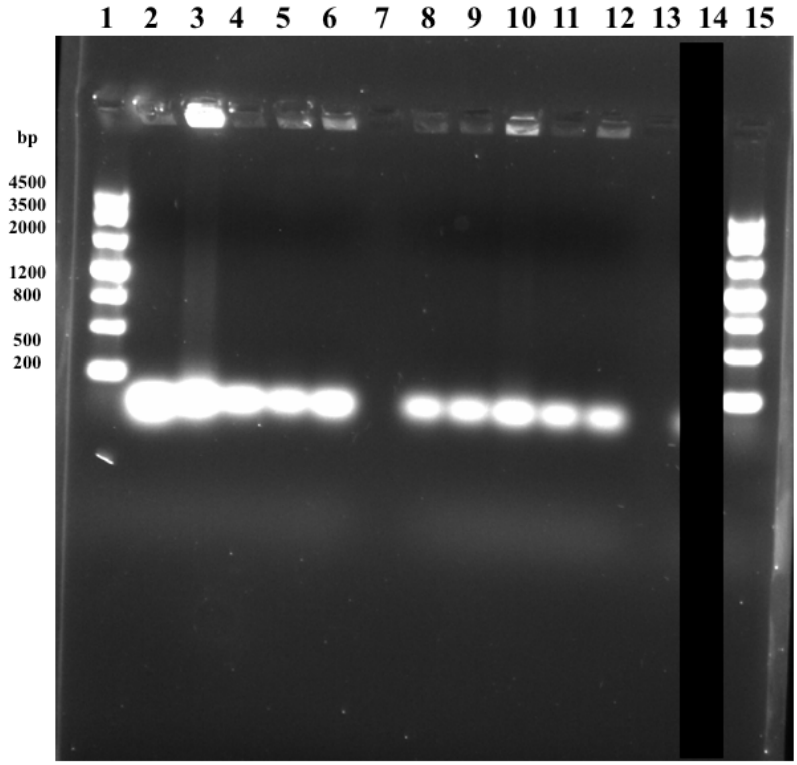

Figure 1: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 1.2 HQ BL21(DE3) 1 (3) Nuc 1.2 HQ BL21(DE3) 2 (4) Nuc 1.2 HQ Top10 1 (5) Nuc 1.2 HQ Top10 2 (6) Nuc 1.2 HQ Top10 3 (8) Nuc 2.1.2 GS BL21(DE3) 1 (9) Nuc 2.1.2 GS BL21(DE3) 2 (10) Nuc 2.1.2 GS BL21(DE3) 3 (11) Nuc 2.1.2 GS Top10 1 (12) Nuc 2.1.2 GS Top10 2 (15) DNA Ladder.

No bands at expected size of approximately 800 bp.

Discussion and Troubleshooting

Since the analysis of the ligation product showed an unsuccessful 3A assembly, the results from the colony PCR was not expected to be positive. It was later discovered that the wrong primers were used (BioBrick primers). Hence, the results of the colony PCR are not reliable.

2 Extraction of pSB1C3-T7-Nuc from transformed Top10 and BL21(DE3) cells with both PCR and digestion analysis

Responsible

Ellinor Lindholm and Oscar He

Protocols used

Plasmid Extraction
PCR Amplification (Taq)
Gel electrophoresis

Modifications and comments to protocols

Plasmid Extraction - Step 1:	centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
Plasmid Extraction - Step 3:	used P2 Lyse Blue
Plasmid Extraction - Step 6:	repeated 6 times
Plasmid Extraction - Step 7:	not performed for samples with Top10 cells
Plasmid Extraction - Step 10:	DNA elution using EB Buffer
PCR Amplification (Taq):	half of the total volume used
	1 μl DNA sample used in PCR mix.
	10x PCR reaction buffer with 15 mM $MgCl_2$ used
PCR Amplification (Taq) primers:	VR (reverse)
	VF2 (forward)

Experimental Setup

Table 2: Volumes of the liquid cultures used for plasmid extraction.

Sample	Volume [μl]
Nuc 1.2 HQ BL21(DE3) 1	1.5
Nuc 1.2 HQ BL21(DE3) 2	1.5
Nuc 1.2 HQ Top10 1	1.5
Nuc 1.2 HQ Top10 2	1.5
Nuc 1.2 HQ Top10 3	1.5
Nuc 2.1.2 GS BL21(DE3) 1	1.5
Nuc 2.1.2 GS BL21(DE3) 2	1.5
Nuc 2.1.2 GS BL21(DE3) 3	1.5
Nuc 2.1.2 GS Top10 1	1.5
Nuc 2.1.2 GS Top10 2	1.5

Table 3: Calculated concentration of sample plasmid in the PCR mix.

Sample	Concentration [ng/μl]
Nuc 1.2 HQ BL21(DE3) 1	3.2
Nuc 1.2 HQ BL21(DE3) 2	3.1
Nuc 1.2 HQ Top10 1	3.0
Nuc 1.2 HQ Top10 2	2.2
Nuc 1.2 HQ Top10 3	2.3
Nuc 2.1.2 GS BL21(DE3) 1	4.1
Nuc 2.1.2 GS BL21(DE3) 2	2.8
Nuc 2.1.2 GS BL21(DE3) 3	4.2
Nuc 2.1.2 GS Top10 1	3.2
Nuc 2.1.2 GS Top10 2	1.5

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \tag{1}$$

Results and Conclusions

Table 4: Concentrations of the extracted plasmid samples.

Sample	Concentration [ng/μl]
Nuc 1.2 HQ BL21(DE3) 1	79.4
Nuc 1.2 HQ BL21(DE3) 2	76.2
Nuc 1.2 HQ Top10 1	75.1
Nuc 1.2 HQ Top10 2	54.6
Nuc 1.2 HQ Top10 3	58
Nuc 2.1.2 GS BL21(DE3) 1	101.3
Nuc 2.1.2 GS BL21(DE3) 2	69.7
Nuc 2.1.2 GS BL21(DE3) 3	105.1
Nuc 2.1.2 GS Top10 1	79.1
Nuc 2.1.2 GS Top10 2	38.3

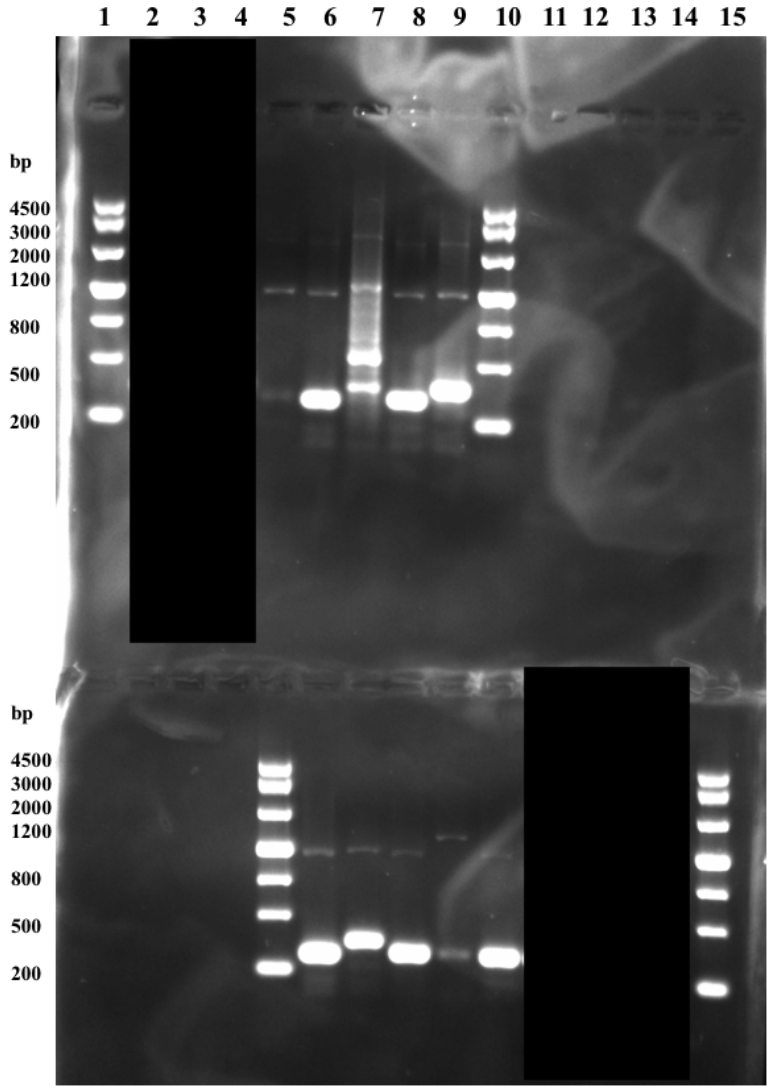

Figure 2: Result of the gel electrophoresis with 1 % agarose at 100 V.

Table 5: Expected and actual size(s) of the PCR products of the extracted plasmids.

Well # (top)	Sample	Expected [bp]	Actual [bp]	Well # (bottom)	Sample	Expected [bp]	Actual [bp]
1	DNA Ladder	-	-	1	-	-	-
2	-	-	-	2	-	-	-
3	-	-	-	3	-	-	-
4	-	-	-	4	-	-	-
5	Nuc 1.2 HQ BL21(DE3) 2	800	2500 1200 300	5	DNA Ladder	-	-
6	Nuc 1.2 HQ BL21(DE3) 1	800	2500 1200 300	6	Nuc 1.2 HQ Top10 3	800	1200 300
7	Nuc 2.1.2 GS BL21(DE3) 3	800	2900 1400 1100 1000 700 600 450 350	7	Nuc 1.2 HQ Top10 2	800	1200 400
8	Nuc 2.1.2 GS BL21(DE3) 2	800	2500 1200 300	8	Nuc 1.2 HQ Top10 1	800	1200 300
9	Nuc 2.1.2 GS BL21(DE3) 1	800	2500 1200 400	9	Nuc 2.1.2 GS Top10 2	800	1600 300
10	DNA Ladder	-	-	10	Nuc 2.1.2 GS Top10 1	800	1200 300
11	-	-	-	11	-	-	-
12	-	-	-	12	-	-	-
13	-	-	-	13	-	-	-
14	-	-	-	14	-	-	-
15	-	-	-	15	DNA Ladder	-	-

No band at expected size of 800-900 bp.

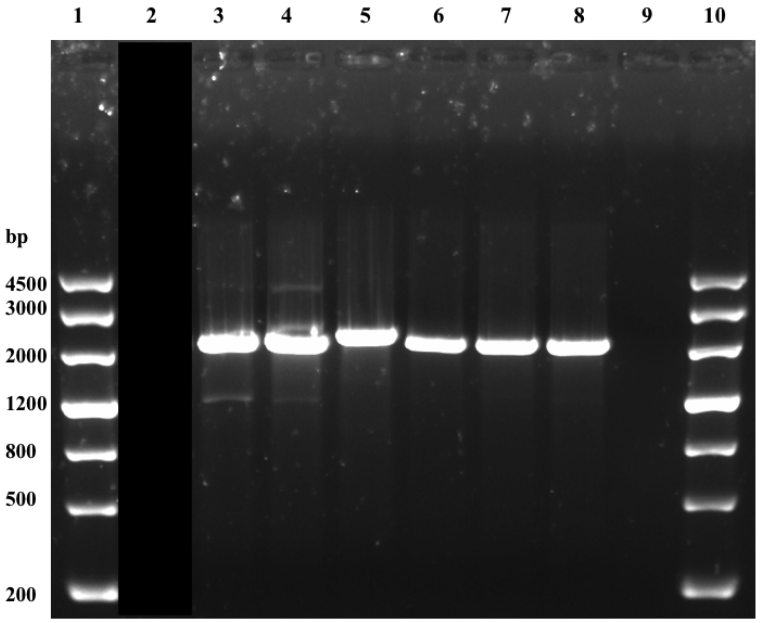

Figure 3: Result of the gel electrophoresis of single digestion of extracted plasmid, with 1 % agarose at 100 V. (1) DNA Ladder (3) Nuc 2.1.2 GS BL21(DE3) 1 (4) Nuc 2.1.2 GS BL21(DE3) 2 (5) Nuc 2.1.2 GS BL21(DE3) 3 (6) Nuc 1.2 HQ Top10 1 (7) Nuc 1.2 HQ Top10 2 (8) Nuc 2.1.2 GS Top10 2 (9) DNA Ladder.

Discussion and Troubleshooting

Nuc 2.1.2 GS BL21(DE3) 3 is a candidate for further processing since its size on the gel (Figure 3) is very close to the expected size of 2800 bp. A sequencing will confirm the identity of the construct. If the construct is correct, the next step is to perform an overhang PCR in order to attach the restriction site BamHI to the 5' end of the nuclease sequence.

3 Addition of BamHI restriction site downstream to the nuclease sequence using overhang PCR

Responsible

Oscar He and Ellinor Lindholm

Protocols used

PCR Amplification (Taq)
Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Taq) primers:	Nuc_R (reverse) VF2 (forward)

Experimental Setup

Table 6: Volumes of the components used in the PCR mix.

Component	Volume [μl]
PCR Grade Nucleotide Mix	0.5
10x Sigma PCR Buffer ($MgCl_2$)	2.5
VF2	1.25
Nuc_R	1.25
Taq DNA Polymerase	0.5
Nuc 2.1.2 GS BL21(DE3) 3	1
ddH_20	18

Results and Conclusions

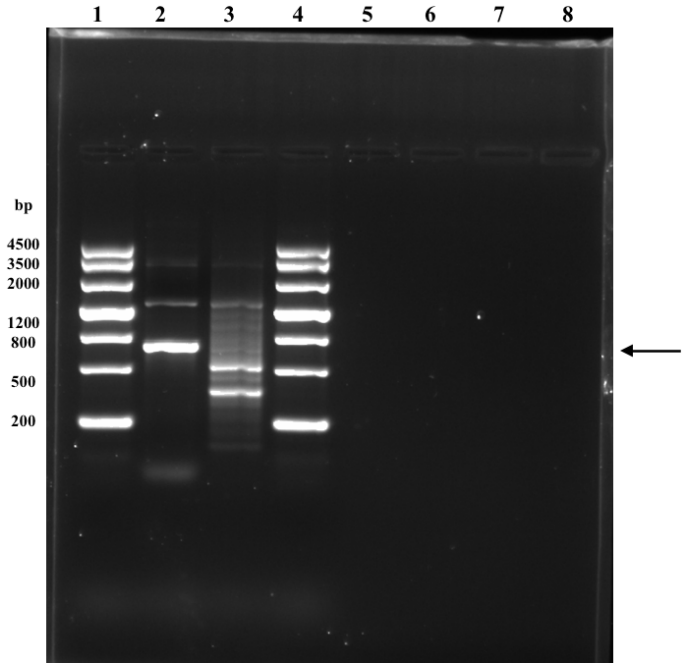

Figure 4: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 2.1.2 GS BL21(DE3) 3 (Nuc_R) (3) Nuc 2.1.2 GS BL21(DE3) 3 (VR) (4) DNA Ladder.

Band at expected size (700 bp) for PCR product using Nuc_R. No band at expected size (800 bp) for PCR product using VR.

Discussion and Troubleshooting

The result are difficult to interpret since the two separate PCR amplifications resulted in completely different products. For the amplification with the Nuc_R primer, an intense band at the expected size (700 bp, upstreams overhang of 100 bp) can be seen. But when the VR primer is used for the same DNA sample, the expected size (800 bp, upstreams overhang 100 bp and downstreams overhang 100 bp) is not present. This indicates a malfunctioning reverse primer. But since such a statement is not reliable without any further troubleshooting, the result of the PCR amplification with Nuc_R can not be trusted without a sequencing analysis.

4 Digestion of pSB1C3-T7-Nuc-BamHI with PCR purification.

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
PCR Purification

Modifications and comments to protocols

3A Assembly:	total volume 50 μl (digestion mix)
3A Assembly - Step 3:	Spin x 2

Experimental Setup

Table 7: Concentration of the pSB1C3-T7-Nuc-BamHI PCR product used for digestion.

Sample	Concentration [ng/μl]
pSB1C3-T7-Nuc-BamHI	504.5

Table 8: Volumes of components in the digestion mix.

Component	Volume [μl]
DNA sample	4.96
10x SuRE/Cut Buffer B	5.0
EcoRI	2.5
BamHI	2.5
ddH_2O	35.04

Table 9: Volume taken for PCR purification.

Sample	Volume [μl]
pSB1C3-T7-Nuc-BamHI	30

Sample Calculation

The volume of DNA sample to add to the digestion mix

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (2)$$

Results and Conclusions

Table 10: The concentration of the sample after PCR Purification, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
pSB1C3-T7-Nuc-BamHI	5.5

Discussion and Troubleshooting

Since no gel electrophoresis analysis was made on this experiment, it is not possible to draw any conclusions whether the digestion was successful or not.

5 3A Assembly (ligation) of pSB1C3-T7-Nuc-BamHI-Tags and pSB1A3-T7-Nuc-BamHI-Tags

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
Gel electrophoresis

Modifications and comments to protocols

	Amount of vector (pSB1A3) per reaction	24.0 ng
	Amount of vector (pSB1C3) per reaction	23.6 ng
	Ratio pSB1A3:NucT7	1:10
3A Assembly:	Ratio pSB1C3:NucT7 (PCR pur.)	1:4.7
	Ratio pSB1A3:Tags1	1:2.5
	Ratio pSB1C3:Tags2	1:2.1
	Incubation:	14 h at 16 °C

Experimental Setup

Table 11: Concentration and size of the parts used for the ligation in the 3A assembly. For this particular experiment, both a non-PCR purified and a PCR purified NucT7 sample was used. Two different samples of Tags were used aswell.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1A3	8.0	2155
pSB1C3	5.9	2070
NucT7	44.9	616
NucT7 (PCR pur.)	5.5	616
Tags1	6.1	84
Tags2	2.5	84

Table 12: Volumes of each component used in the ligation mix.

Assemble part	pSB1A3 construct [μl]	pSB1C3 construct [μl]
Vector	3	4
NucT7	5.4	-
NucT7 (PCR pur.)	-	20
Tags1	10	-
Tags2	-	20
T4 DNA Ligase	1.5	2
10x T4 DNA Ligase Buffer	3	4
ddH_2O	0	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3/pSB1C3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{3}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ insert\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{4}$$

Results and Conclusions

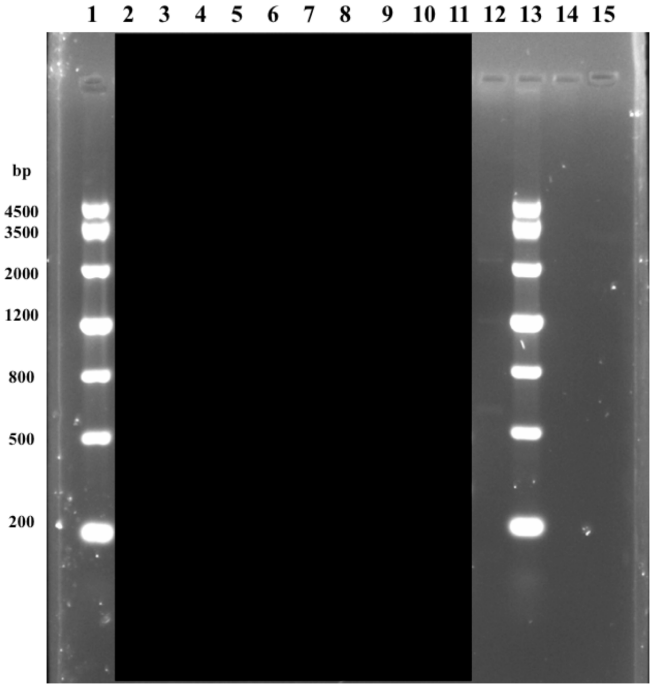

Figure 5: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (12) pSB1C3-T7-Nuc-BamHI-Tags (13) DNA Ladder.

Discussion and Troubleshooting

No band at the expected size can be seen on the gel leading to the conclusion of a probably unsuccessful ligation. Several contaminations at smaller sizes than the expected indicates non-ligated parts.

6 Transformation of pSB1C3-T7-Nuc-BamHI-Tags and pSB1A3-T7-Nuc-BamHI-Tags in Top10 and BL21(DE3) cells with Colony PCR analysis and inoculation in liquid media

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Transformation
PCR Amplification (Taq)
Gel electrophoresis
Colony picking

Modifications and comments to protocols

PCR Amplification (Taq):	half of the total volume used
	1 μl DNA sample used in PCR mix.
	10x PCR reaction buffer with 15 mM $MgCl_2$ used
PCR Amplification (Taq) primers:	VR (reverse)
	VF (forward)

Experimental Setup

Table 13: Volumes of liquid culture for each taken colony.

Sample	Volume [μl]
NucTagCmR Top10 1	5
NucTagCmR Top10 2	5
NucTagCmR Top10 3	5
NucTagCmR Top10 4	5
NucTagCmR Top10 5	5
NucTagCmR BL21(DE3) 1	5
NucTagCmR BL21(DE3) 2	5
NucTagCmR BL21(DE3) 3	5
NucTagCmR BL21(DE3) 4	5
NucTagCmR BL21(DE3) 5	5

Results and Conclusions

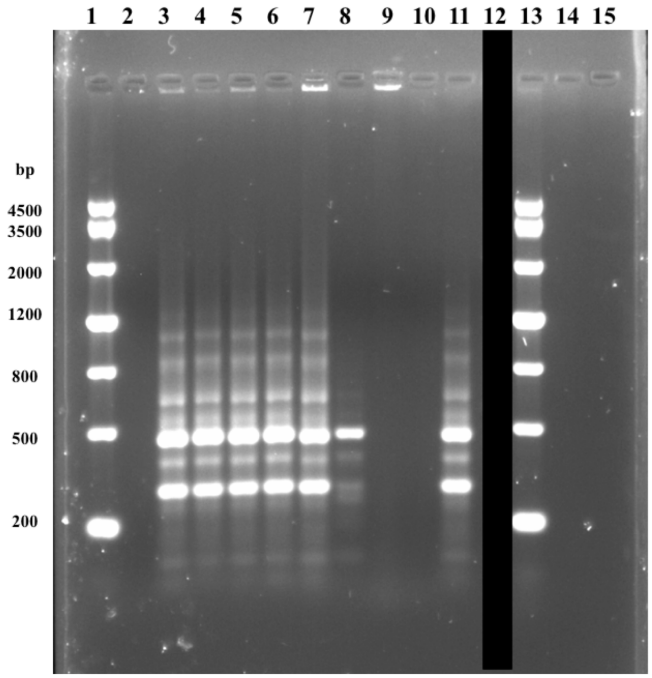

Figure 6: Result of the gel electrophoresis with 1 % agarose at 100 V.

Discussion and Troubleshooting

No clear evidence of a successful ligation and transformation can be seen in the gel electrophoresis analysis since the expected band can not be seen. Furthermore, a lot of contaminations is instead visible. The contaminations are of the same kind in almost every of the samples, indicating that the cells contain the same type of plasmid and that the ligation was unsuccessful, resulting in a unknown plasmid. Another reason to the gel result relates to the results from the PCR analysis of the pSB1C3-T7-Nuc-BamHI construct, showing the expected band when using the Nuc_R reverse primer but an unclear result with the VR primer. The VF2 primer is suspected to be to some extent unfunctional, either as an individual primer or in mixture with one or several of the other components in the PCR Amplification mixture. Further troubleshooting has to be done before a conclusion can be drawn.

7 Extraction of pSB1C3-T7-Nuc-BamHI-Tags

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Plasmid Extraction

Modifications ans comments to protocols

Plasmid Extraction - Step 1:	centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
Plasmid Extraction - Step 6:	repeated 6 times
Plasmid Extraction - Step 7:	not performed for samples with Top10 cells
Plasmid Extraction - Step 10:	DNA elution using EB Buffer

Experimental Setup

Table 14: Volumes of the liquid cultures used for plasmid extraction.

Sample	Volume [μl]
NucTagCmR Top10 1	1.5
NucTagCmR Top10 2	1.5
NucTagCmR Top10 3	1.5
NucTagCmR Top10 4	1.5
NucTagCmR Top10 5	1.5
NucTagCmR BL21(DE3) 1	1.5
NucTagCmR BL21(DE3) 2	1.5
NucTagCmR BL21(DE3) 3	1.5
NucTagCmR BL21(DE3) 4	1.5
NucTagCmR BL21(DE3) 5	1.5

Results and Conclusions

Sequencing results showed an unsuccessful assembly of the desired construct.