Defensin

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 Transformation of pSB1C3-T7-Def in Top10 and BL21(DE3) with Colony PCR analysis with inoculation in liquid media

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Transformation
Gel electrophoresis
Colony picking

Results and Conclusions

One colony for transformed Top10 cells. Transformation into BL21(DE3) was unsuccessful.

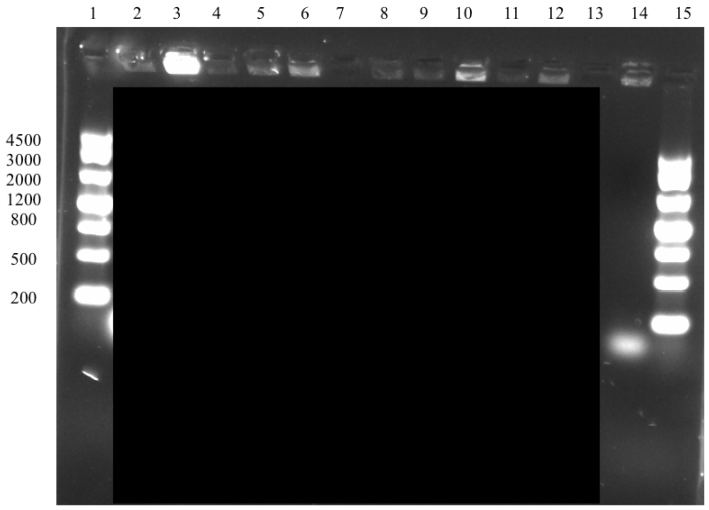

Figure 1: Result of the gel electrophoresis of Colony PCR product, with 1 % agarose at 100 V. (1) DNA Ladder (14) Def Top10 (15) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

Discussion and Troubleshooting

The transformation was unsuccessful according to the gel. However, the one colony of Top10 was picked for inoculation in liquid media and the extracted plasmid was then analysed to confirm the result from the Colony PCR.

2 Extraction of pSB1C3-T7-Def with PCR and single digestion analysis

Responsible

Oscar He and Ellinor Lindholm

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 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):	1 μl DNA sample used in PCR mix. 10x PCR reaction buffer with 15 mM $MgCl_2$ used
PCR Amplification (Taq) primers:	VF2_R (reverse) VF2_F (forward)

Experimental Set Up

Table 1: Calculated concentration of sample plasmid in the PCR mix.

Sample	Concentration [ng/μl]
pSB1C3-T7-Def	2.54

Table 2: Concentration of sample for digestion analysis.

Sample	Concentration [ng/μl]
pSB1C3-T7-Def	63.5

Table 3: Volumes of components in the digestion mix.

Component	Volume [μl]
DNA sample	7.87
10x SuRE/Cut Buffer B	1.0
EcoRI	0.5
ddH2O	0.6

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \quad (1)$$

Results and Conclusions

Table 4: Concentration of extracted plasmid sample measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
pSB1C3-T7-Def	63.5

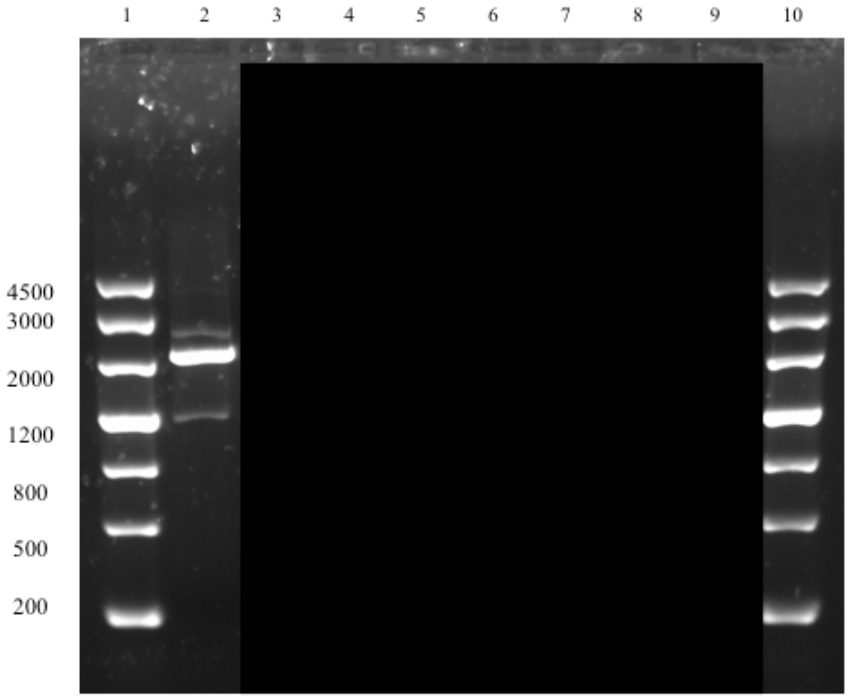

Figure 2: Result of the gel electrophoresis of single digested product, with 1 % agarose at 100 V. (1) DNA Ladder (2) Def Top10 single digested (10) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

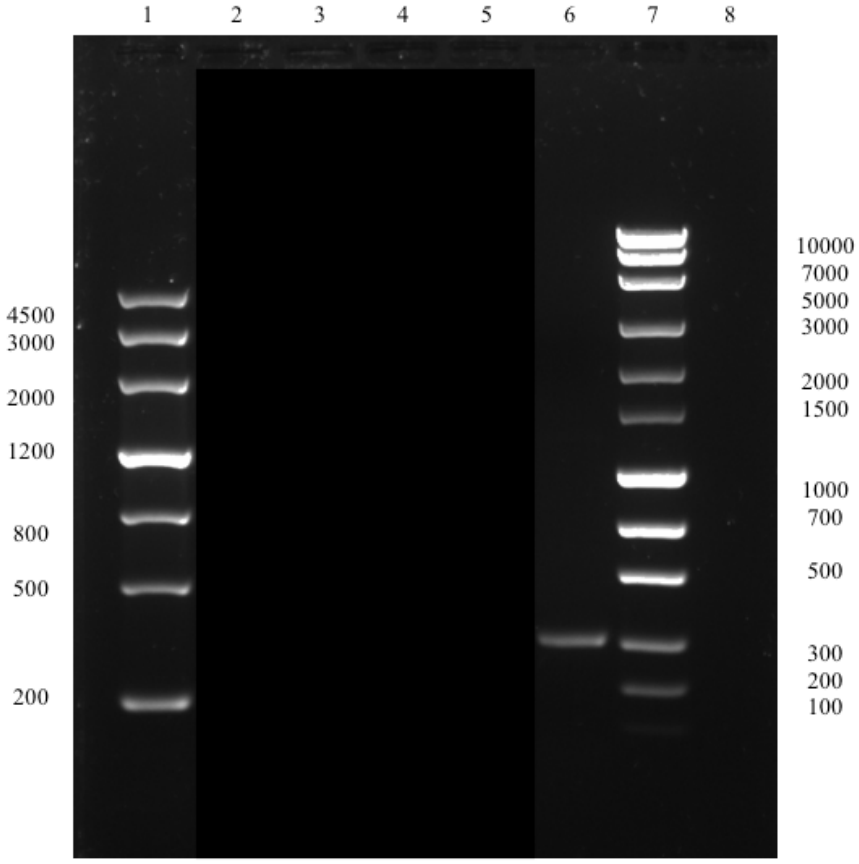

Figure 3: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 100 V. (1) DNA Ladder (6) Def Top10 (7) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

Discussion and Troubleshooting

The PCR analysis of the ligation product and the extracted plasmid was not convincing enough to draw the conclusion of a successful ligation. Thus, a single digestion of the PCR amplified extraction product was performed which showed a clear band at the expected size of 2264 bp. Another PCR analysis was later done with the Def_R primer instead of VR. This analysis showed clearly the presence of the correct ligation product, thus confirming the previous results.