Sortase

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.

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1 Digestion and Ligation of pSB1C3-Gb1-SortA

Responsible

Aman Mebrahtu

Protocols used

- Digestion and Ligation

Modifications and comments to protocols

The amount of enzyme used was adjusted to the concentration of the DNA sample.

Experimental Set Up

The experimental set up for the digestions are stated in the tables below. A reaction volume of 10 μl was used for both of the digestions.

Table 1: Concentrations of DNA to be digested

Sample	Concentration [ng/μl]
pSB1C3	41.0

Table 2: Digestion reaction of pSB1C3

Reaction	Volume [μl]
DNA	5
Buffer SH 10 X	1.0
Pst1	0.2
EcoR1	0.2
Sterile water	3.6

In this ligation, we decided to go forward with an insert:vector molar ratio of 10:1. The calculations shown on the tables below were adjusted so that each reaction contains 50 ng of backbone and the inserts are adjusted according to the desired molar ratio. Since the concentrations of the purified DNA fragments were relatively low, this resulted in an overload of reaction volume which is indicated by a minus sign for sterile water.

Table 3: Ligation Reaction of pSB1C3-Gb1-SortA

Component	Volume
Gb1-SortA	12.89
pSB1C3	4.17
T4 DNA Ligase	0.5
T4 DNA Ligase Buffer 10X	2.5
Sterile water	4.95

Results and Conclusions

The result of the ligation is to be confirmed with transformation.

2 Transformation of pSB1C3-GB1-SortA

Responsible

Reskandi Rudjito and Maren Maanja

Protocols used

- Transformation

Experimental Set Up

The ligation was performed overnight at a temperature of 16°C was later transformed into both TOP 10 and BL21 cells.

Table 4: Re-Transformation of pSB1C3-T7-Lys

Sample	Comment
pSB1C3-GB1-SortA Top10	Transformed into Top10 cells
pSB1C3-GB1-SortA BL21 (DE3)	Transformed into BL21 (DE3) cells
positive control Top 10	No antibiotics
positive control BL21	No antibiotics

Results and Conclusions

No colonies were observed indicating that the previous ligation procedure had been unsuccessful.

3 Digestion of Gb1-SortA

Responsible

Reskandi Rudjito and Maren Maanja

Protocols used

- Digestion

Experimental Set Up

Table 5: Concentrations of DNA to be digested

Sample	Concentration [ng/μl]
Gb1-SortA	21.9

Table 6: Digestion of Gb1-SortA

Reaction	Volume [μl]
DNA	8
Buffer SH 10 X	2.0
Pst1	0.2
EcoR1	0.2
Sterile water	1.6

The digested products were subjected to purification and to maximize the amount of DNA eluted, we performed re-elution of DNA from the purification columns.

Results and Conclusions

The result of the digested Gb1-SortA is shown in the figure below (lane 5). The expected size of the digested fragment is 669 bp.

Table 7: Concentrations of digested products after purification

Sample	Concentration [ng/μl]
Gb1-SortA	4.0
Gb1-SortA (re-ellution)	2.0

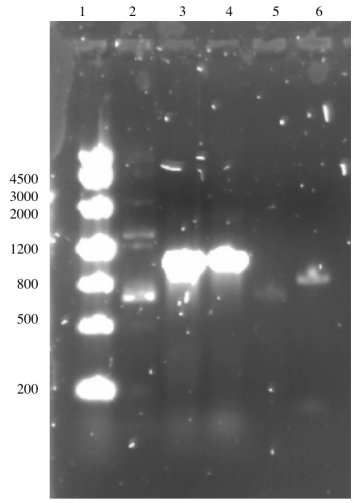

Figure 1: Digested pSB1C3. (1) Ladder (2)-(4) Not of interest (5) Digested GS (6) Not of interest

4 Ligation and Transformation of pSB1C3-Gb1-SortA

Responsible

Reskandi Rudjito

Protocols used

- Ligation
- Transformation

Experimental Set Up

In order to perform the ligation, the two DNA fragments consisting of the Gb1-SortA and pSB1C3 backbone are digested and purified prior to ligation. The concentrations of each DNA fragment are stated in Table 8.

Table 8: Concentrations DNA fragments for Ligation

Sample	Concentration [ng/μl]	Size [bp]
Digested Gb-SortA	4.0	669
Digested pSB1C3	5.9	2200

In this ligation, we have decided to go forward with an insert:vector molar ratio of 10:1. The calculations shown on the tables below are adjusted so that each reaction contains 50 ng of backbone and the inserts are adjusted according to the desired molar ratio.

Table 9: Ligation Reaction of pSB1C3-Gb1-SortA

Component	Volume
Gb1-SortA	17.76
pSB1C3	4.24
T4 DNA Ligase	0.5
T4 DNA Ligase Buffer 10X	2.5
Sterile water	0

The ligation was performed overnight at a temperature of 16 °C was later transformed into both TOP 10 and BL21 cells.

Table 10: Re-Transformation of pSB1C3-T7-Lys

Sample	Comment
pSB1C3-GB1-SortA Top10	Transformed into Top10 cells
pSB1C3-GB1-SortA BL21 (DE3)	Transformed into BL21 (DE3) cells
positive control Top 10	No antibiotics
positive control BL21	No antibiotics

Results and Conclusions

The ligation product was subjected to gel electrophoresis, as shown in the figure below.

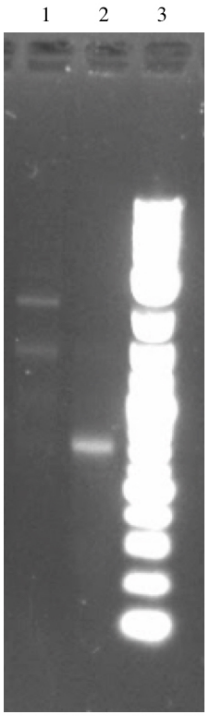

Figure 2: Digested pSB1C3. (1) Ligated pSB1-C3-Gb1-SortA (2) Digested pSB1C3 (3) Ladder

Discussion and Troubleshoot

Lane 1 from Figure 2 shows that there are several bands is the upper part of gel which could possible indicate ligated plasmids.

5 Transformation of pSB1C3-Gb1-SortA

Responsible

Aman Mebrahtu and Reskandi Rudjito

Protocols used

- Transformation

Experimental Set Up

Transformation was done on both TOP 10 cells and BL21 cells to improve efficiency.

Table 11: Re-Transformation of pSB1C3-T7-Lys

Sample	Comment
pSB1C3-GB1-SortA Top10	Transformed into Top10 cells
pSB1C3-GB1-SortA BL21 (DE3)	Transformed into BL21 (DE3) cells
positive control Top 10	No antibiotics
positive control BL21	No antibiotics

Results and Conclusions

Transformation was unsuccessful.

Discussions and Troubleshoot

We have been getting negative result in our transformation procedures. Thus, to come around this problem, we will try to purify the ligation product before transformation and also try electro-competent cells instead of chemically competent cells.

6 Re-Transformation of pSB1C3-Gb1-SortA using purified ligation product and electro-compotent cells

Responsible

Reskandi Rudjito and Aman Mebrahtu

Protocols used

- PCR Purification
- Transformation

Experimental Set Up

In this experiment we tried both chemically competent and electro-competent cells with purified ligation products.

Table 12: Re-Transformation of pSB1C3-T7-Lys

Sample	Comment
pSB1C3-GB1-SortA	Transformed into Top10 cells
pSB1C3-GB1-SortA	Transformed into BL21 (DE3) cells
pSB1C3-GB1-SortA	Transformed into electro-competent BL21 (DE3) cells
positive control Top 10	No antibiotics
positive control BL21	No antibiotics

Results and Conclusions

As a result we saw several positively transformed colonies.

Table 13: Result of Transformation

Sample	Number of colonies
Chemical TOP 10 cells	0
Chemical BL21 (DE3) cells	2
Electro-competent BL21 (DE3) cells	1

7 Colony picking and Colony PCR of Transformed colonies containing pSB1C3-Gb1-SortA

Responsible

Reskandi Rudjito and Aman Mebrahtu

Protocols used

- Colony picking
- PCR

Experimental Set Up

The PCR was done with verification primers (VF2 and VR). Table 8 shows the composition used for the PCR and Table 9 shows the PCR conditions used in the thermocycler. The PCR reaction used for each sample was 25 μl.

Table 14: PCR Reaction

Master Mix	Component	Volume [μl]
1	PCR Grade Nucleotide mix	1
	R_Primer (VR)	5
	F_primer (VF2)	5
	Template DNA	2
	Sterile water	14
	Total Volume	25
2	PCR Reaction buffer 10 x	5
	Taq DNA Polymerase	0.5
	Sterile water	19.5
	Total Volume	25

Table 15: PCR condition using Taq polymerase

Step	Cycles	Temperature {°C}	Time
Initial Denaturation	1	94	2 min
Denaturation	30	94	30 secs
Annealing		52	1 min
Extension		72	1 min
Final Extension	1	72	7 min
Hold	indefinitely	4	-

Results and Conclusions

The result of the gel electrophoresis is shown below. The amplicon containing Gb1-SortA should be 941 bp in size.

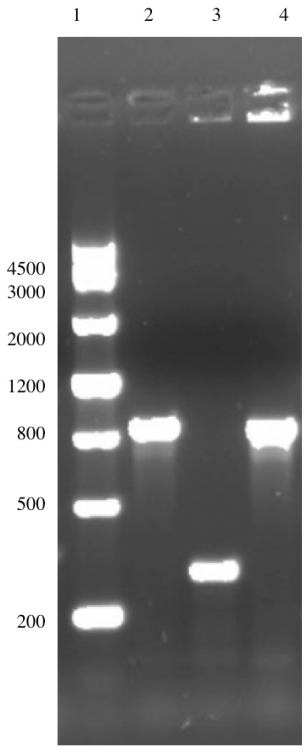

Figure 3: Colony PCR of Gb1-Sortase. (1) Ladder (2) Colony 1 (3) Colony 2 (4) Colony 3 - Electroporation

Discussions and Troubleshoot

The result of the colony PCR shows that colony 1 and 3 contains the right insert. This is confirmed by the fact that the colony PCR show bands of above 800 bp and the expected size is around 941 bp. Colony 2 however most likely contained a contaminating sequence that was successfully cloned into the backbone.

8 Glycerol Stocks and Plasmid Extraction of pSB1C3-Gb1-SortA

Responsible

Reskandi Rudjito and Aman Mebrahtu

Protocols used

- Glycerol stocks
- Plasmid extraction

Experimental Set Up

Colony 1 and 3 containing the pSB1C3-Gb1-SortA was cultured in LB containing chloramphenicol. Samples of the two cultures were saved as glycerol stocks and stored in the -80°C freezer. The liquid culture was also used for plasmid extraction, where the extracted plasmid was subjected to sequence analysis.

Results and Conclusions

The concentrations of the extracted plasmids are summarized below.

Table 16: Concentration of Plasmid

Sample	Concentration [ng/μl]
pSB1C3-Gb1-SortA (Colony 1)	322.1
pSB1C3-Gb1-SortA (Colony 3)	180.4