

Lysostaphin

Week 15

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 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - third attempt

Responsible

Reskandi Rudjito and Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Lys_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) components:	GC Buffer and DMSO
PCR Amplification (Phusion) extension time:	75 seconds
PCR Amplification (Phusion) annealing temperature:	55 and 57 °C

Experimental Set Up

Table 1: Concentration of samples used for the overhang PCR amplification.

Sample	Concentration [ng/ μ l]
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	10

Table 2: Volumes of components in PCR mix.

Component	Volume [μ l]
10 μ M Suffix_F	1
10 μ M Lys_R	1
5X Phusion GC Buffer	4
Phusion DNA Polymerase	0.2
2 mM dNTP mix	2
Template DNA	1
DMSO	0.6
ddH ₂ O	10.2

Table 3: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[° C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		55/57	30 secs
Extension		72	1 min 20 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Sample Calculation

No further calculations were done.

Results and Conclusions

The PCR was unsuccessful, no bands were observed on the gel.

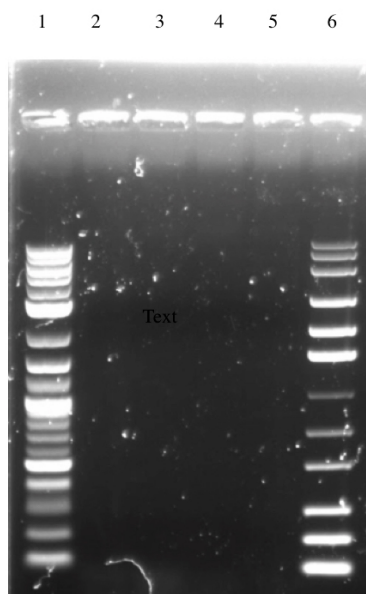


Figure 1: Overhang PCR of pSB1C3-T7-Lys third attempt. (1) Ladder (2)-(3) Not of interest (4) Lys at annealing of 55 °C (5) Lys at annealing of 57 °C

Discussion and Troubleshooting

The extension time could have been too long.

2 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - fourth attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Lys_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	GC Buffer
PCR Amplification (Phusion) extension time:	75 seconds
PCR Amplification (Phusion) annealing temperature:	65 and 70 °C

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 4: Concentration of samples used for the overhang PCR amplification.

Sample	Concentration [ng/ μ l]
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	10

Table 5: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.8
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 6: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[° C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		65/70	30 secs
Extension		72	75 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

There is no product at an annealing temperature of 65°C and 70°C .

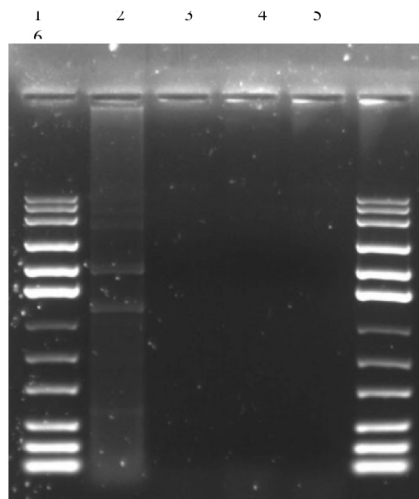


Figure 2: Overhang PCR of pSB1C3-T7-Lys fourth attempt. (1) Ladder (2) Not of interest (3) Lys at annealing of 65 °C (4) Not of interest (5) Lys at annealing of 70 °C

Discussion and Troubleshooting

The annealing temperature was possibly too high

3 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - fifth attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Lys_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	GC Buffer
PCR Amplification (Phusion) extension time:	90 seconds
PCR Amplification (Phusion) annealing temperature:	60 °C

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 7: Concentration of samples used for the overhang PCR amplification.

Sample	Concentration [ng/ μ l]
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	10

Table 8: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.8
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 9: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		60	30 secs
Extension		72	90 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

Several unspecific bands appeared. No bands appeared at the desired size of 2852 bp.

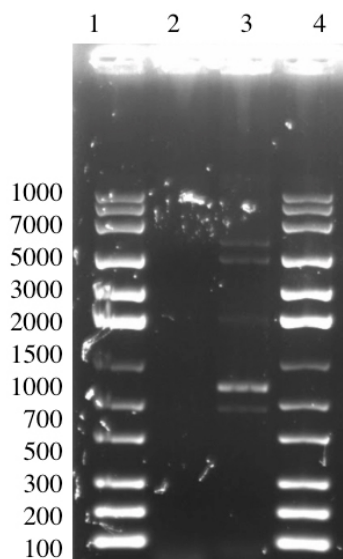


Figure 3: Overhang PCR of pSB1C3-T7-Lys fifth attempt. (1) Ladder (2)Not of interest (3) Lys at annealing of 60 °C (4) Ladder

Discussion and Troubleshooting

The primer could have annealed at unspecific sites thus resulting in the emergence of several bands.

4 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - sixth attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Lys_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	GC Buffer
PCR Amplification (Phusion) extension time:	75 seconds
PCR Amplification (Phusion) annealing temperature:	65 and 70 °C
PCR Amplification (Phusion) cycles:	35

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 10: Concentration of samples used for the overhang PCR amplification.

Sample	Concentration [ng/ μ l]
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	10
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	45

Table 11: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.8
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 12: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		57	30 secs
Extension		72	60 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

There is no product at an annealing temperature of 65°C and 70°C .

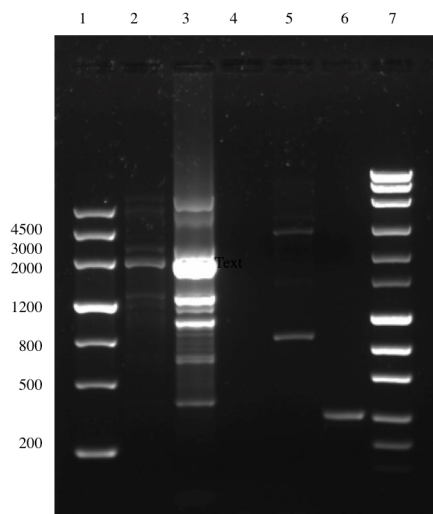


Figure 4: Overhang PCR of pSB1C3-T7-Lys sixth attempt. (1) Ladder (2)-(3) Not of interest (4) Lys at 10 ng/ μ l (5) Lys at 45 ng/ μ l (6) Ladder

Discussion and Troubleshooting

The annealing temperature was possibly too high

5 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - seventh attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers: Lys_R (reverse)
 Suffix_F (forward)
 PCR Amplification (Phusion) buffer: GC Buffer
 PCR Amplification (Phusion) annealing temperature: 60 °C

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 13: Concentration of samples used for the overhang PCR amplification.

Sample	Concentration [ng/ μ l]
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	10
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	45
pSB1C3-T7-Lys (diluted from 180 ng/ μ l)	90

Table 14: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.8
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 15: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		60	30 secs
Extension		72	60 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

Several bands emerged, one of which was the desired band of around 3000 bp.

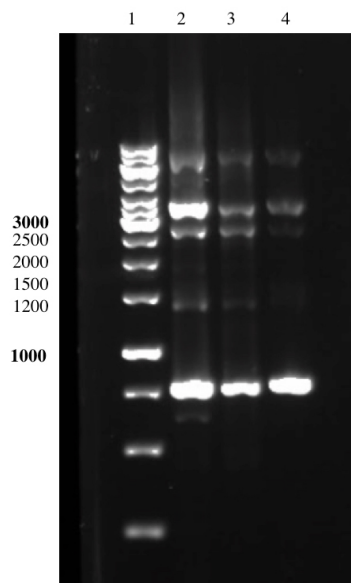


Figure 5: Overhang PCR of pSB1C3-T7-Lys seventh attempt. (1) Ladder (2) Lys 90 ng/ μ l (3) Lys 45 ng/ μ l (4) Lys 10 ng/ μ l

Discussion and Troubleshooting

With this condition and also with the increase of the template concentration, we observed faint bands of the desired size, that being around 3000 bp. However, we still saw some unspecific bands which were difficult to avoid.

6 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - eighth attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:

Lys_R (reverse)
Suffix_F (forward)

PCR Amplification (Phusion) buffer:

GC Buffer

PCR Amplification (Phusion) extension time:

60 seconds

PCR Amplification (Phusion) annealing temperature:

66 °C

PCR Amplification (Phusion) template concentration:

90 ng/ μ l

PCR Amplification (Phusion) reaction: the PCR reaction was done with and without DMSO

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 16: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.8
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 17: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		66	30 secs
Extension		72	60 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

Faint unspecific bands was observed on the gel. The use of DMSO had no significant difference.

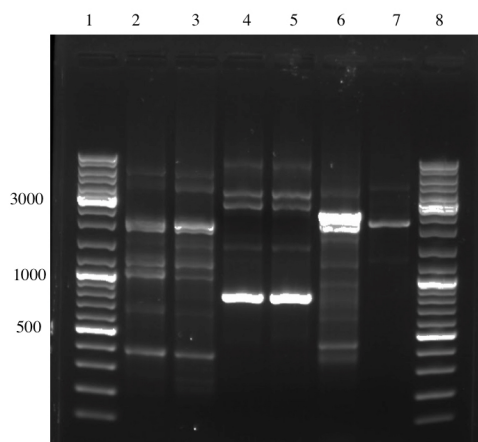


Figure 6: Overhang PCR of pSB1C3-T7-Lys eighth attempt. (1) Ladder (2)-(3) Not of interest (4) Lys without DMSO C (5) Lys with DMSO (6)-(7) Not of interest (8) Ladder

Discussion and Troubleshooting

At this point we could conclude that the design of the primers were not optimal for this PCR.

7 Addition of BamHI restriction site downstream to the lysostaphin sequence and amplification of the whole plasmid, using overhang PCR - ninth attempt

Responsible

Oscar He

Protocols used

- PCR Amplification (Phusion)
- Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Lys_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	GC Buffer
PCR Amplification (Phusion) extension time:	60 seconds
PCR Amplification (Phusion) annealing temperature:	66 °C
PCR Amplification (Phusion) template concentration:	90 ng/ μ l

Experimental Set Up

The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 18: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μ l]
Autoclaved water (23.5 ng/ μ l)	10.2
5X Phusion GC Buffer	4.0
2 mM dNTPs	2.0
10 μ M Forward Primer	1.0
10 μ M Reverse Primer	1.0
Template DNA	1.0
DMSO	0.6
Phusion DNA Polymerase	0.2

Table 19: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		65/70	30 secs
Extension		72	75 secs
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

Unspecific bands were still observed on the gel.

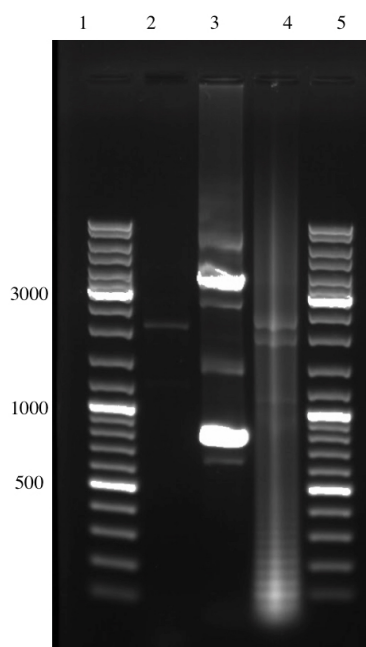


Figure 7: Overhang PCR of pSB1C3-T7-Lys ninth attempt. (1) Ladder (2)Not of interest (3) Lys with DMSO (4) Not of interest (5) Ladder

Discussion and Troubleshooting

Possibly redesign of new primers.