Lysostaphin

Week 14

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

Responsible

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Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

The purpose of the overhang PCR is to amplify the whole pSB1C3-T7-Lys construct with the addition of a BamHI restriction sequence after the Lys coding sequence. This BamHI site serves as a place of attachment for linker tags, which will be added later on. Thus, the amplified region is 2852 bp in length. To maintain accuracy of the PCR performed, we chose to work with Phusion High Fidelity DNA Polymerase. The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 1: Primer sequences for overhang PCR of lysostaphin

Primer	Sequence
Suffix F	5'TACTAGTAGCGGCCGCT'3
Lys R	5'CGCGGATCCCTTTATAGTTCCCCAAAGAACACCTAAAG'3

Table 2: PCR reaction of Overhang Lysostaphin

Reaction	Volume [μl]
Autoclaved water (23.5 ng/μl)	10.8
5X Phusion HF	4.0
2 mM dNTPs	2.0
10 μlM Forward Primer	1.0
10 μlM Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

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
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Sample Calculation

No further calculations were done.

Results and Conclusions

The PCR product was visualized with gel electrophoresis as shown below. Based on this figure, there was no PCR product at an annealing temperature of 55°C. At 57°C we saw several bands, one of which has the desired size of around 300 bp.

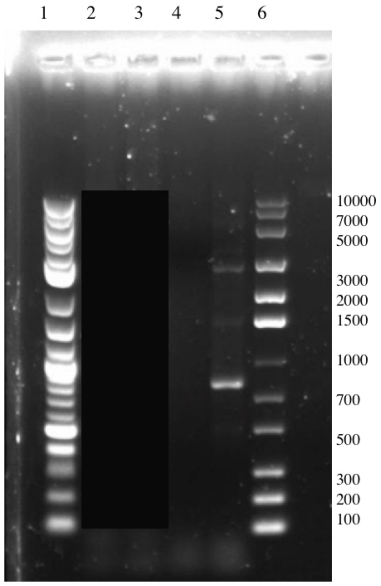

Figure 1: Overhang PCR of pSB1C3-T7-Lys. (1) Ladder (2) Nuc Sample - not shown (3) Nuc Sample - not shown (4) Lys at annealing of 55°C (5) Lys at annealing of 57°C

Discussion and Troubleshooting

The inefficient PCR could have been caused by several factors such as unspecific binding of primers, incompatible buffer system and PCR conditions that are not optimized. Thus, with intentions of improving the PCR result, we plan to change the buffer to GC buffer which is more suitable for DNA strands with high a GC content. The other approach would also be to increase the annealing temperature to reduce the chances of unspecific binding.

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

Responsible

Reskandi Rudjito

Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

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

Table 4: PCR reaction of Overhang Lysostaphin

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

Table 5: 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
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

There is no product at an annealing temperature of 55°C. At 57°C we again see unspecific bands but at a more intense concentration.

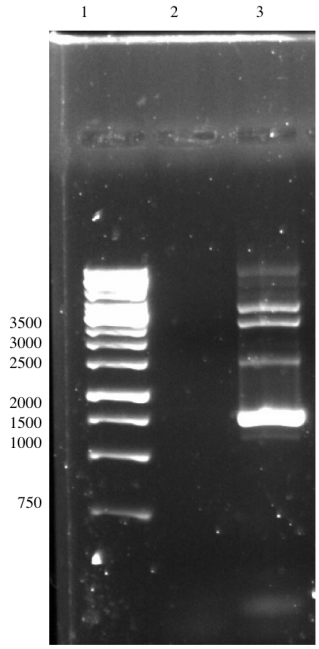

Figure 2: Overhang PCR of pSB1C3-T7-Lys. (1) Ladder (2) Lys at annealing of 55°C (2) Lys at annealing of 57°C

Discussion and Troubleshooting

As a second attempt to perform the overhang PCR, we changed the buffer to GC buffer and kept the others parameters the same as in the previous experiment. The change of buffer resulted in a higher intensity of the DNA bands observed. However, we still clearly see the various-sized bands as an indication of unspecific binding. To overcome this we can try to use DMSO or redesign the primers from scratch.