iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin/silk **Project title:** Cloning Thuricin S with Silk-overhangs into pSB1C3 **Sub project:** Clone Thuricin S with ED overhangs into pSB1C3	**Creation date:** 2016.09.22 **Written by:** Pernille Vigsø Rasmussen **Performed by:** Pernille Vigsø Rasmussen, Brian Kenn Baltzar, Astrid Sophie Pejstrup Honoré.

1. SOPs in use.

SOP number: SOP0007_v01 LA plates with antibiotic

SOP number: SOP0022_v01 Competent cell - freeze-stock

SOP number: SOP0023_v01 Ca^{++} transformation

SOP number: SOP0009_v01 TSB transformation

Plasmid purification kit

SOP number: SOP0001_v01 ON culture of *E.coli*

SOP number: SOP0004_v01 bacterial freeze stock

SOP number: SOP0017_v01 Fast digest

SOP number: SOP0015_v01 Ligation

Gel purification kit

SOP number: SOP0010_v01 Phusion PCR

SOP number: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To clone Thuricin S with silk-overhangs into pSB1C3

3. Overview.

Day	SOPs	Experiments
1	SOP0010_v01 Phusion PCR	Adding proper silk-overhangs with correct restriction sites to the bacteriocin
2	Gel purification kit	Purifying bacteriocin with silk-overhangs using gel purification kit on the unloaded sample
2	NanoDrop	Determination of concentration of the purified Thuricin S with silk-overhangs
3	SOP0017_v01 Fast digest	Cut Thuricin S and standard plasmid (pSB1C3) with EcoRI and SpeI
3	SOP number: SOP0015_v01 Ligation	Ligate Thuricin S with silk-overhangs and pSB1C3 together.
4	SOP number: SOP0009_v01 TSB transformation	Transform recombinant plasmid into TOP10 *E.coli* strain
5	SOP number: SOP0021_v01 Colony PCR with MyTaq	Quality test transformation with VF2 and VR to check if they contain the recombinant plasmid
	SOP number: SOP0001_v01 ON culture of *E.coli*	Make overnight culture of colonies containing correct plasmid
5	Plasmid purification kit	Purify the correct plasmids
7	SOP number: SOP0004_v01 bacterial freeze stock	Take sample from coloni that contains the recombinant plasmid and make freezestock
8	Sequencing	Send plasmids containing Thuricin S with silk-overhangs to sequencing

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-40 5-0	
Glycerol	50 %	AppliChem	Anne Mette, RT	
LB		Anne-Mette		
LA	1% Tryptone 1% NaCl 0.5% Yeast extract 1.5% agar	Oxoid Sigma-Aldrich Merck Difco agar from BD	Anne-Mette Or V18-40 5-0	
Water	Demineralised milli-Q autoclaved water	Milli-Q water purification system (Millipore)	RT	Water
5X HF or GC Buffer		Agilent Technologies	Freezer at 1. Floor	
dNTPs (2 mM each dNTP)		Agilent Technologies	Freezer at 1. Floor	
DNA template			Freezer at 1. Floor	
Primers	A-reverse, D-forward, D-reverse, E-forward, E-reverse.		Freezer at 1. Floor	
Phusion® High-Fidelity DNA Polymerase		New England Biolabs	Freezer at 1. Floor	
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	Ligase
FastDigest enzyme	EcoR31I, EcoRI og SpeI	Agilent Technologies	Freezer at 1. Floor	

Fast digest green / 10 x FastDigest Buffer		Agilent Technologies	Freezer at 1.	
$CaCl_2$	0.1M		Chem room	
$MgCl_2$	0.1M		Chem room	$MgCl_2$
liquid nitrogen	liquid nitrogen	liquid nitrogen	liquid nitrogen	
Fast digest green		Agilent Technologies	Freezer at 1.	
6x DNA Loading Dye		GeneRuler	fridge floor 1	
Fort. LB		the new Anne-Mette	Autoclave room	
Polyethylene glycol (PEG) 3.350		Sigma Aldrich	Micro Chemical room	
Magnesiumchloride (MgCl2) 1M	1M	The New Anne-Mette	Autoclave	
MyTaq™HS Red Mix	http://www.bioline.com/documents/product_inserts/MyTaq%E2%84%A2%20HS%20Red%20Mix.pdf#zoom=130	Bioline	V18-405 a-2	
Reverse primer	Made specific to the template: VR	Sigma-Aldrich		
Forward primer	Made specific to the template: VF2	Sigma-Aldrich		

5. Other

As competent cells, LB and LA media was used by all parts of our project and not just this protocol the dates for use of these SOPs are not added. this comment deal with SOP number: SOP0007_v01 and SOP0022_v01

Gel Electrophoresis is set at 75 V for 30-45 minutes, dependent on the gel percent.

6. Experiment history.

<table>
<tr><th>Date (YY.MM.DD)</th><th>SOPs</th><th>Alterations to SOPs and remarks to experiments</th></tr>
<tr><td>16.08.19</td><td>SOP0010_V01 pPCR</td><td>Got correct silk-overhangs ED on Thuricin S gene
<table><tr><td>Temperature (degree)</td><td>Time (min)</td></tr><tr><td>98</td><td>2:00</td></tr><tr><td>98</td><td>0:30</td></tr><tr><td>70</td><td>0:30</td></tr><tr><td>72</td><td>0:15</td></tr><tr><td>72</td><td>5:00</td></tr><tr><td>20</td><td>hold</td></tr></table>The steps at 98-0:30 to 72-0:15 cycled 34 times.</td></tr>
<tr><td>16.08.24</td><td>Gel purification kit</td><td>Purified Thuricin S with silk-overhangs, without loading our sample on a gel.</td></tr>
<tr><td>16.08.26</td><td>Nanodrop</td><td>Measured the concentration of our PCR product</td></tr>
<tr><td>16.08.29</td><td>SOP number: SOP0017_v01 Fast digest</td><td>Fast digested PCR: Thurcin S with silk-overhangs and pSB1C3 with EcoRI and SpeI. Sample was cut 2 h with SpeI and 30 min with EcoRI</td></tr>
<tr><td>16.08.29</td><td>SOP number: SOP0015_v01 Ligation</td><td>Ligated pSB1C3 together with Thuricin S with silk-overhangs

Different ligation ratio of insert(sample=10) and vector(sample=BR16) was made to estimate no. of religation: 1:0, 1:3, 1:5 and 1:10</td></tr>
<tr><td>16.08.30</td><td>SOP number: SOP0009_v01 TSB transformation</td><td>Transformed plasmid into TOP10 E.coli</td></tr>
<tr><td>16.08.31</td><td>SOP number: SOP0021_v01 Colony PCR with MyTaq</td><td>Quality tested our transformations to verify that the transformation had been successful</td></tr>
</table>

		Temperature (degree)	Time (min)
		95	2:00
		95	00:15
		60	00:15
		72	00:15
		72	2:30
		4	hold
		The steps at 95-0:15 to 72-0:15 cycled 34 times.	
16.08.31	SOP number: SOP0001_v01 ON culture of *E.coli*	Made overnight culture of cultures containing the recombinant plasmid	
16.09.01	SOP number: SOP0004_v01 bacterial freeze stock	Made freeze stock of those colonies containing the plasmid	
16.09.01	Plasmid purification kit	Purified the correct plasmid containing Thuricin S with silk-overhangs	
16.09.01	Sequencing	Sent samples for sequencing to confirm the correct plasmid was present	

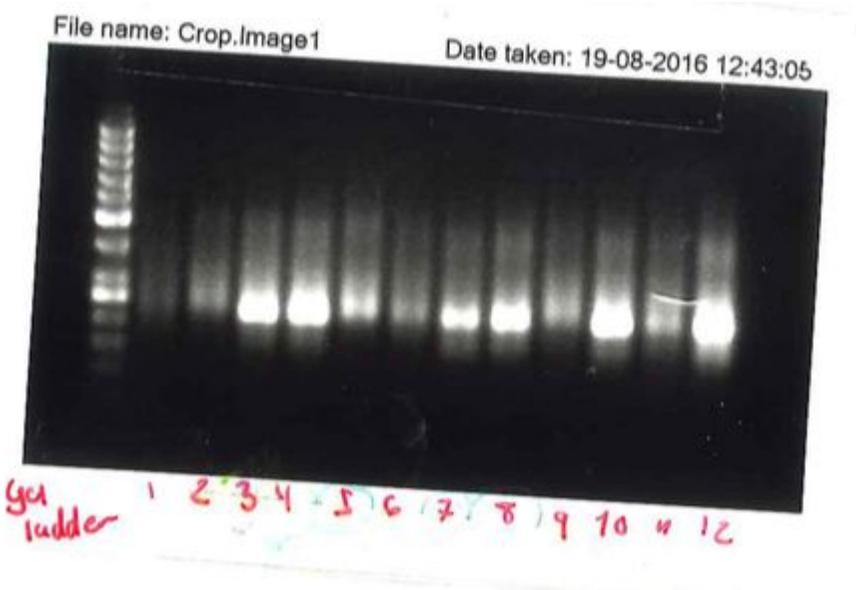
7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
10	PCR product of k2018011(Thuricin S) with ED silk overhangs	IDT	Cloning into pSB1C3 /Fridge floor 1
BR16	pSB1C3	IDT	Vector for cloning / Fridge floor 1

15	k2018011 with ED overhangs	IDT	Final part / Fridge floor 1

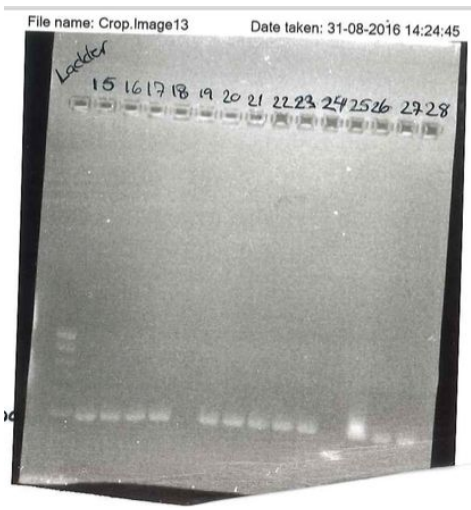
8. Remarks on setup.

9. Results and conclusions.



Lane marked no. 4 and 4 are Thuricin with ED-overhangs and is expected to be 217 bp.

Fermentas Generuler 50 kb ladder plus was used which is why it can be expected that we succeeded at getting silk-overhangs on Thuricin S.



The samples 1-10 are from colonies containing plasmids with Thuricin S with ED overhang.

The samples 11-17 are from colonies containing plasmids with Thuricin S with DA overhang

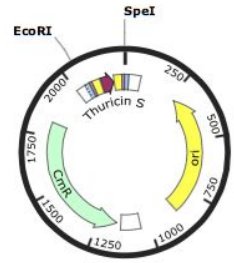
The samples 18-24 are from colonies containing plasmids with Thuricin S with EA overhang

The samples 25- 28 are from colonies containing plasmids with Thuricin S with DE overhang

The Thuricin S with silk-overhang together with primer overhang (VF2 and VR) is 517 bp, *Fermentas Generuler 1 kb ladder plus* was used, so from these pictures it can verified that Thuricin S with ED overhangs have the right length.

10. Appendixes

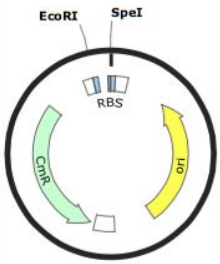
Below it can be seen the path towards inserting Thuricin S with DA overhangs into pSB1C3. The mechanism is the same for all silk-overhangs used.



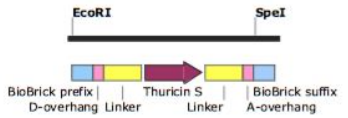
k2018011 DA cloned into pSB1C3
2236 bp

INSERT FRAGMENT Replace EcoRI (2049) – SpeI (2)

Insert EcoRI (5) – SpeI (194)

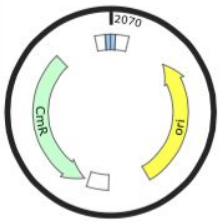


pSB1C3
2125 bp

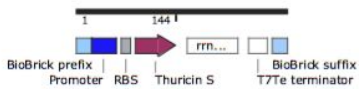


K2018011_DA
217 bp

INSERT BASES Insert at 2070



PCR Amplify 1 .. 144 using:
Primer 1
Primer 2



BBa_K2018011
302 bp