iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin	**Creation date:** 2016.08.10
Project title: Cloning composite part into iGEM standard plasmid.	**Written by:** Astrid Sophie Pejstrup Honoré & Brian Kenn Baltzar
Sub project: Insertion of k2018015 (Lacticin Q-Lacticin Z) into pSB1C3 plasmid	**Performed by:** Pernille Vigsø Rasmussen, Brian Kenn Baltzar, Astrid Sophie Pejstrup Honoré, Cathrine Høyer Christensen

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: SOP0010_vo1 Phusion PCR

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: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To insert composite part: k2018015 (Lacticin Q-Lacticin Z), into iGEM standard plasmid; pSB1C3.

3. Overview.

Day	SOPs	Experiments
1	SOP0023_v01	Ca^{++} transformation
2	SOP0001_v01	ON culture of *E.coli*
3	Miniprep kit	Plasmid purification
3	SOP0004_v01	Bacterial freeze stock
4	Phusion PCR	Phusion PCR
4	SOP0017_v01	Fast digest
4	SOP0015_v01	Ligation
5	SOP0023_v01	Ca^{++} transformation
6	SOP0021_v01	Colony PCR with MyTaq
6	SOP0001_v01	ON culture of *E.coli*
7	Gel purification kit	Gel purification not on Gel but PCR product
7	SOP0004_v01	Bacterial freeze stock

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
MyTaq^{TM}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	Sigma-Aldrich		
Forward primer	Made specific to the template	Sigma-Aldrich		
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	Ligase
FastDigest enzyme		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	
dH_2O		Contact Lab-manager	Micro Storage	
5X Phusion HF or GC Buffer		Agilent Technologies	Freezer at 1. Floor	
dNTPs (10 m mM each dNTP)		Agilent Technologies	Freezer at 1. Floor	

Primer #1 (10µM)		Freezer at 1. Floor
Primer #2 (10µM)		Freezer at 1. Floor
Phusion® High-Fidelity DNA Polymerase	New England Biolabs	Freezer at 1. Floor
PCR/gel purification kit		Micro storage

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.

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
16.07.26		resuspended the received 4µg of plasmid with 40µl dH2O
16.07.26	SOP0023_v01 Ca++ transformation	Their where used 2.5 µl instead of the normal 1µl
16.07.27	SOP0001_v01 ON culture of *E. coli*	
16.07.28	miniprep kit	50 µl were used for elution step, made in 3 samples and are after Nanodrop, mixed into one, and a new nanodrop concentration is stated.

16.07.28	SOP0004_v01 Freeze stock	Name; #52
16.07.29	Phusion PCR	This is made to replace a defective promoter, Primer; k2018015_IDT

H_2O	32.5µl
5 x HFbuffer	10µl
dNTPs	1µl
Primer; k2018015_IDT	2.5µl
Suffix-Primer	2.5µl
Polymerase	0.5µl

Program:

STEP	**Temp ºC**	**Time (m)**
1	95	02:00
2	95	00:10
3	72	00:16
4	72	00:15
5	72	10:00
6	20	HOLD

Step 2-4 was repeated 34 times.

16.07.29	Gel electrophoresis	1kb plus DNA ladder(Green) was used, in a 2% gel and 5µl sample
16.07.29	SOP0017_v01	Fast digest with Xbal and Pst1 - 15 hours
16.07.29	SOP0015_v01 Ligation	At the ligation step following ratios of vector and DNA were used: 1:0(negative control), 1:5 and 1:10, BR16(positive control). For cPCR most cultures are taken from both ratio, and 3 replicates, to minimise the risk of getting religation.

Ratio	**1:0**	**1:5**	**1:10**
Ligase buffer	2µl	2µl	2µl

		Ligase	1µl	1µl	1µl
		BG78	0µl	5µl	10µl
		BG17	1µl	1µl	1µl
		H_2O	17µl	12µl	7µl
16.08.01	SOP0023_v01 Ca++ transformation	Their where used 2.5 µl instead of the normal 1µl			
16.08.02	SOP0021_v01 cPCR with MyTaq	Made on 23 colonies			

Segement	Step	Temperature	Duration
1	Initial denaturation	98 °C	2 min
2	34 cycles	98 °C	10 sec
		72 °C	15 sec
		72 °C	15 sec
3	Final extension	72 °C	5 min
4	Keep the sample cold	12 °C	HOLD

16.08.02	Gel electrophoresis on cPCR product	1 kb DNA ladder(green) was used
16.08.02	SOP0001_v01 ON culture of *E. coli*	
16.08.03	miniprep kit	Name; BR82
16.08.03	Freeze stock	Name; #62

7. Sample specification.

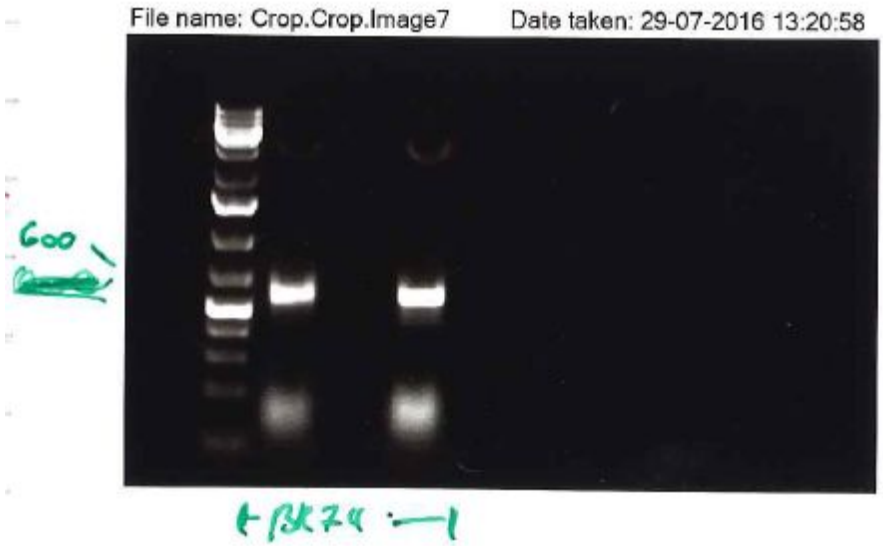
Sample name	Sample content	From	Used for / Saved where
BR72	pUCIDT-AMP:k2018015	IDT	transferred to *E. coli* / saved in coolbox
BR74	pUCIDT-AMP:k2018015	IDT	Purifeid BR72, used to Cut with restriction enzymes and ligated with the vector/saved in coolbox and on Freeze stock (#52)
BG17	pSB1C3:k748002	iGEM	Vector cut with pst1 and Xbal / cool box
BY77	k2018015	IDT	PCR product / Saved in coolbox
BG78	k2018015		BY77 cut with pst1 and Xbal. /Saved in coolbox
BB79	pSB1C3:k2018015		vector and composite part ligated and transferred to *E.coli.* /Saved in coolbox
BR82	pSB1C3:k2018015		purified and transferred BB79 / freeze stock in *E.coli* (#62)

8. Remarks on setup.

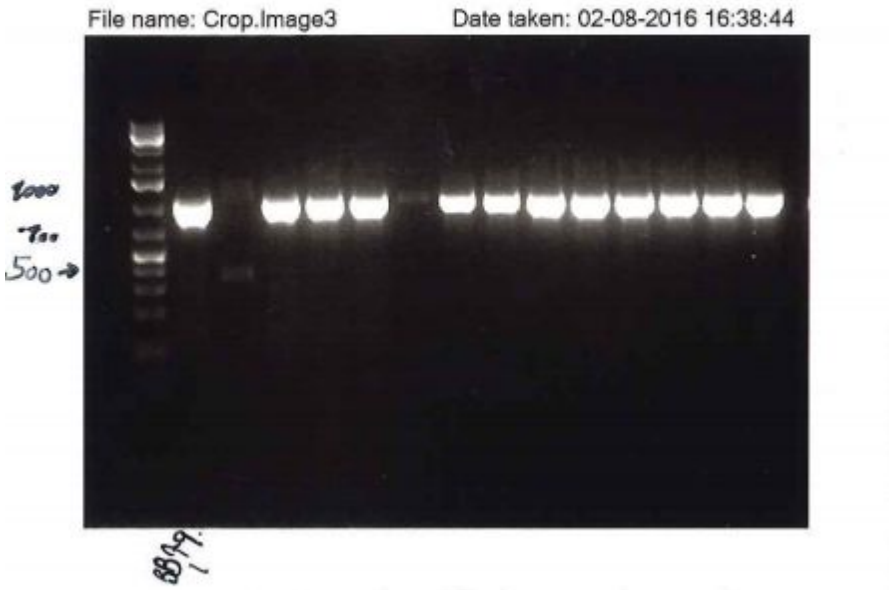
9. Results and conclusions.

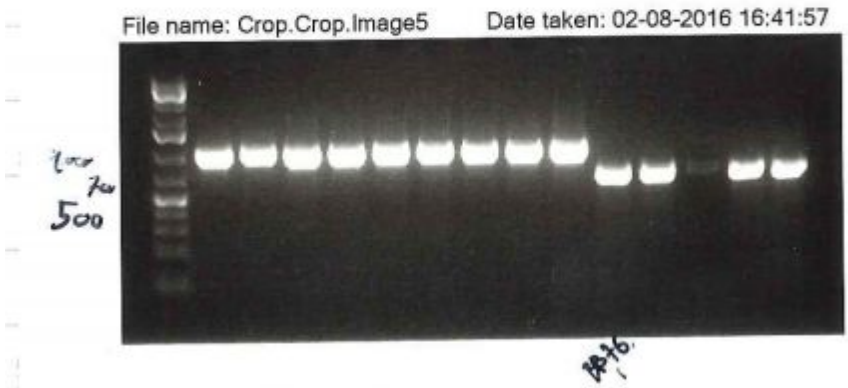
Form the Gel photo under this part, is the result from the pPCR shown. This is made to replace a defective promoter, with the correct one. To solve this, is the Primer; k2018015_IDT used. As it can be seen from the photo, is first the ladder, here the green one. and then sample BR74. The gene is ___ bp. and it is therefore possible from the gel to assume that the pPCR is succeed.

Note: There is loaded 5 µl of each sample.



The Gel Photo under this, shows on the first Gel from the left the ladder; in this gel is used Green ladder. the following bands are BB79, 1 to 14, on the other gel is the Ladder, and then BB79 sample 15to 23. BB79 is the ligation of the vector BG17 and the insert BG78. From the photo it can be expect that all the samples, except; 2 and 6, are perfect ligated. and one of the colonies are therefore used to make ON for the plasmid purification and Freeze stock.





10. Appendixes