iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin **Project title:** Cloning composite part into iGEM standard plasmid. **Sub project:** Insertion of k2018012 (Lacticin Q) into pSB1C3 plasmid	**Creation date:** 2016.08.10 **Written by:** Astrid Sophie Pejstrup Honoré **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

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: k2018012 (Lacticin Q), 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
3	SOP0017_v01	Fast digest
4	Gel purification kit	Gel purification
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™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	

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; #51
16.07.28	SOP0017_v01 Fast digest	Fast digest enzymes, pst1 and Xbal over the nigth at 37 ºC.
16.07.29	Gel electrophoresis	1kb plus DNA ladder(Green) was used, in a 2% gel
16.07.29	Gel purification kit	30 µl were used for elution step
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
BG75	0µl	5µl	10µl
BG17	1µl	1µl	1µl
H_2O	16µl	11µl	6µl

		The samples had stranded at room temperature in 30 minutes and after are the ligases inactivated at 65 ºC in 10 minutes.
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 5 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; BR81
16.08.03	Freeze stock	Name; #61

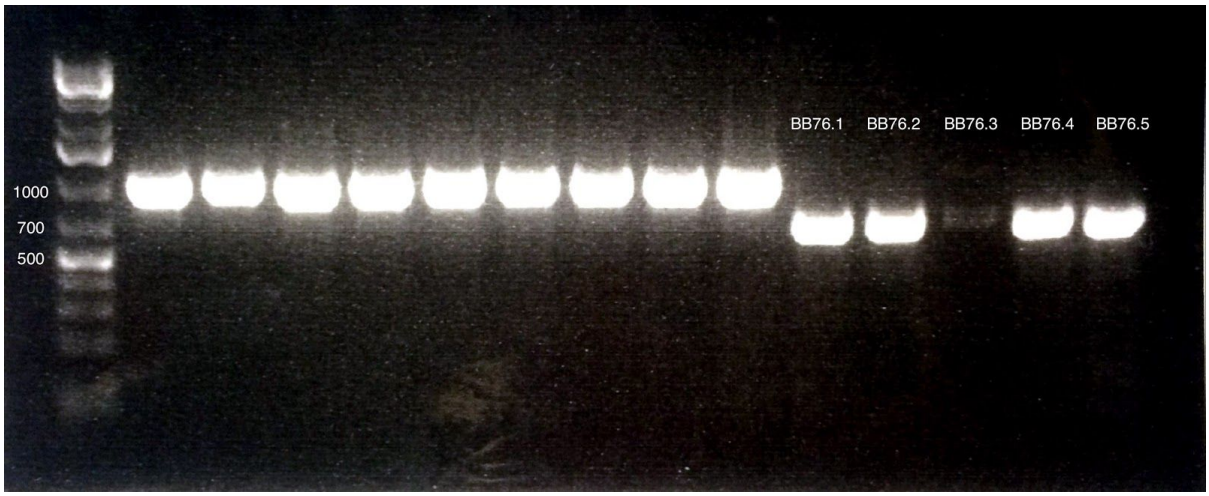
7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
BR71	pUCIDT-AMP:k2018012	IDT	transferred to *E. coli* / saved in coolbox
BR73	pUCIDT-AMP:k2018012	IDT	Purifeid BR71, used to Cut with restriction enzymes and ligated with the vector/saved in coolbox and on Freeze stock (#51)
BR17	pSB1C3:k748002	iGEM	Vector cut with pst1 and Xbal / cool box
BG75	k2018012	IDT	Cuted BR73, cuted with pst1 and Xbal / Saved in coolbox
BB76	pSB1C3:k2018012		vector and composite part ligated and transferred to *E.coli.* /Saved in coolbox
BR81	pSB1C3:k2018012		purified and transferred BB76 / freeze stock in *E.coli* (#61)

8. Remarks on setup.

9. Results and conclusions.

On the following gel picture shows that we successfully cloned our part K2018012 into the standard iGEM plasmid, pSB1C3.



We made a colonyPCR with the VF2 and VR primers, resulting in a PCR product on 675 bp, so we expected the band be just below the 700 bp mark, which it is.

10. Appendixes