iGEM 2016 – Microbiology – BMB – SDU

Project type: Bacteriocin **Project title:** Cloning composite part into iGEM standard plasmid. **Sub project:** Insertion of k2018019 (Pyocin S) 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

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: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To insert composite part: k2018019 (Pyocin S), 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	SOP0001_v01	ON culture of *E.coli*
4	SOP0004_v01	Bacterial freeze stock
5	SOP0017_v01	Fast digest
5	Gel purification kit	Gel purification
6	SOP0015_v01	Ligation
6	SOP0009_v01	TSB Transformation
7	SOP0021_v01	Colony PCR with MyTaq
7	SOP0001_v01	ON culture of *E.coli*
8	Gel purification kit	Gel purification
8	SOP0001_v01	ON culture of *E.coli*
9	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	Oxoid Sigma-Aldrich Merck	Anne-Mette Or	

	1.5% agar	Difco agar from BD	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	
Fort. LB		the new Anne-Mette	Autoclave room	
Polyethylene glycol (PEG) 3.350		Sigma Aldrich	Micro Chemical room	

Dimethylsulfoxid (DMSO)		Sigma Aldrich	Micro Chemical room
Magnesiumchloride (MgCl2) 1M	1M	The New Anne-Mette	Autoclave

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**
16.07.14		resuspended the received 4µg of plasmid with 40µl dH2O
16.07.14	SOP0023_v01 Ca++ transformation	Their where used 2.5 µl instead of the normal 1µl
16.07.16	SOP0001_v01 ON culture of *E. coli*	
16.07.17	miniprep kit	50 µl were used for elution step
16.07.18	SOP0004_v01	Name; #32
16.07.21	SOP0017_v01 Fast digest	Fast digest enzyme(pst1) cut in 2 hours at 37 ºC and with Fast digest enzymes(EcoR1) cut in 30 min. After 30 min. are the enzymes denaturated at 80 ºC in 10 min.
16.07.21	Gel electrophoresis	1kb plus DNA ladder(Green) was used
16.07.21	Gel purification kit	30 µl were used for elution step

16.07.22 SOP0015_v01

ligation

At the ligation step following ratios of vector and DNA were used: 1:0, 1:5 and 1:10. 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
BG59	0µl	5µl	10µl
BG60	1µl	1µl	1µl
H_2O	16µl	11µl	6µl

16.07.22 SOP0009_v01

TSB transformation

5 µL plasmid is used for every sample

16.07.25 SOP0021_v01

cPCR with MyTaq

Made on 14 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

16.07.25 Gel electrophoresis

1 kb DNA ladder(green) was used

	on cPCR product	
16.07.26	SOP0001_v01 ON culture of *E. coli*	
16.07.27	miniprep kit	Name; BR70
16.07.27	Freeze stock	Name; #50

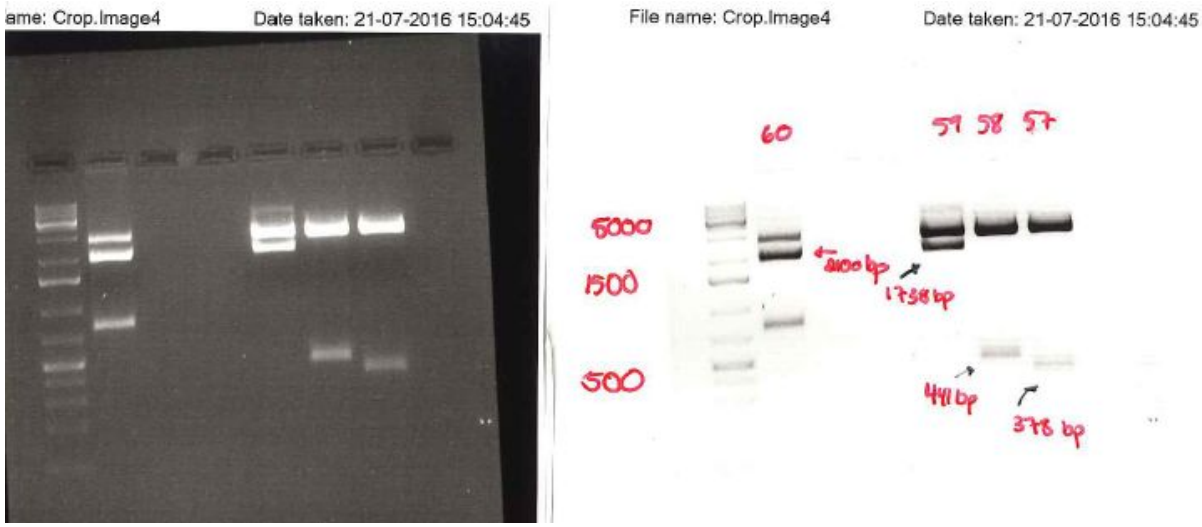
7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
BR36	pUCIDT-AMP:k2018019	IDT	transferred to *E. coli* / saved in coolbox
BR40	pUCIDT-AMP:k2018019	IDT	Purifeid BR36, used to Cut with restriction enzymes and ligated with the vector/saved in coolbox and on Freeze stock (#32)
BR60	pSB1C3:k748002	iGEM	Vector cut with pst1 and EcoR1 / cool box
BG59	k2018019	IDT	Cuted BR40, cuted with pst1 and EcoR1 / Saved in coolbox
BB63	pSB1C3:k2018019		vector and composite part ligated and transferred to *E.coli.* /Saved in coolbox
BR70	pSB1C3:k2018019		purified and transferred BB63 / freeze stock in *E.coli* (#50)

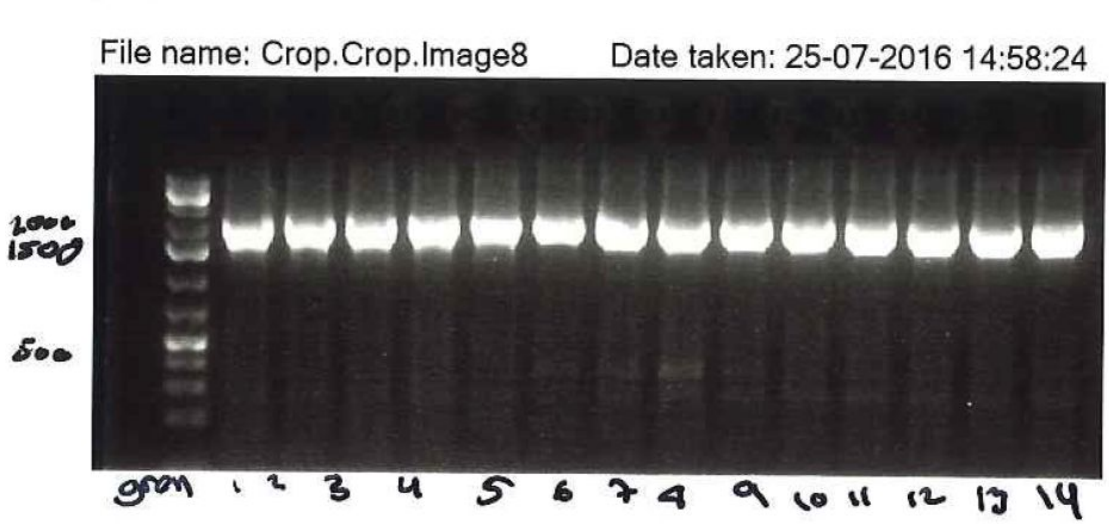
8. Remarks on setup.

9. Results and conclusions.

The Gel Photo below here shows the results from cutting the BR40 with Pst1 at 2 hours with 37 ºC and EcoR1 at 30 minutes, and after that called BG59 (At the photo named; 59). The photos below here, is the same gel in two picture. The composite part is 1739 bp. It is possible from the Gel photo to assume that the DNA is cut correctly. And from this gel is the BG59 band with around 2000 bp purified, for a ligation with BR60, the vector.



The Gel Photo under this, shows from the left the ladder; in this gel is used Green ladder. the following bands are BB59, 1 to 14, there is the ligation of the vector BR60 and the insert BG59. From the photo it can be expect that all the samples are perfect ligated. and one of the colonies are therefore used to make ON for the plasmid purification and Freeze stock.



10. Appendixes