iGEM2013 – Microbiology – BMB – SDU	
Project type: USER cloning **Project title:** USER cloning of pSB1A3 for Composite Production System only composed of Prenyl tranferase **Sub project:**	**Creation date:** 13.08.01 **Written by: PRA** **Performed by:** PRA, SIS, MH, MHK

1. SOPs in use

SOP0006_v01 PCR protocol for USER cloning
SOP0014_V01 Gel Purification

2. Purpose

To perform USER PCR to amplify the plasmid backbone to be ligated together with arabinose promoter and prenyl transferase

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006	PRA	USER PCR of pSB1A3
2	SOP0006	SIS, MH	USER PCR of pSB1A3
3	SOP0014 SOP0006 SOP0014	MHK SF, MHK MHK	Gel purification of USER PCR of pSB1A3 USER PCR of pSB1A3 Gel purification of USER PCR of pSB1A3
4	SOP0006	PRA	USER PCR of pSB1A3
5	SOP0014	SF	Gelpurification
6	SOP0006	MH	Touchdown gradient PCR of pSB1A3
7	SOP0014	SF	Gelpurification

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 39+49	10uM	Sigma Aldrich		

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
13.08.01	USER PCR	A USER PCR with primer 39+49, arabinose promoter on pSB1A3 as template, and 5x25 uL total reaction was performed with the following program: 95 deg 2 min 95 deg 30 sec 45, 49, 51, 55, 60 deg 30 sec 30 cycles 72 deg 2 min 10 sec

13.08.02	USER PCR	A USER PCR with primer 39+49 was performed using template: Arabinose promoter on pSB1A3. 5x25 uL total reaction was performed. Sample 1 was run as an ordinary USER PCR with annealing temp 53,6 deg. Four samples (2-5) were run with a touchdown with the following program: 95 deg 2 min 95 deg 30 sec Touch down from 55 deg 30 sec 20 cycles 72 deg 2 min 10 sec 53,6 deg 30 sec 30 cycles 72 deg 2 min 10 sec
13.08.05	Gel purification Nano drop USER PCR Gel purification Nano drop	The sample was purified according to the SOP. The concentration of pSB1A3 in the sample was 5,8ng/ µL. A USER PCR was done with primer 39+49. Template was sample Grøn 102, the PCR product from the gel purification. 3 PCR reactions were prepared in each their own tube. 95 deg 2 min 95 deg 30 sec 55 deg 30 sec 68 deg 2 min 10 sec 30 cycles 72 deg 10 min (final elongation) The sample was purified according to the SOP. The concentration of pSB1A3 in the sample was 24,2 ng/ µL.
13.08.19	USER PCR	A USER PCR was done with primer 39+49. Template was sample Grøn 102, the PCR product from the gel purification. 4 PCR reactions were prepared in each their own tube. 95 deg 2 min 95 deg 30 sec 55 deg 30 sec 68 deg 2 min 10 sec 30 cycles 72 deg 10 min (final elongation)
13.08.20	Gelpurification	The bands were cut out and purified. The spins were performed at 14,5 g.
13-09.06	Touchdown gradient PCR	MH
13.09.06	Gelpurification	SF

7. Sample specification

Sample name	Sample content	Concentration	Used for / Saved where
Grøn 102	pSB1A3 for CPS prenyltransferase only from primers 39+49	5,8 ng/µL	iGEM fridge
Grøn 103	pSB1A3 for CPS prenyltransferase only from primers 39+49	24,2 ng/µL	iGEM fridge
Green 169	pSB1A3 for CPS prenyltransferase only from primers 39+49	7,5 ng/µL	iGEM Fridge
Green 170	pSB1A3 for CPS prenyltransferase only from primers 39+49	**9,8 ng/µL**	**iGem fridge**

8. Remarks on setup

9. Results and conclusions

13.08.02

Results for USER PCR run over night: 25 uL was loaded in each well on a 1,5% agarose gel. Ladder: red.

No bands appeared. A new PCR was performed.

Results for todays ordinary PCR:
25 uL was loaded in well 2 on a 1,5% agarose gel.

No bands could be seen on the picture that was taken. Which was the case for our eyes as well.

Results for todays touchdown PCR:
25 uL was loaded in well 2-5 on a 1,5% agarose gel. Well 1 contains red ladder.

There were 4 small bands around 2000bp, which were cut out and purified (bad picture quality).

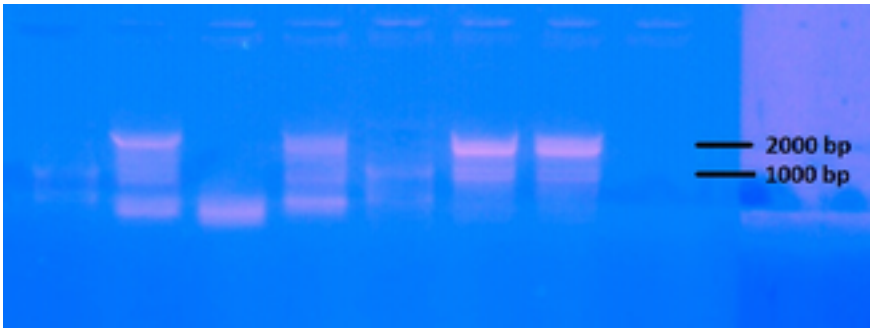
13.08.05

The product of the gel purification was sample Grøn 102 with a concentration of 5,8ng/µL.

Results for PCR:

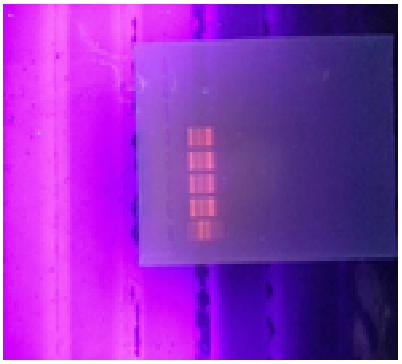
50 uL was loaded in well 2, 3 and 4 on a 1% agarose gel. Bands appeared around 2000 bp for well 2 and 4. These bands were cut out and purified together as sample Grøn 103 with a concentration of 24,2 ng/µL. Well 3 showed a band around 300 bp.

The ladder is 100 bp plus, loaded in well 1 and 5.



13.08.20

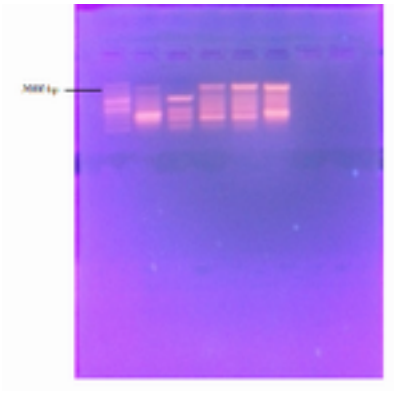
pSB1C3 with light blue ladder.



The upper bands are app. 2000 bp long. They were cut out and purified yielding a concentration of 7,5 and 9,8 ng/µL.

13,09.06

Blue ladder. pSB1A3 with primers 66+67.



The bnds were cut out and purified.

10. Appendices