iGEM2013 – Microbiology – BMB – SDU	
Project type: USER cloning **Project title:** USER cloning of Ara promoter for Composite Production System and prenyl transferase part only **Sub project:**	**Creation date:** 13.08.01 **Written by: PRA** **Performed by:** PRA, SIS, MH

1. SOPs in use

SOP0006_v01 PCR protocol for USER cloning
SOP0014_V01 Gel Purification

2. Purpose

To perform USER PCR to amplify the arabinose promoter to be used in CPS and prenyl transferase only part

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006	PRA	USER PCR of Ara promoter
2	SOP0014 SOP0006	SIS MH, SIS	Gel purification USER PCR on Ara promoter PCR product

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 40+41	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 340+41, 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	Gel purification USER PCR	Gel purification of USER PCR. Eluted in 30 uL water. USER PCR on PCR product from 13.08.01 (green 99). Primer: 40+41. 1 samples of 25 uL was performed with the following program: 95 deg 2 min 95 deg 30 sec 53,6 deg 30 sec 30 cycles 72 deg 2 min

7. Sample specification

Sample name	Sample content	Concentration	Used for / Saved where
Green 98	USER PCR on Arabinose promoter for prenyltransferase in CPS	5,0 ng/uL	Green box in the fridge.
Green 99	USER PCR on Arabinose promoter for prenyltransferase in CPS	15,0 ng/uL	Green box in the fridge.

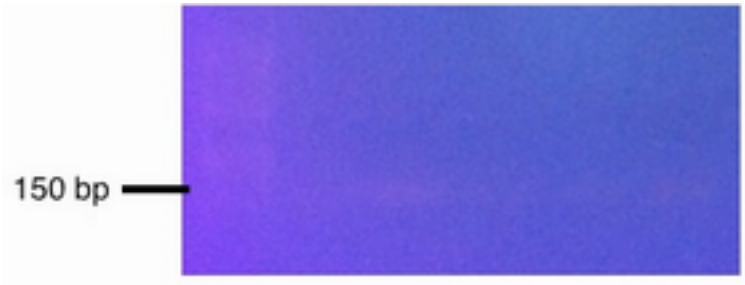
Green 100	USER PCR on Arabinose promoter for prenyltransferase in CPS	5,2 ng/uL	Green box in the fridge.

8. Remarks on setup

9. Results and conclusions

31.08.01

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



Very small bands appeared in all five wells around 150 bp. The bands were cut out and purified give concentrations of 5 ng/uL and 15 ng/uL.

Result for USER PCR on PCR:
50 uL was loaded in well 2 on a 1,5% agarose gel.

Picture were of too bad quality, but a clear band around 150 bp were cut out and purified (green 100).

10. Appendices