iGEM2013 – Microbiology – BMB – SDU	
Project type: USER Cloning **Project title:** USER cloning of SDM DXS (B. sub) for Composite Production System **Sub project:**	**Creation date:** 31/7-13 **Written by: MH** **Performed by:** MH, MHK

1. SOPs in use

SOP0006_v01 PCR protocol for USER cloning

2. Purpose

To perform USER cloning of Dxs (b. sub), which has undergone site directed mutagenisis, for use in CPS.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006_v01 SOP0014_v01	MHK, MH, SIS, MHK	USER PCR of Dxs (b. sub) Gel purification

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
31/07-13	SOP0006_v01	A gradient PCR was performed at the following annealing temperatures: 49.8 52.1 54.7 56.8 Otherwise, the SOP was followed, and the samples mixed in the PCR tubes. Template: 68 Primers: 46, 47
	SOP0014_v01	Gel purification All four bands of DXS were cut from the gel and purified. The bands from well no 1 and 2 were purified in the same tube and the bands from well no 3 and 4 were purified in the same tube.
	SOP0013_v01	The concentrations of the gel purifications were measured.

7. Sample specification

Sample name	Sample content	From	Used for / Saved where
Grøn 90	**USER DXS PCR product from primers 46 and 47**		**iGEM fridge**
Grøn 91	**USER DXS PCR product from primers 46 and 47**		**iGEM fridge**

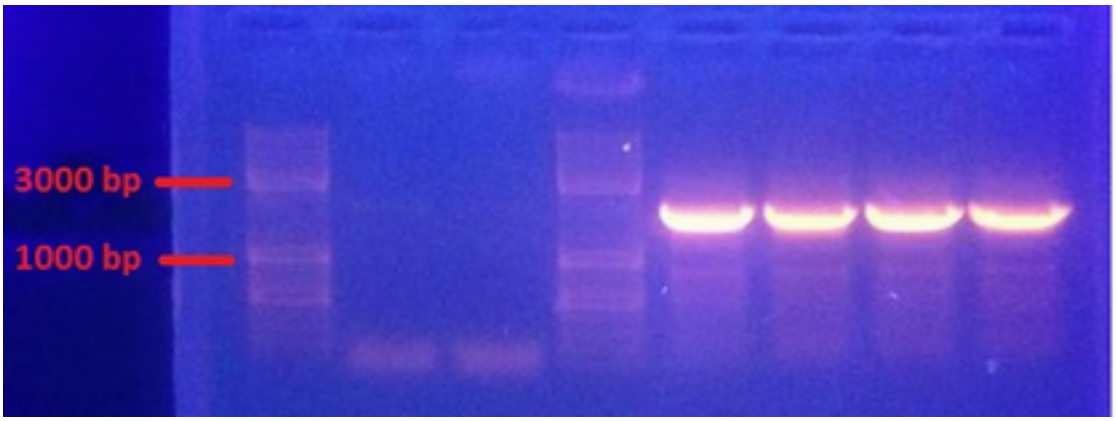
8. Remarks on setup

9. Results and conclusions

31.07.13

Results for USER PCR. 50 µL was loaded in each well on a 1% agarose gel. Ladder: red.
Order of well load:

Red ladder
1) pSB1C3 til CPS
2) pSB1C3 til CPS
Red ladder
4) DXS B.sub til CPS with annealing temp. 49.8 deg
5) DXS B.sub til CPS with annealing temp. 52.1 deg
6) DXS B.sub til CPS with annealing temp. 54.7 deg
7) DXS B.sub til CPS with annealing temp. 56.8 deg



Bands appeared in well 4-7. The bands were cut out and purified.

31.07.13

Results for gel purification. The concentration of DXS in sample Grøn 90 was 95,7 ng/µL and in sample 91 was 65,7 ng/µL.

10. Appendices