iGEM2013 – Microbiology – BMB – SDU	
Project type: USER PCR **Project title:** USER PCR of DXS (E. coli) **Sub project:**	**Creation date:** 23.07.13 **Written by:** SF **Performed by:** SF, SIS

1. SOPs in use

SOP0006_v01

2. Purpose

3. Overview

Day	SOPs	Persons	Experiments
1	SOP006 SOP006	SF, SIS AK, PRA	PCR of DXS (E. coi) for ligation with plasmid and GFP. PCR of DXS (E. coi) for ligation with plasmid and GFP.
2	SOP006	SIS, MH	PCR of DXS (E. coi) for ligation with plasmid and GFP.

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
23.07.13	**SOP0006_v01**	A 5X mastermix was prepared. The template was extracted from the kitplate and dilluted 100X. A gradient PCR was made with annealingtime of 30 sec. and the temperatures: 1) 39,9 2) 40,4 3) 41.7 4) 43.5 5) 51.5.deg. C. Elongation time was was 2 min. primers: 29 and 30.
	USER PCR	A 2X mastermix was prepared. The template was extracted from the kitplate and dilluted 100X. PCR program: 95 deg 2 min 95 deg 30 sec 38, 40 deg 30 sec 5 cycles 72 deg 2 min 95 deg 30 sec 55 deg 30 sec 30 cycles 72 deg 2 min 4 deg Overnight

13.07.24	**SOP0006_v01**	A 5x mastermix was prepared. Template: 100x diluted sample from kit plate. Primere: 30+29 PCR program: 95 deg 2 min 95 deg 30 sec 35-50 (gradient) deg 30 sec 5 cycles 72 deg 2 min 95 deg 30 sec 55 deg 30 sec 30 cycles 72 deg 2 min 4 deg hold The specific annealing temp: 37.6 deg, 39.3 deg, 41.3 deg, 43,3 deg and 45.3 deg

7. Sample specification

Sample name	Sample content	From	Used for / Saved where

8. Remarks on setup

9. Results and conclusions

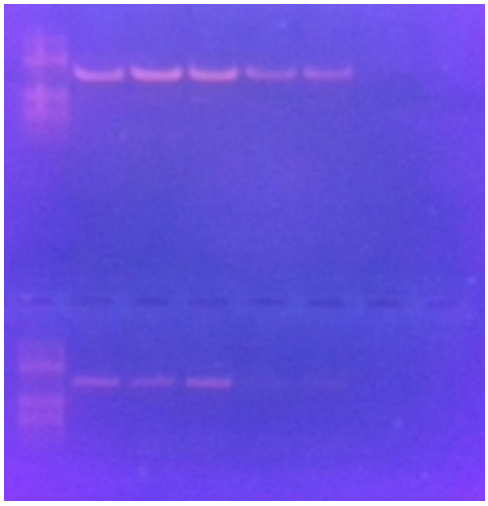
The USER PCR was run on an 1% agarose gel

There were no bands and the PCR was done again.

24.07.13

No bands appeared and the PCR was done again.

Result for new PCR reaction:



50 µL was loaded in each well on a 1 % agarose gel. Ladder: red.The upper line is the PCR result for this protocol. There appeared band in all wells around 2000 bp. The bands were cut out and purified from gel.

Overview of the well loads:

Well:	1	2	3	4	5	6

Low annealing temp:	Red ladder	37.6 deg	39.3 deg	41.3 deg	43,3 deg	45.3 deg

10. Appendices