iGEM2013 – Microbiology – BMB – SDU	
Project type: **Project title:** SDM DXS (E. coli) amilCP **Sub project:**	**Creation date:** 13.08.08 **Written by: MHK** **Performed by:** SF, MHK

1. SOPs in use

SOP0010 Phusion PCR

SOP0014 Gel purification

2. Purpose

To remove stop-codon in DXS-amilCP construct.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0010 SOP0014 SOP0010	SF, MHK SF, MHK MHK	SDM Phusion PCRs Gel purification SDM Phusion PCR
2	SOP0014	MHK	Gel purification of the 2nd PCR from day 1
3	SOP0015	HWJ	Ligation of LacI into the plasmid

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 4+63			iGEM fridge	
Primer 5+62			iGEM fridge	
Blå 36			iGEM fridge	

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
13.08.07	Phusion PCRs	Two phusion PCRs of each 2 reactions were carried out. Both had the template Blå 26 and annealing temperatures of 55 deg. In the one phusion PCR the primers were primer 4 + 63 and the other primer 5 + 62.
	Gel purification	For the PCRs with the same primers, the bands were purified together. The concentrations were 49,4 ng/µL for the PCR product with primer 4 + 63 and 99,3 ng/µL for the PCR product with primer 5 + 62.
	Phusion PCR	4 PCRs were carried out. For all of them the primers were 4 + 5 and the templates were Grøn 113 and Grøn 114. The annealing temperature was 55 deg. Template and water content in PCRs: PCR1: ½µL of 10ng/µL Grøn 113 and Grøn 114, 12,4 µL water PCR2: 1µL of 10ng/µL Grøn 113 and Grøn 114, 11,4 µL water PCR3: 2µL of 10ng/µL Grøn 113 and Grøn 114, 9,4 µL water PCR4: 1µL of 49,4ng/µL Grøn 113, 1µL of 99,3 ng/µL Grøn 114, 11,9 µL water

13.08.08	**Gel purification**	Gel cut-outs purified according to SOP.
13.08.14	**Digestion and Ligation**	The SOPs were followed. Digestion with EcoRI and PstI plasmid from red 58 was used. Ligations were made: 10fmol plasmid to 0fmol, 20fmol and 40fmol insert.
13.08.15	**Transformation**	The SOP was followed.

7. Sample specification

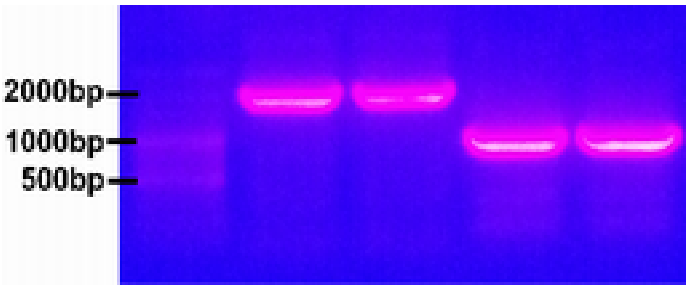
Sample name	Sample content	From	Used for / Saved where
Grøn 113	49,4 ng/ µL SDM PCR product, primers used: 4+63		**iGEM fridge**
Grøn 114	99,3 ng/ µL SDM PCR product, primers used: 5+64		**iGEM fridge**

Grøn 119	~69ng/µL DXS (*E. coli*) amil PCR product, primers used: 4+5		**iGEM fridge**

8. Remarks on setup

9. Results and conclusions

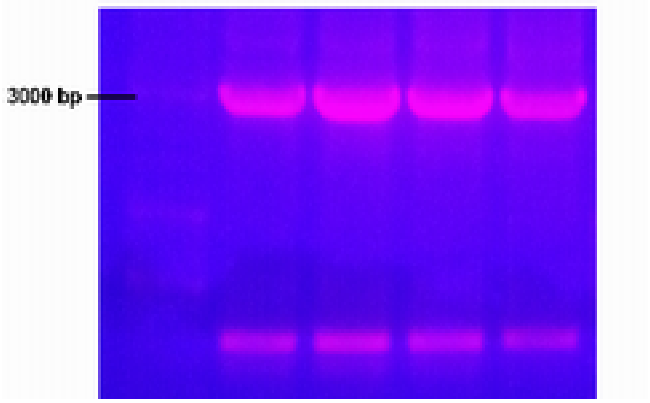
13.08.07 PCR and gel purification results



Well 2+3: primer 4+63
Well 4+5: primer 5+62

The bands had the appropriate lengths (around 1900bp and 1000bp) and were cut out, purified and stored as samples Grøn 115 and Grøn 116. See 7. sample specification for details.

13.08.08 PCR results



Well loads (see experiment history 13.08.07 for details):

Red Ladder, PCR1, PCR2, PCR3, PCR4

Bands appeared in all four wells just below 3000. Bands were purified and concentration estimated at 69ng/µL using nanodrop.

10. Appendices