iGEM2013 – Microbiology – BMB – SDU	
Project type: USER cloning **Project title:** USER cloning of pSB1C3 and AmilCP **Sub project:**	**Creation date:** 6/8-13 **Written by:** MHK and SF **Performed by:** MHK and SF

1. SOPs in use

SOP0006_v01_USER_cloning

2. Purpose

To compare the colonies of E. coli containing just AmilCP with the colonies containing AmilCP and DXS in the pSB1C3 plasmid.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006_ v01	MHK, SF SF	USER PCR of pSB1C3 and AmilCP USER PCR of Amil
2	SOP0014_ V01	SF, MHK	Gelpurification of ON PRC of Amil and Plasmid
3	SOP0007_ v01 SOP0009_ v01	SF SF	Excision and ligation of PCR products in USER cloning TSB Trnsformation

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 7				
Primer 13				
Primer 52				
Primer 53				
Grøn 24				
Grøn 26				

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
13.08.06	**SOP0006_v01**	The PCR program was altered from the SOP version to the program used for succesful plasmid PCR: 95 deg 2 min 95 deg 30 sec* 48 deg 30 sec* 68 deg 2:30 min* 72 deg 10 min *37 cycles Amil Template: grøn 24 Primers: 13+52 Plasmid Template: Grøn 26 Primers: 7+53 50 µL was loaded in each well on the gel.

13.08.06	**SOP0006_v01**	The PCR program: 95 deg 2 min 95 deg 30 sec* 38 deg 30 sec* 72 deg 1:00 min* 72 deg 10 min *30 cycles amil Template: grøn 24 Primers: 13+52 ON
13.08.06	**SOP0006_v01**	The PCR program was altered from the SOP version to the program used for succesful plasmid PCR: 95 deg 2 min 95 deg 30 sec* 48 deg 30 sec* 68 deg 2:30 min* 72 deg 10 min *37 cycles Plasmid band 1-3 Template: Grøn 26 Primers: 7+53 Plasmid band 4-6 Template: Green 106 Primers: 7+53 ON

13.08.08	**SOP0007_v01**	The reaction mixtures were: 10 fmol plasmid : 20 fmol amil 2 µL buffer 1 µL USER enzyme water to 20 µL and: 10 fmol plasmid : 50 fmol amil The rest as above Control: 10 fmol plasmid : 0 fmol amil The rest as above. Incubated for 30 min at 37 deg. C. Incubated for 30 min at 25 deg. C.
13.08.08	**SOP0009_v01**	0,55 µL culture was used for ech sample. 10 µL of each sample from above was used. 1 µL RFP (on plasmid) was used. One sample was pure bacteria. 55 min Phenotypical expression.

7. Sample specification

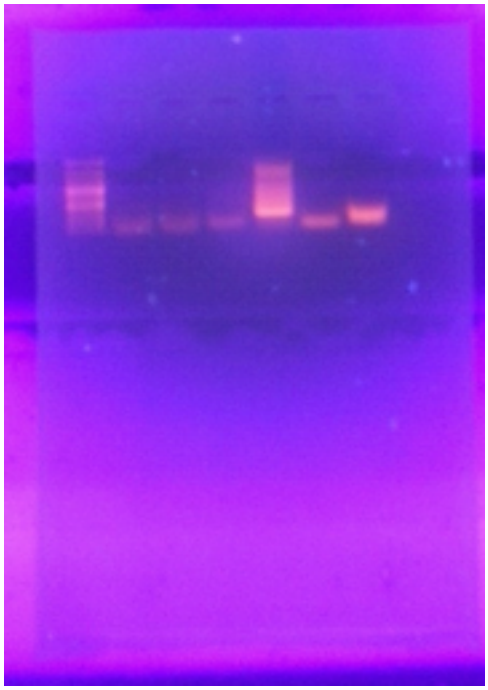
Sample name	Sample content	From	Used for / Saved where
Green 106	**pSB1C3 USER**	**USER PCR from first PCR**	**iGEM fridge**
Green 107	**pSB1C3 USER**	**USER PCR from second PCR**	**iGEM fridge**
Green 108	**pSB1C3 USER**	**USER PCR from second PCR**	**iGEM fridge**
Green 109	**AmilCP USER**	**USER PCR from second PCR**	**iGEM fridge**
Green 110	**AmilCP USER**	**USER PCR from second PCR**	**iGEM fridge**

8. Remarks on setup

9. Results and conclusions

06.08.13

PCR was run on 1% agarose gel.



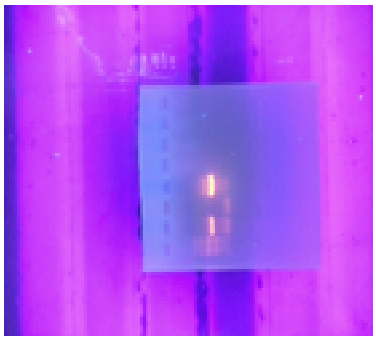
Well 2-4= amil, well 5-7=plasmid. Upper band in well 5 was cut out and purified.

Nanodrop:

Green 106: 3,3 ng/µL

13.08.07

PCR Amil:



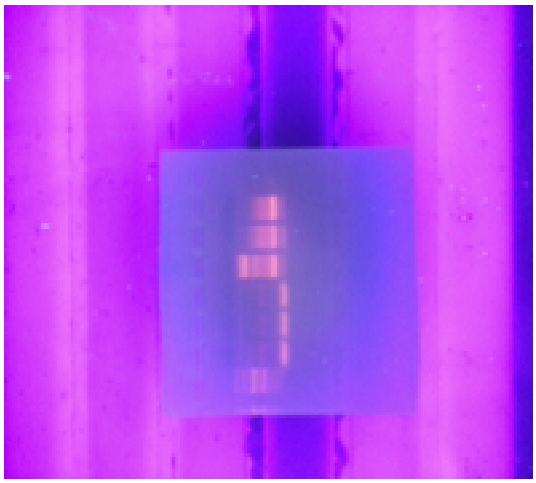
the two bands were cut out and purified.

Nanodrop:

Grøn 109: 26,4

Grøn 110: 82,4.

PCR Plasmid:



The slight bands in well 2-4 was purified together. The band in well 5 was also purified.

Nanodrop:

Grøn 107: 9,3 ng/µL

Grøn 108: 5,0 ng/µL

10. Appendices