iGEM2013 – Microbiology – BMB – SDU	
Project type: USER Cloning	Creation date: 13.07.31
Project title: USER cloning of pSB1C3 for Composite Production System	Written by: MH Performed by: MH, MHK
Sub project:	renomica by. will, willix

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	МНК, МН	USER PCR of pSB1C3
2	SOP0006	PRA	USER PCR of pSB1C3
3	SOP0006	MH, SIS	USER PCR of pSB1C3
4	SOP0006 SOP0014	SF, MHK MHK	USER PCR of pSB1C3 Gel purification

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 38+39	10uM	Sigma Aldrich		

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments	
13.07.31	SOP0006_v01	A USER PCR was performed with the following program:	
		Annealing: 55 deg Elongation: 2 min 30 sek, 68 deg Cycles: 37	
		Template: 22	
		Primers: 38, 39	
13.08.01	USER PCR	A USER PCR with primer 38+39, E.coli DXS on pSB1C3 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	USER PCR	A USER PCR with primer 38+39 was performed using template: blue 68. 5x25 uL total reaction was performed. Sample 1 was run as an ordinary USER PCR with annealing temp 53,6 deg. Four samples (2-5) were run with a touchdown with the following program: 95 deg 2 min 95 deg 30 sec Touch down from 55 deg 30 sec 2 min 10 sec 53,6 deg 30 sec 30 cycles 72 deg 2 min 10 sec
13.08.05	USER PCR	A USER PCR was done with primer 39+49. Template was sample Grøn 26. 3 PCR reactions were prepared in each their own tube. 95 deg 2 min 95 deg 30 sec 55 deg 30 sec 68 deg 2 min 10 sec 30 cycles 72 deg 10 min (final elongation)

7. Sample specification

Sample name	Sample content	Concentration	Used for / Saved where
Grøn 101	PCR of pSB1C3. Length around 1500bp and not 2000bp!	2.2 ng/uL	iGEM fridge
Grøn 104	PCR of pSB1C3, correct band length	58,9 ng/μL	iGEM fridge

8. Remarks on setup

9. Results and conclusions

31.07.13

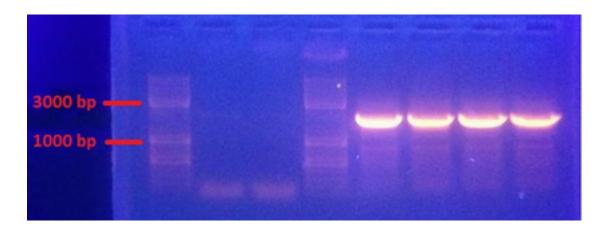
Results for USER PCR. 50 μ L was loaded in each well on a 1% agarose gel. Ladder: red. Order of well load:

Red ladder

- 1) pSB1C3 til CPS
- 2) pSB1C3 til CPS

Red ladder

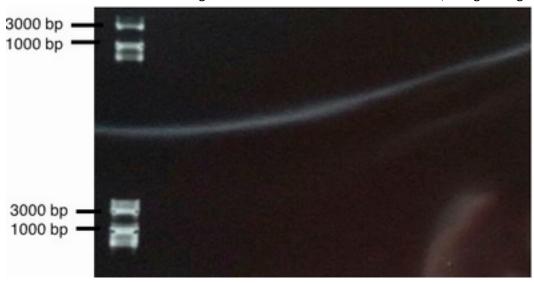
- 4) DXS B.sub til CPS with annealing temp. 49.8 deg
- 5) DXS B.sub til CPS with annealing temp. 52.1 deg
- 6) DXS B.sub til CPS with annealing temp. 54.7 deg
- 7) DXS B.sub til CPS with annealing temp. 56.8 deg



No bands appeared. A new USER PCR was performed over night.

13.08.02

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



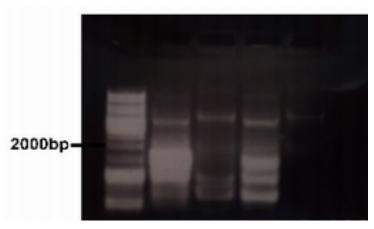
No bands appeared. A new PCR was performed.

Results for todays ordinary PCR:

25 uL was loaded in well 3 on a 1,5% agarose gel. No bands could be seen on the picture that was taken. Though, there was a band around 1500 bp and 3000 bp.

Results for todays touchdown PCR:

25 uL was loaded in well 2-5 on a 1,5% agarose gel. Well 1 contains red ladder.



None of the bands had the appropriate length around 2000 bp.

13.08.05

Results for PCR:

50 uL was loaded in well 6, 7 and 8 on a 1% agarose gel. Bands appeared around 2000 bp for well 6 and 7. These bands were cut out and purified together as sample Grøn 104 with a concentration of 58,9 ng/ μ L. Well 8 showed a band around 300 bp (cannot be seen because of poor picture quality). The ladder is 100 bp plus, loaded in well 1 and 5.



10. Appendices