iGEM2013 – Microbiology – BMB – SDU	
Project type: USER PCR **Project title:** USER PCR of Lac promoter-Dxs (E. coli) without reporter gene **Sub project:**	**Creation date:** 23.07.13 **Written by:** SF **Performed by:** SIS, SF, MH, PRA, AK, MHK

1. SOPs in use

SOP0006_v01 PCR protocol for USER cloning

2. Purpose

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006 SOP0006	SIS, SF, MH AK, PRA	USER PCR on dxs (E.coli) from sample green 63 without reportergene. USER PCR on dxs (E.coli) from sample green 63 without reportergene.
2	SOP0006	SIS, MH	USER PCR on dxs (E.coli) from sample green 63 without reportergene.
3	SOP0007 SOP0009		USER cloning TSB transformation
4	SOP0021		Colony PCR in order to check if the insert is of the right lenght.
5	SOP0019	MH	Plasmid miniprep on ONC in order to make a sample for sequencing.
5		MH	Senquencing mixture prepared
6	SDM		Site directed mutagenesis was performed in order to remove stopcodons in the sequence. See protocol iGEM2013_031_SDM_Lac_DXS(E.coli).
7	SOP0017 SOP0014 SOP0015	SIS	Fast digest of lac-dxs(E.coli) (green 121) with EcoRI and PstI. Gel purification Ligation of digested lac-dxs(E.coli) and distested pSB1C3.
8	SOP0019	SIS	Plasmid Miniprep of ONC in order to digest with EcoRI and PstI (to check for the right insert size) and EcoRI alone (to linearize the plamid).

9	SOP0017	MHK	Fast digest of Blue 162 (lac-DXS (*E. coli*)
10		MH	Preparation of sequencing mixture for the expected sequence: lac-dxs(coli).
11	SOP0014	SIS, MH	TSB transformation of blue 162 into MG1655 and KG22 cells. NOTE: doubt has arisen as to the notation of the petri dishes. Transformation should be redone (MH).
12	SOP0014	MH	TSB transformation of blue 162 into MG1655 and KG22.
13	SOP0019	SF	Plasmid Miniprep

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

13.07.23	SOP0006_v01 USER PCR	Template: samble Green 63. Mastermix for 5 PCR samples was made. The PCR was made with a annealing temp. gradient: 1) 48,4 deg 2) 51,1 deg 3) 53,8 deg 4) 56,2 deg 5) 58,1 deg Elongation time: 2 min. The primers were: 29 and 32. 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: 32 and 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

13.07.25	USER reaction TSB transformation	20 µL total reaction with pfu 10x buffer 10 fmol pSB1C3 to each reaction: 1:0:0 control: no insert 1:2:2: 20 fmol DXS, 20 fmol Lac promoter 1:5:5: 50 fmol DXS, 50 fmol Lac promoter 30 min at 37 degrees 30 min at RT (Green 80-82) TSB transformation was done as described, except no LB was added during resistance expression. 15 of the 20 µL USER reaction was used. 1.5 hour resistance expression.
13.07.26	SOP0021	Colony PCR with Mytaq was performed on two colonies from the transformation plate 1:2:2 and 1:5:5. Annealing temp: 60 deg. Elongation time 30 sec. A mastermix containing water, primere (004 and 005) and Mytaq HS Red Mix was performed.
13.07.29	SOP0019	Plasmid miniprep on ONC in order to make a sample for sequencing.
13.07.29		Sample was prepared for sequencing using 14 µl of Blue 89 mixed with 1 µl of either primer 4, 5 or 1. **Sample sent to sequencing.**
13.08.16	SOP0017 SOP0014 SOP0015	Fast digest of Lac-dxs(E.coli) PCR product from mutagenesis (green 121) with EcoRI and PstI. Gel purification was performed. Eluated in 30 uL water. Ligation of red 58 and red 151. Ligation samples: 1:2 (plasmid:lac-dxs) 1:5 (plasmid:lac-dxs) All performed with 10 fmol plasmid. Unfortunately no negative control (1:0) was performed since the red 58 sample run out.
13.08.17	TSB transformation	SOP followed, 1.5 h of phenotypical expression.
13.08.18	Colony PCR Colony PCR	Colony PCR with MyTaq and verification primers 3uL H2O 5uL MyTaq Colony 1-4 No control plate Colony PCR with MyTaq and verification primers 0.25uL of each primer 4.5 uL H2O 5uL MyTaq Colony 1-4 No control plate

13.08.19	Plasmid miniprep Fast digest	Plasmid Miniprep of ONC were performed in order to digest with EcoRI and PstI (to check for the right insert size) and EcoRI alone (to linearize the plamid). Two minipreps were performed (both eluated in 50 uL water), but they gave around the same nanodrop so they were pooled. Blue 162 was digested with EcoRI and EcoRI + Pst. 10x FastDigest green buffer was used and the products were loaded directly on a gel.
13.08.20	Sequencing mixture	3 mixtures from blue 162 were prepared of 17 ul volume with 2 ul of the following primers: 4, 5, and 63 given the corresponding numbers: 38, 39, and 40
13.08.26	TSB transformations	TSB transformation of blue 162 into MG1655 and KG22 cells. Expression time: 1 hour. Samples were disposed of.
13.08.27	TSB transformation	TSB transformation of blue 162 into MG1655 and KG22 redone. Expression time: 1 hour.
13.08.29	Plasmid Miniprep	Miniprep of blue 162.

7. Sample specification

Sample name	Sample content	Concentration	Used for / Saved where
Red 151	Digested lac-dxs(E.coli) from SDM with EcoRI and PstI.	5,5 ng/uL	Used to ligate together with pSB1C3 (red 58).

Blue 162	Miniprep of pSB1C3-Lac-dxs(E.coli)	250,9 ng/uL	To be digested with EcoRI and PstI and EcoRI alone. Stored in the iGEM frigde.
Blue 188	Miniprep pSB1C3-Lac-dxs(E.coli)	79,4 ng/µL	To be used by Andreas at Teknikum?
Blue 189	Miniprep pSB1C3-Lac-dxs(E.coli)	69,6 ng/µL	To be used by Andreas at Teknikum?

8. Remarks on setup

9. Results and conclusions

23.07.13:

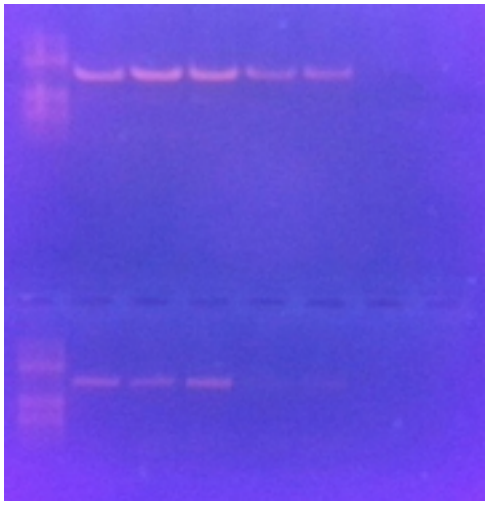
The USER PCR was run on an 1% agarose gel

There were no bands and the PCR was done again.

24.07.13

The user PCR was run on an 1% agarose gel

Results for the new PCR reaction:

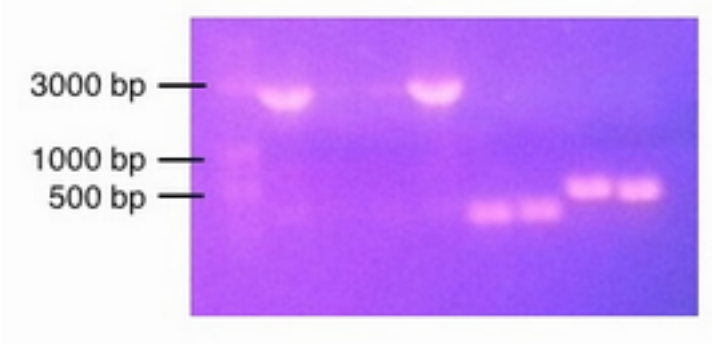


50 µL was loaded in each well on a 1 % agarose gel. Ladder: red. The lower line is the PCR result for this protocol. There appeared clear band around 2000 bp in well 2-4 and small bands in well 5 and 6. The bands were cut out and purified from gel.
Overview of the well loads:

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

13.07.24
The transformation was successful.

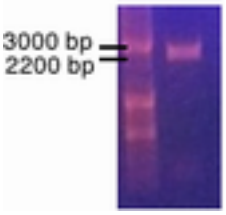
13.07.25
Results for the colony PCR on transformations: 10 µl was loaded in each well. Ladder: red.



Well 1-4 contains colony PCR of cells transformed with dxs (E.coli) in pSB1C3. Bands around 2200 bp appeared in all four wells, but the strongest bands is in well 1 and 4 (colony 1 and 4).

29.07.13
Miniprep was performed and eluted in 200 µl. Concentration was estimated at 79.2 ng/µl using nanodrop.

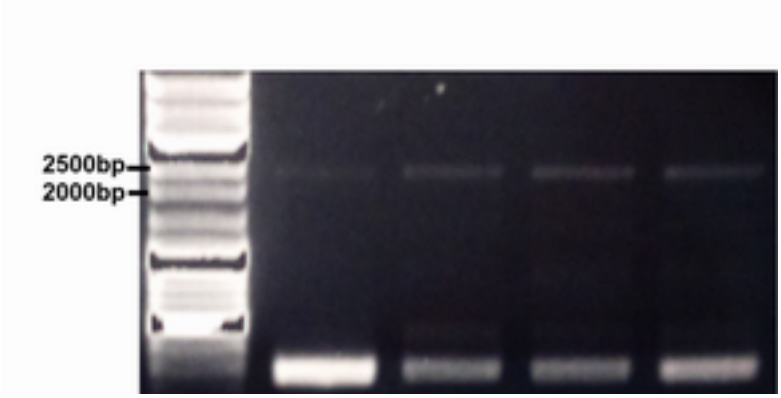
16.08.13
30 uL was loaded in a well on a 1% agarose gel. Ladder: red.



Band appeared around the right lenght (2200 bp). The band were cut out and purified giving a concentration of 5,5 ng/uL.

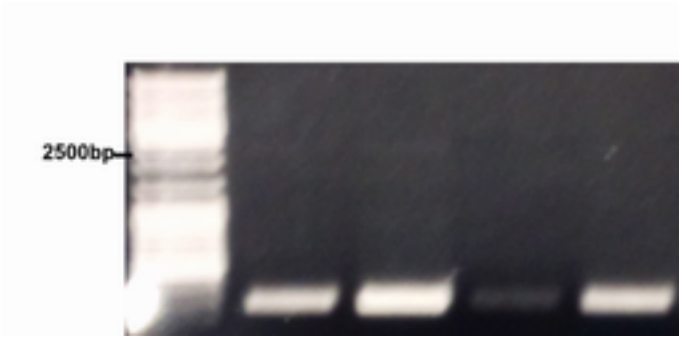
Ligation was kept at 16 deg for the next day.

13.08.18
Colony PCR 1



Bands is seen with the right length between 2000bp and 2500bp

Colony PCR 2

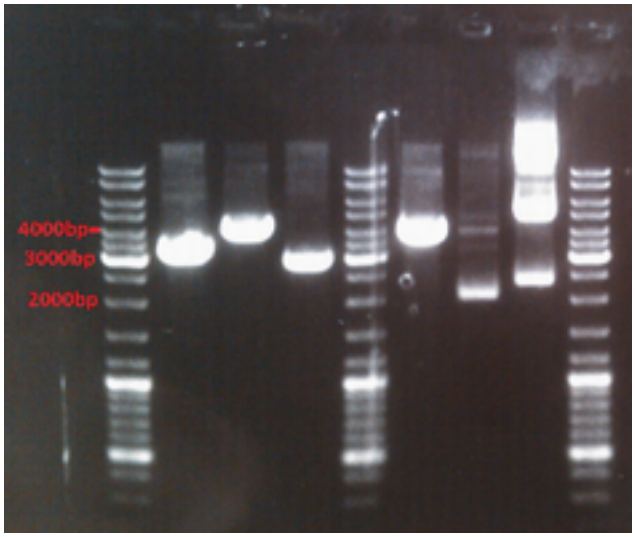


Weak bands is seen between 2000bp and 2500bp

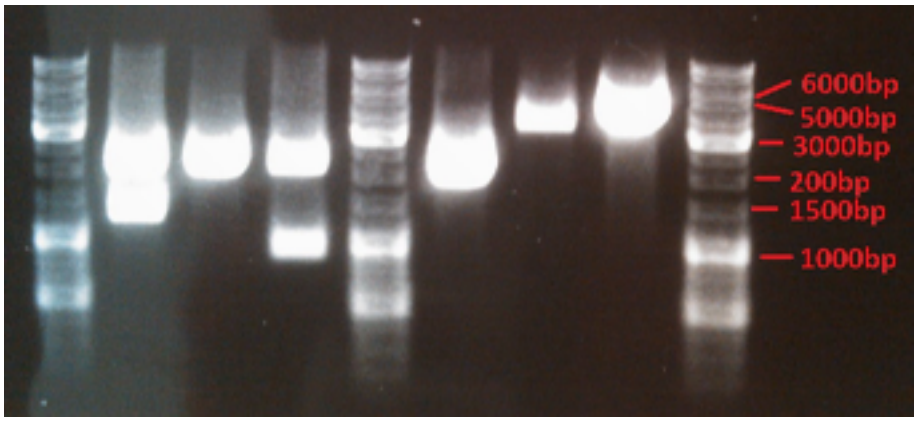
13.08.19

Nanodrop results for the miniprep: 250,9 ng/uL.

Below: Fast digest of Blue 162 with EcoRI loaded on a 1% agarose gel. Red ladder is used.
Blue 162 is loaded in well number 3. A thick band appeared around 4000bp which was as expected (pSB1C3 ~2000bp, lac ~200bp, DXS (*E. coli*) ~2000bp).



Below: Fast digest of Blue 162 with EcoRI and PstI loaded on a 1% agarose gel. Red ladder is used.
Blue 162 is loaded in well number 3. A thick band appeared a bit above 2000bp. The expected lengths are ~2000bp for both fragments (pSB1C3 ~2000bp, lac + DXS (*E. coli*) ~2200bp).



13.08.29

Nanodrop of plasmmidmiiniprep - see Sample specifications.

10. Appendices