

iGEM2013 – Microbiology – BMB – SDU	
Project type: Conventional cloning Project title: LacI-Lac-Rbs-dxs(col) Sub project:	Creation date: 26-08-13 Written by: Hwj Performed by: Hwj

1. SOPs in use

SOP0017_Fastdigest

SOP0009_Transformation

SOP0014_Gel purification

SOP0015_ligation

SOP0021_Colony PCR

2. Purpose

Clone LacI into lac-dxs

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0017	HWJ	Digestion of blue 162 and Green 122 for conventional cloning
2	SOP0017	HWJ	Digestion of Blue 139.
	SOP0014	SF	Gelpurification of digested 162 pSB1C3 -pLac-dxs(col)
	SOP0015	HWJ	Ligation of digested blue 162 with green 122.
3	SOP0009	SIS, MH	TSB transformation into MG1655
4	SOP0019	MH	Miniprep of ONC of koloni 15 with the purpose to digest to test the expected sequence: pSB1C3-lacI-Plac-Dxs(col)

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Blue 162	pSB1C3 - Lac-RBS- dxs(coli) 250,9 ng/ μl		iGEM fridge	
Green 122	LacI		iGEM fridge	
Blue 139	LacI 8,1ng/μl		iGEM fridge	

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
13.08.23	SOP0017	Digestion of Blue 162 with EcoRI and XbaI 0,5 μl phosphatase Digestion of green 122 with EcoRI and SpeI, should be 1200bp long, was 1500bp.

7. Sample specification

Sample name	Sample content	From	Used for / Saved where
Red 178	pSB1C3 - Lac-RBS-dxs(col) digested with EcoRI and XbaI (and fast AP)	Blue 162	iGEM fridge
Red180	digested LacI with EcoRI and SpeI	green 122	iGEM fridge
red 181	pSB1C3 - Lac-RBS-dxs(col) digested with EcoRI and XbaI (and fast AP)	from blue 162	iGEM fridge
Blue 185	pSB1C3-lacI-Plac-Dxs(col)?	Colony 15	127.8 ng/ul

8. Remarks on setup

9. Results and conclusions

13.08.23

after digestion the samples were run on a gel. Blue 162 looked to be fine, and LacI from sample 122 was about the right length, but to be sure a new digestion with another template will be made.

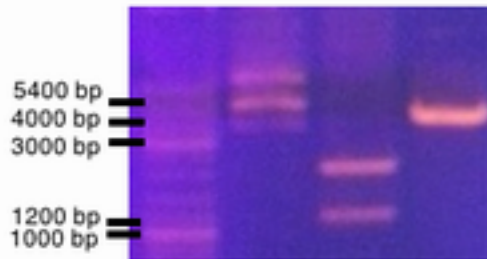
13.08.26

File name: iGEM2013_0037_Protocol_LacI_Lac_Dxs(col)

Digestion of blue 139 showed no bands on the gel.

Result for new fast digestion:

20 uL was loaded on a 1% agarose gel with red ladder. Well two contains pSB1C3-LacI-PLac-dxs(B.sub) digested with E and X. Well three contains IspG digested with E and S and well four contains pSB1C3-Lac-dxs(E.coli) digested with E and X.



The right band around 4200 bp appeared in well four. The band was cut out and purified.

Result for gel purification: Green 122 = 10,4 ng/μl and blue 162 = 9,2 ng/μl

Nanodrop measurements of digested 162 pSB1C3-pLac-DXS(col): 9,2 ng/μL (red 178).

13.08.28

Colony PCR results: 10 uL was loaded on a 1% agarose gel. Ladder red. Order of load: 10-18.



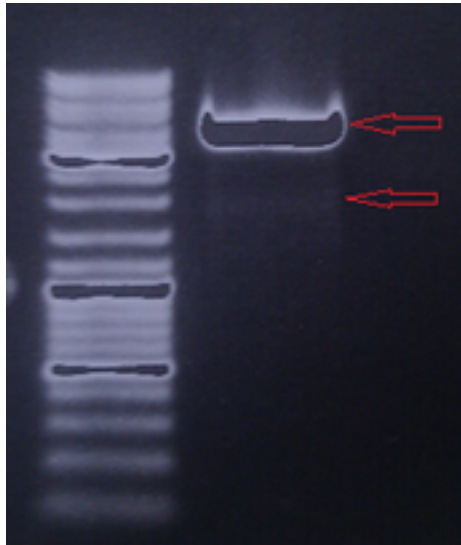
The band of colony 15 lies around 1200 bp beneath the other bands (religations) and not over as expected, so a ONC is performed in order to miniprep the plasmid from the colony and test digest it with EcoRI and PstI in order to check the insert size.

13.08.29

Test digestion of blue 185: expected bands:

insert: ~4000bp

plasmid: ~2000bp



10. Appendices