

iGEM2013 – Microbiology – BMB – SDU	
Project type: Conventional cloning of IspG together with PLac-dxs(B.sub) in pSB1C3. Project title: PLac-dxs(B.sub) in pSB1C3 Sub project:	Creation date: 5/9-13 Written by: Hwj, MHK, SF Performed by: Hwj, MHK, SF

1. SOPs in use

iGEM2013_SOP0017_v01_Fast digest

iGEM2013_SOP0014_v01_Gel purification

iGEM2013_SOP0015_v01_Ligation

2. Purpose

Cloning of IspG together with PLac-dxs(B.sub) in pSB1C3.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0017 SOP0014 SOP0015	Hwj SF	Digest of blue 105 with Spel, PstI and Fast AP Gelpurification Ligation
2	SOP0009	SF, MHK	TSB transformation
3	SOP0021	HWJ	Mytaq colony PCR
4	SOP0021	HWJ	Mytaq colony PCR
5?	SOP0009	HWJ, MHK	TSB transformation of pSB1C3-PLac-dxs(B)-IspG into MG1655
6	SOP0021	SIS	Mytaq colony PCR
7	SOP0019	SF	Plasmid miniprep

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Blue 105				

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
13.09.05	SOP0017 SOP0014 SOP0015	Fast digest of blue 105 with PstI and SpeI with Fast AP. The SOP was followed. Nanodrop was measured. Ligations were made with 10 fmol pSB1C3-pLaC-dxs(b. sub) and 0, 20 and 50 fmol IspG and left overnight.
13.09.06	SOP0009	The SOP was followed, 1 hour phenotypical expression. Plated on LA plates with CM.
13.09.06	Mytaq Colony PCR	Mytaq Colony PCR: Mytaq colony PCR was performed on 9 colonies from the transformation plates 1:0 (colony 9), 1:2 (colony 1-4) and 1:5 (colony 6-9) with VR and VF primers.

7. Sample specification

Sample name	Sample content	From/concentration	Used for / Saved where
Red 216	pSB1C3-pLaC-dxs(b. sub) digested with p+s+phosphate	Blue 105	iGEM fridge
Blue 250	pSB1C3-pLaC-dxs(sub)- ISP G	48,4	iGEM fridge

8. Remarks on setup

9. Results and conclusions

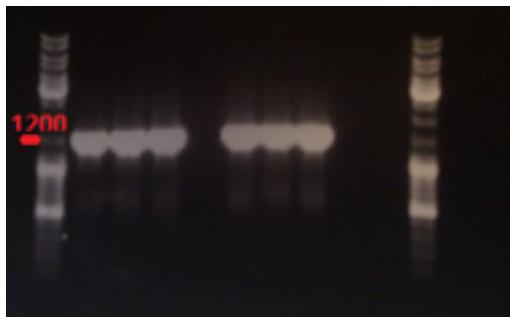
The digest showed the expected lenght ~4200bp



The band was cut out and purified yielding a concentration of 5,1 ng/µL.

13.09.07

Colony PCR with Mytaq: There is no band in the religation well, and no bands with the correct lenght.



#1-->9, 9 was the religation

13.09.09

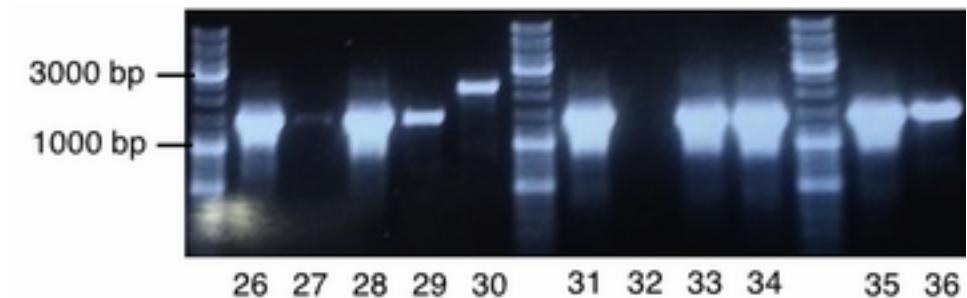
#19-25 in well 2-8. Well 2 is the religation



13.09.16

Result for the colony PCR with mytaq and VR and VF primers: 10 μ L was loaded in each well. Ladder: red. Colony 22-29 are PLac-dxs(B.sub)-ISPG and colony 30 is a negative control.



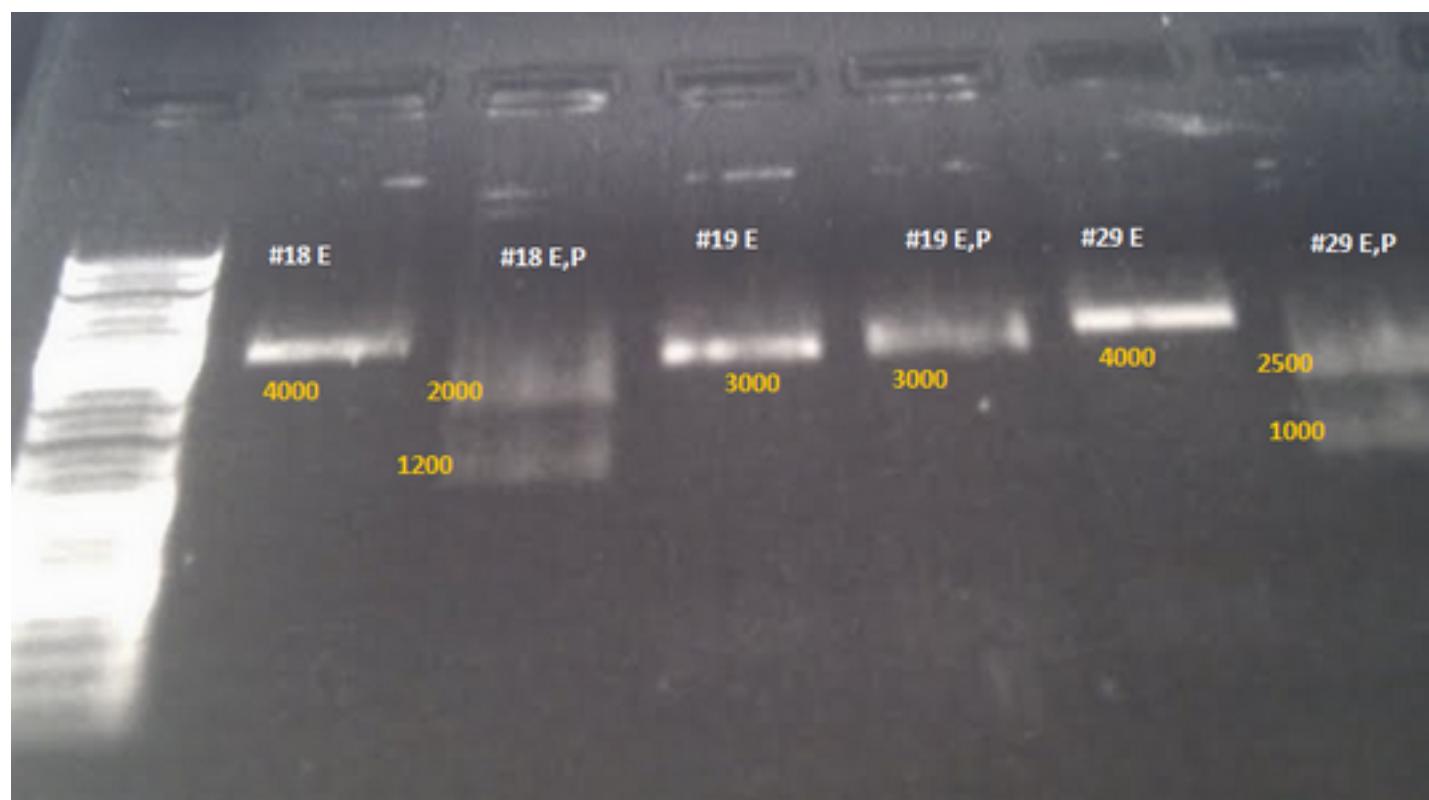


For colony 22, 26, 27, 28 and 29 bands of the right length appeared. Colony 23 and 25 has bands of the same length as the negative control, so might be religations. A ONC of colony 29 was performed in order to check the insert size with a test digestion.

13.09.17

miniprep of colonie 29 from d. 15/9-13: blue 250 see table.'

Test digest showed that pLac-dxs has been cut out and IspG is the only insert. (bad picture see real one in the folder)



10. Appendices