iGEM2013 – Microbiology – BMB – SDU	
Project type: BioBrick creation **Project title:** BioBrick Creation of lacI without LVA composite part **Sub project:**	**Creation date:** 13.09.13 **Written by: PRA** **Performed by:** PRA, SF, MH, MHK, HWJ

Status:

1. SOPs in use

iGEM2013_SOP0014_v01_Gel purification

iGEM2013_SOP0012_v01_Restiction_digest

iGEM2013_SOP015_v01_ ligation

iGEM2013_SOP0019_v01_ Plasmid Miniprep

iGEM2013_SOP0009_v01 TSB transformation

iGEM2013_SOP0017_v01_Fast_digest

2. Purpose

To create a BioBrick device containing natural lacI, a constitutively active promoter and a terminator.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0010 SOP0010	PRA SF	Phusion PCR gradient Phusion PCR gradient
2	SOP0010 SOP0014 SOP0012 SOP0015	PRA PRA PRA PRA	Phusion PCR gradient Purification of PCR product Digest of PCR product and pSB1C3 Ligation of pSB1C3 and lacI device
3	SOP0009	MHK HWJ	TSB transformation of promotor-RBS-LacI(natural)-stop

4	SOP0021	SIS	Mytaq colony PCR
5	SOP0019	SF	Plasmid Miniprep Test digest
6	SOP0019	MH	Plasmid Miniprep Test Digest
7	SOP0010	MH, SIS	Phusion PCR

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

13.09.13	SOP0010	The SOP was followed. Template was? Primers were 72+73. The program: 98 deg 1 min 98 deg 10 sec* 50, 53, 55.4, 60 deg 20 sec* 72 deg 45 sec* *30 cycles 72 deg 5 min
	SOP0010	The SOP was followed. Template was colony with lacI Primers were 72+73. The program: 98 deg 1 min 98 deg 10 sec* 50, 52.8, 55.4, 58.8, 59.9 deg 20 sec* 72 deg 45 sec* *30 cycles 72 deg 5 min
	Gel Purification	Gel purification was done according to SOP.
	Phusion PCR	The SOP was followed. Template was colony with Green 231 Primers were 72+73. The program: 98 deg 1 min 98 deg 10 sec* 60, 63, 67, 70 deg 20 sec* 72 deg 45 sec* *30 cycles 72 deg 5 min
	Gel purification	Purified according to SOP
	Digest 1	Digested with EcoRI and SpeI for 25 min at 37°C.
	Digest 2	Digested with EcoRI and SpeI for 30 min at 37°C.
	Ligation	Ligation of digested green ?? (sample red ??) with digested pSB1C3(sample red ??) 10 fmol plasmid 1:0 no insert 1:1 10 fmol insert 1:2 20 fmol insert
13.09.15	TSB transformation	Transformation of pSB1C3-promotor-RBS-LacI(natural)-stop 1:0, 1:1 and 1:2 into MG1655. Expression time 1h.

13.09.16	Mytaq colony PCR	Mytaq colony PCR with VR and VF primere was performed on four colonies from transformation plate 1:1 (colony 35-36)
13.09.17	Plasmid Miniprep	Miniprep of coloni nr. 35 from transformation d. 15/9 with correct lenght. 10 mL ONC was used. The SOP was followed. Test digest of colony #35 with E and E,P wierd result so tested again.
13.09.18	Plasmid miniprep Test digest	Miniprep of coloni nr. 36 from transformation d. 15/9 with correct lenght. 15 mL ONC was used. The SOP was followed. Test digest of colony 36 (253) with E and E,P. 20 minute digest.
13.09.21	Phusion PCR	Phusion PCR performed using sample blue 251 as template, and primer 72 og 73. The program: 98 deg 1 min 98 deg 10 sec* 60 deg 20 sec* 72 deg 45 sec* * x 30 72 deg 5 min The PCR was run over night

7. Sample specification

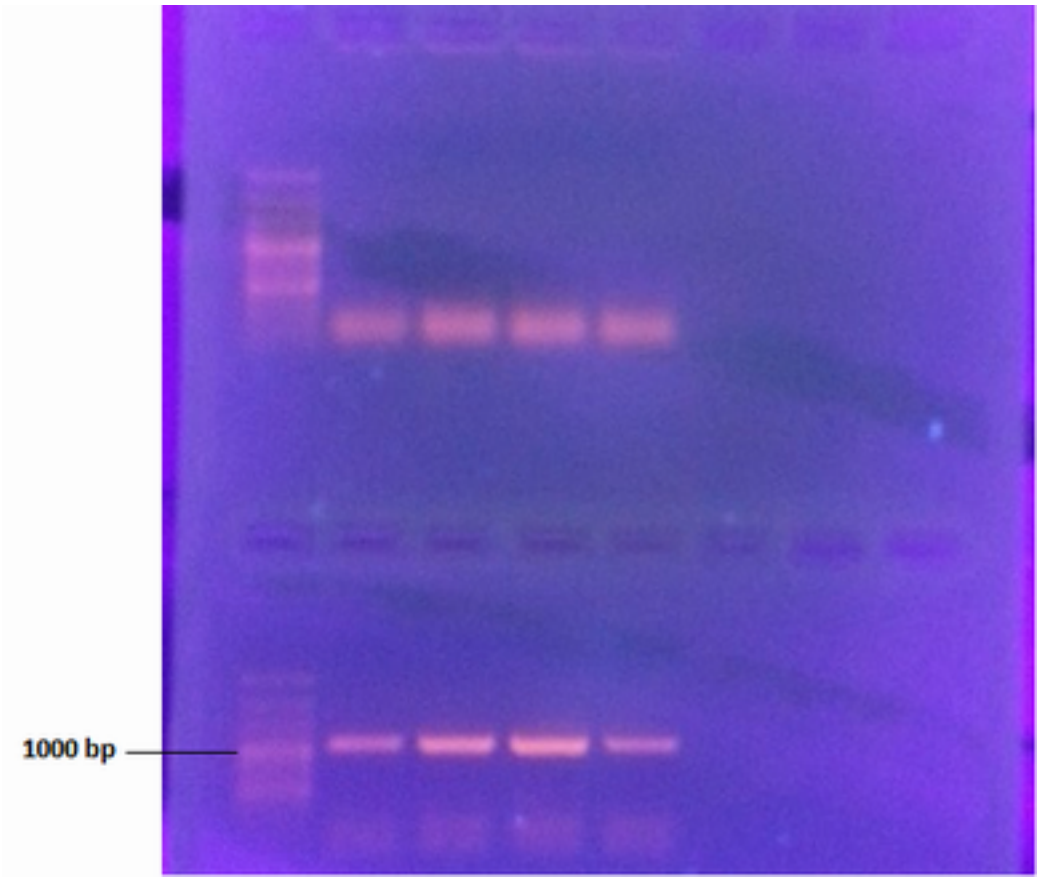
Sample name	Sample content	Concentration	Used for / Saved where
Green 230	Purified PCR product of lacI device	2.8ng/uL	template for another round of PCR
Green 231	Purified PCR product of lacI device	5.4ng/uL	template for another round of PCR
Green 237	Purified PCR product of lacI device	49ng/uL	To be ligated into pSB1C3 and pSB1C3-Plac-dxs(B.sub)-GFP
Blue 252	pSB1C3-LacI(no LVA) Device	49,0	iGEM fridge
Blue 253	pSB1C3-LacI(no LVA) Device	65,5	

8. Remarks on setup

9. Results and conclusions

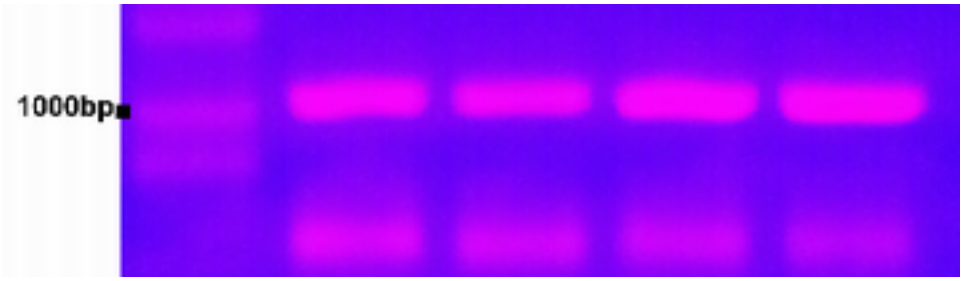
13.09.13

Gradient PCR run on gel. Light blue ladder, 1% agarose. Device in the top. No bands appeared.



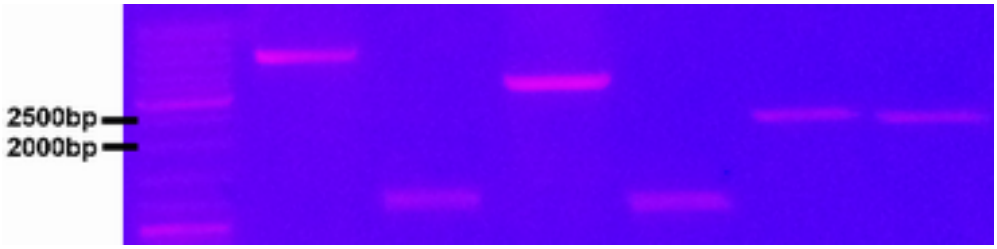
13.09.14

Phusion PCR:



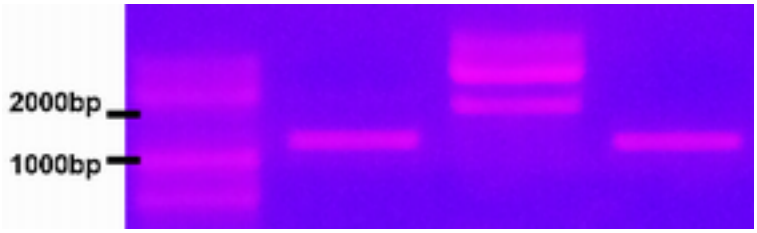
Bands appeared around the right length in all lanes. The bands were cut out and purified.

Digested PCR product (lane 3) and digested blue 135 (pSB1C3-lacILVA; lane 4):



The plasmid was not digested (only one band just above 3000bp). Digested PCR product was not purified.

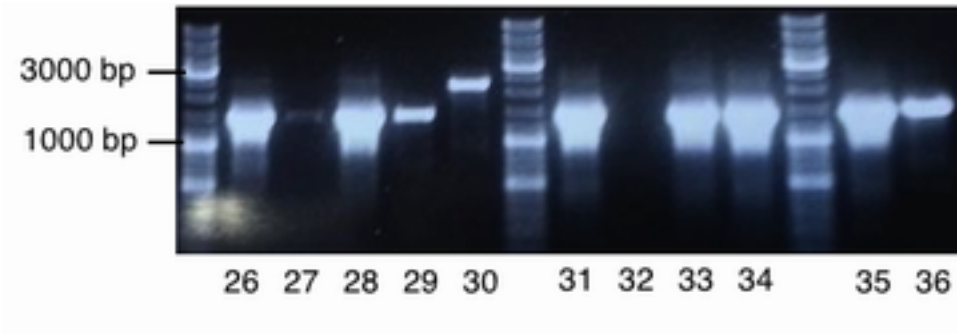
Digested PCR product (lane 2) and digested blue 174 (pSB1C3-lacI-Plac-dxs; lane 3):



The plasmid was cut and the PCR product and plasmid (band around 2000bp) was cut out and purified.

13.09.16

Result for the colony PCR with Mytaq and VR and VF primers: 10 µL was loaded in each well. Ladder: red. Colony 35-36 are constitutive promoter-LacI(without LVA).



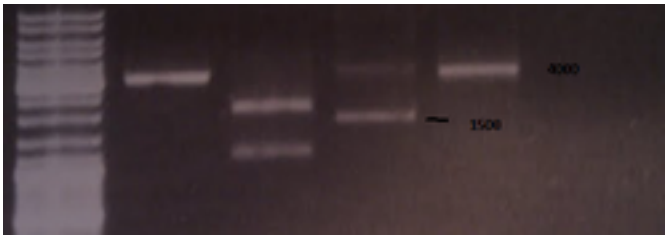
Bands of the right length (1500 bp) appeared for both colonies. A ONC of colony 35 was performed in order to double check the insert size with a test digestion.

13.09.17

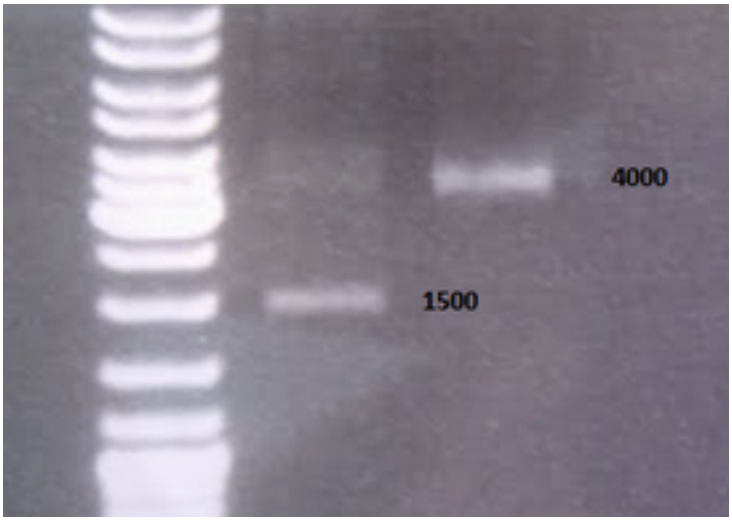
Miniprep of colony 35 see blue 252 in table.

Testdigest of #35

1. wierd lengths so we digest again

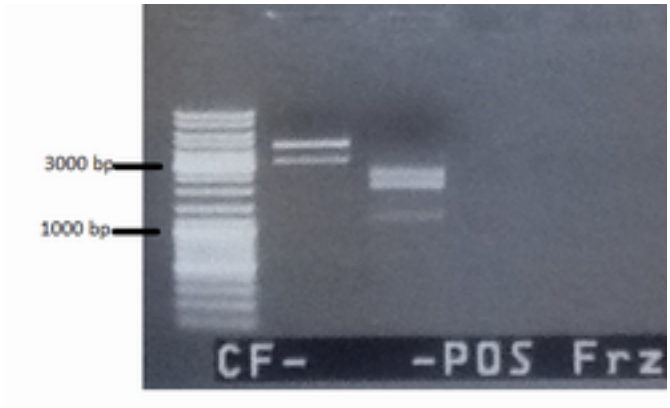


2. gives the same results as digest 1. so an ON of #36 is made so we can test digest that.



19.09.13

The digest showed the following colony #36:



First load is only digested with E, while the second with both E and P. It would appear that an EcoR1 site has emerged. Also, the insert is much larger than would be expected. However, when disregarding the upper two bands in each load, the results fit nicely to the expected lengths.

10. Appendices