iGEM2013 – Microbiology – BMB – SDU	
Project type: Creation of Biobrick with IspG **Project title:** Biobrick_IspG **Sub project:**	**Creation date:** 13.07.23 **Written by: PRA** **Performed by:** PRA, SF, SIS, MH

1. SOPs in use

iGEM2013_SOP0010_v01_Phusion PCR

iGEM2013_SOP0014_v01_Gel purification

iGEM2013_SOP0012_v01_Restiction_digest

iGEM2013_SOP015_v01_ Ligation

iGEM2013_SOP0019_v01_ Plasmid Miniprep

2. Purpose

To create a biobrick with IspG

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0010 SOP0012 SOP0015	PRA PRA PRA	Phusion PCR to amplify IspG from the chromosome of *E. coli* Digest of PCR product (IspG) to be ligated into pSB1C3 Ligation of EcoRI and PstI digested IspG into pSB1C3
2	SOP0009	SF	TSB Transformation
3	SOP0021	SIS, MH	Colony PCR with Mytaq using VF2 and VR primere in order to check the size of the insert in pSB1C3.
4	SOP0019	MH	Plasmid miniprep on ONC in order to make a sample for sequencing.
5		PRA	Prepared for shipping

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Primer 035	10μM	Sigma aldrich	iGEM fridge	
Primer 036	10μM	Sigma aldrich	iGEM fridge	
Primer 004	10μM	Sigma aldrich	iGEM fridge	
Primer 005	10μM	Sigma aldrich	iGEM fridge	

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments

13.07.23	Phusion PCR	MG1655 were used as template in 25µL total reaction phusion PCR with primer 035 and 036 PCR program: 98 deg 2 min 98 deg 10 sec 55.5, 57 60, 62 deg 15 sec 30 cycles 72 deg 30 sec
	Phusion PCR	MG1655 were used as template in 25µL total reaction phusion PCR with primer 035 and 036 PCR program: 98 deg 2 min 98 deg 10 sec 55 deg 30 sec 5 cycles 72 deg 1 min 98 deg 10 sec 65 deg 15 sec 30 cycles 72 deg 1 min
	Restriction digest	Green 75 was digested with EcoRI and PstI for 30 min at 37°C
	Ligation	Red 77 was ligated into pSB1C3. 10 fmol pSB1C3 to 20 and 50 fmol Red 77. The ligation was left at 16°C overnight
13.07.24	SOP0009 - Trasformation	Two transformations - Plasmid:Red77 1:2 and 1:5. The TSB buffer was premade. 0,95mL culture and 10 µL plasmid were used. Phenotpical expression for 1 hr.
13.07.25	SOP0021	Colony PCR with Mytaq was performed on two colonies from the transformation plate 1:2 and 1:5. 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	Sample was prepared for sequencing using 14 µl of Blue 87 mixed with 1 µl of either primer 4, or 5. **Sample sent to sequencing.**
13.08.06		10 uL of a concentration of 25ng/uL of Blue 87 was prepared for shipping. PCR tube#1

7. Sample specification

Sample name	Sample content	Concentration	Used for / Saved where
Green 75	Phusion PCR product of IspG	12 ng/µl	To be digested and inserted into pSB1C3. Stored in the iGEM fridge.
Red 77	EcoRI and PstI digested Green 74	5.5 ng/µl	To be inserted into pSB1C3. Stored in the iGEM fridge.
Blue 87	pSB1C3-IspG	67.1 ng/µl	

8. Remarks on setup

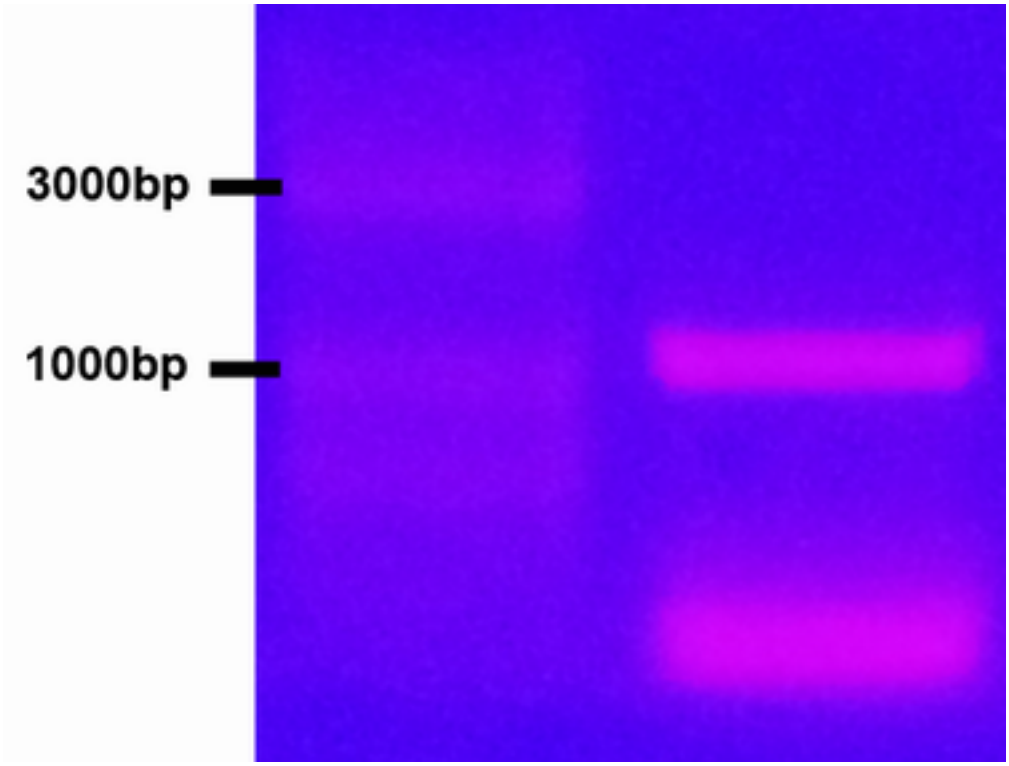
9. Results and conclusions

13.07.23

Phusion PCR with primer 035 and 036 to amplify IspG

No bands were seen and the PCR was done again

Phusion PCR with primer 035 and 036 to amplify IspG



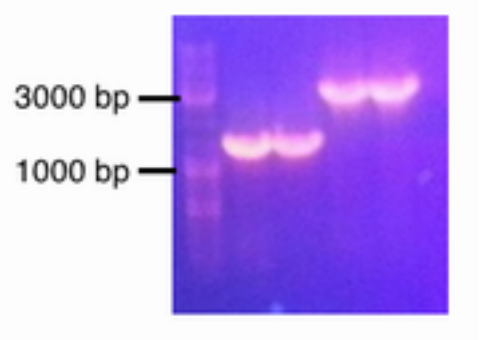
The band with the right length just above 1000bp were cut out and purified.

13.07.24

Transformation was successful

13.07.25

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



Only in well 1 and 2 (colony 9 and 10) bands of the right length (ca. 1400 bp) appeared. The bands in well 3 and 4 is around 3000 bp, so these does not have the right length.

29.07.13

Miniprep was performed and elution was done in 200 µl. The concentration was estimated at 67.1 ng/µl using nanodrop.

10. Appendices