iGEM 2015 – Microbiology – BMB – SDU	
Project type: Creation of a biobrick **Project title:** Linker-GFP **Sub project:** Assembly of a biobrick that contains the Linker-GFP gene	**Creation date:** **Written by:** ADK; EMT, CEM **Performed by:** ADK, EMT

1. SOPs in use

iGEM2015_SOP010_v01_Phusion PCR
iGEM2015_SOP0014_v01_Gel purification
iGEM2015_SOP0017_v01_Fast digest
iGEM2015_SOP015_v02_ ligation
iGEM2015_SOP009_v01_ TSB transformation

2. Purpose

Assembly of a biobrick that contains the Linker-GFP gene.

3. Overview

Date (YY.MM.DD)	**Person(s) (initials)**	**Experiments**	**SOPs**
15.04.02 15.04.02	ADK, JSP	Phusion PCR x3 Gel purification	iGEM2015_SOP010_v01_Phusion PCR iGEM2015_SOP0014_v01_ Gel purification
15.04.03 15.04.03	ADK, JSP ADK, JSP, TBA, AC	Gel purification Digest DNA-pieces	iGEM2015_SOP0014_v01_ Gel purification iGEM2015_SOP015_v02_ ligation
15.04.04	TBA, JSP, KBS	Transformation	iGEM2015_SOP009_v01_ TSB transformation

15.04.05 15.04.05	ADK, AC, KBC, JSP KBC	Ligation (2nd attempt) Transformation	iGEM2015_SOP015_v02_ ligation iGEM2015_SOP009_v01_ TSB transformation
15.04.06	ADK	Colony PCR with my taq	iGEM2013_SOP0021_v01 Colony PCR with my taq
15.04.11 15.04.11	TBA, AC TBA, AC	Fast digest Phusion PCR	 iGEM2015_SOP010_v01_P husion PCR
15.04.12	AC	Gel purification	iGEM2015_SOP0014_v01_ Gel purification
15.04.19	TBA, AC, JSP	Fast Digest	iGEM2015_SOP0017_v01_ Fast digest
15.04.19	TBA, AC, JSP	Gel purification	iGEM2015_SOP0014_v01_ Gel purification

4. Materials required.

Materials in use

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

5. Experiment history

Date (YY.MM.DD)	**Person(s) (initials)**	**Alterations to SOPs and remarks to experiments**	**SOPs**
2015.04.02	ADK, JSP ADK, JSP JSP, TBA, AC	**First PCR attempt:** - Template: Linker_GFP - Primer: Linker-GFP_F + Con-mCherry_R - Optimised Temperature gradient: 55°C-64,9°C - Buffer:HF - Number of bp: about 1000 **Second PCR attempt**: - Template: Linker_GFP - Primer: Linker-GFP_F + Con-mCherry_R - Optimised Temperature gradient: 55°C-64,9°C - Buffer: 5 sample with HF and 4 sample with GC, - Number of bp: about 1000 **Gel purification:** Used 15µl distilled water to elute the DNA **Third PCR attempt**: - Template: Linker_GFP - Primer: Linker-GFP_F + Con-mCherry_R. Instead 2,5µl of each, we used 1,25µl of each - Optimised Temperature gradient: 55°C-64,9°C - Buffer: GC	- iGEM2015_SOP010_v01_Phusion PCR - iGEM2015_SOP0014_v01_Gel purification

		- Number of bp: about 1000	
2015.04.03		**Gel purification:** Used 15µl distilled water to elute the DNA **Fast digest** <u>PCR-product:</u> - Buffer: FastDigest - PCR product: [Linker-GFP]=24,8ng/µl, used 8µl - FastDigest enzyme: 1µl PstI + 1µl XbaI - Sterile water: 17µL <u>Plasmid DNA:</u> Plasmid: pSB1C3, 114 ng/µl: 9 µl Reaction time: approx. 30 min **Ligation of pieces of DNA:** 3 samples: 1) <u>approx. 0 fmol Linker-GFP</u> - Plasmid (psB1C3 (R1)), 10 fmol/µl: 1µl - Linker-GFP: 0µl - H_2O: 16µl - DNA ligase buffer: 2µl - DNA ligase: 1µl 2) <u>approx. 20 fmol Linker-GFP</u> - Plasmid (psB1C3 (R1)), 10 fmol/µl: 1µl - Linker-GFP, 14 fmol/µl: 1,4µl - H_2O: 14,6µl - DNA ligase buffer: 2µl - DNA ligase: 1µl 3) <u>approx. 50 fmol Linker-GFP</u> - Plasmid (psB1C3 (R1)), 10 fmol/µl: 1µl - Linker-GFP, 14 fmol/µl: 3,6µl	iGEM2015_SOP0014_v01_ Gel purification iGEM2015_SOP0017_v01_ Fast digest iGEM2015_SOP0015_Ligation of pieces of DNA

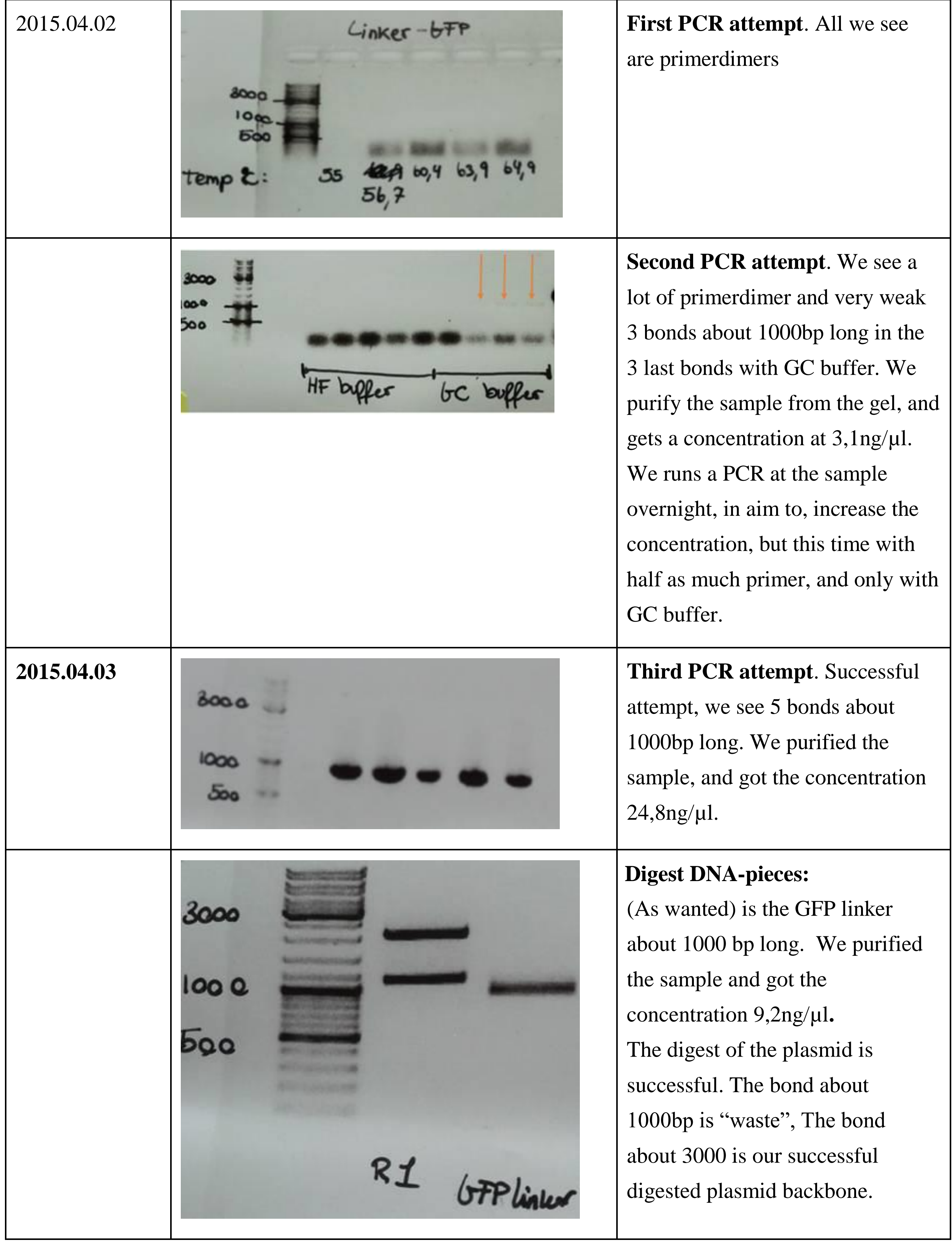
		- H_2O: 12,4µl - DNA ligase buffer: 2µl - DNA ligase: 1µl Leave the mixture overnight at 16°C	
2015.04.04	TBA, KBC, JPS	**Transformation:** Plasmid: linker-GFP with pSB1C3 backbone (0, 20, 50 fmol linker-GFP insert) E. coli wild type: MG1655 Did not spin: approx. 100 µl was directly added on agar plate (Cml antibiotic)	- iGEM2015_SOP009_v01_ TSB transformation
2015.04.05	ADK, AC, KBC, JPS	*Same alterations as ligation made on 2015.04.03*	iGEM2015_SOP015_v02_ ligation
2015.04.06	KBC	**Transformation:** E. coli wild type: MG1655 Plasmid: linker-GFP with pSB1C3 backbone (0, 20, 50 fmol linker-GFP insert): 15 µl added Approx. 100 µl was directly added on agar plate (Cml antibiotic)	- iGEM2015_SOP009_v01_ TSB transformation
2015.04.07	ADK	**Colony PCR with My Taq** - 8 reaction in total, 5µl Taq, 2*1µl primer, 3µl steril water pr. reaction	iGEM2013_SOP0021_v01 Colony PCR with my taq
15.04.11 15.04.11 15.04.11	TBA, AC TBA, AC TBA, AC	**Fast Digest** of linker-GFP from G8 cut with BamH1 and Pst1 **Gel purification**, reveals that Fast digest of linker-GFP was unsuccessful. **Fast digest (2nd attempt)** cut with BamH1 and Pst1 **Gel purification (2nd attempt)**	iGEM2015_SOP0017_v01_ Fast digest iGEM2014_SOP0014_v01_ Gelpurification iGEM2015_SOP0017_v01_ Fast digest

		Successful Fast digest. **Phusion PCR** from G7, using UF-buffer and half amount of primers. temp gradient from 55 degC to 65 degC and left overnight	iGEM2015_SOP010_v01_Phusion PCR
15.04.12	AC	**Gel purification**	iGEM2014_SOP0014_v01_Gelpurification
15.04.19 15.04.19	TBA, AC, JSP	**Fast digest x3** 11,5 µL of G9 for total volume of 30 µL. Using BamH1 and Pst1, incubating for 30 min @37 degC runing a gel for 35 min **Gel purification x3** eluted in 50 µL water, stored as Y12 = linker-GFP; 7,3 ng/µL Y13 = linker-GFP; 8,7 ng/µL Y14 = linker-GFP; 11,2 ng/µL	iGEM2015_SOP0017_v01_Fast digest

15-05-06	AC	Ligation GFP with T18 and T25. See two hybrid protocol for further information	iGEM2015_SOP015_v02_ligation

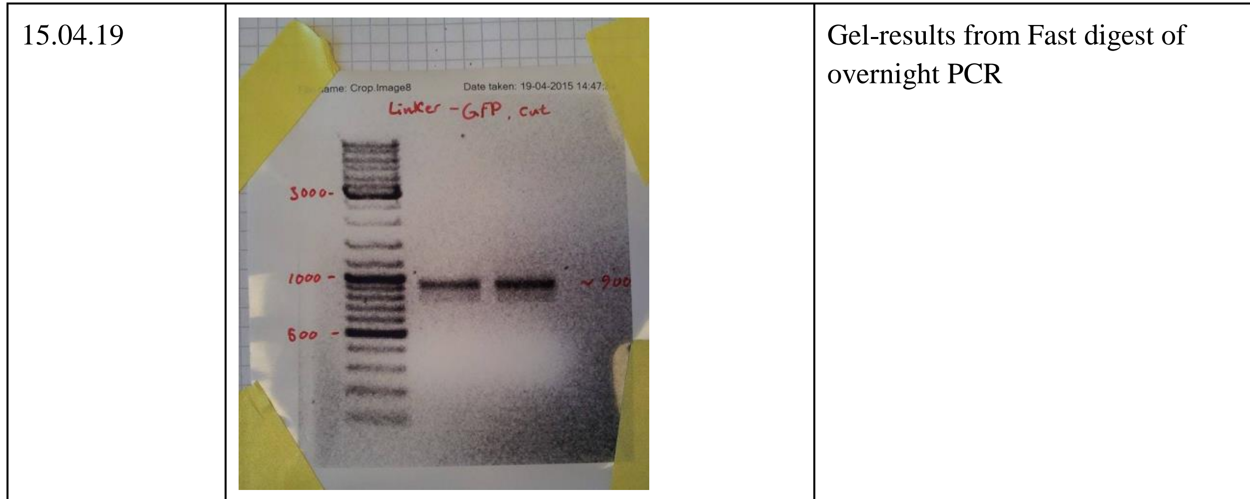
6. Results

Date **(YY.MM.DD)**	**Picture**	**Comments**



2015.04.02		**First PCR attempt**. All we see are primerdimers
		Second PCR attempt. We see a lot of primerdimer and very weak 3 bonds about 1000bp long in the 3 last bonds with GC buffer. We purify the sample from the gel, and gets a concentration at 3,1ng/µl. We runs a PCR at the sample overnight, in aim to, increase the concentration, but this time with half as much primer, and only with GC buffer.
2015.04.03		**Third PCR attempt**. Successful attempt, we see 5 bonds about 1000bp long. We purified the sample, and got the concentration 24,8ng/µl.
		Digest DNA-pieces: (As wanted) is the GFP linker about 1000 bp long. We purified the sample and got the concentration 9,2ng/µl**.** The digest of the plasmid is successful. The bond about 1000bp is “waste”, The bond about 3000 is our successful digested plasmid backbone.

2015.04.05		**Transformation:** No colony was seen
2015.04.06		**Transformation (from yesterday):** Red and white colonies at all agar plates. The white colonies at the 0-sample are religations.
		Colony PCR. The first bond didn't work (colony 1). The second bond (colony 2) was under 500bp, which shows it is a religation. The bonds 3-8 shows about 1200bp, which fit the length of the plasmid backbone and GFP-linker. TJECK the length of GFP linker!
15.04.11		Gel-results from 2nd Fast Digest, Succesful

15.04.19		Gel-results from Fast digest of overnight PCR

7. Appendices