iGEM 2015 – Microbiology – BMB – SDU

Project type: Interlab study. **Project title:** iGEM interlab study of GFP. **Sub project:** Measurement of GFP	**Creation date:** 23/6 2015 **Written by:** CEM, AC **Performed by:** AC

1. SOPs in use:

iGEM 2014 SOP0009 - TSB Transformation.

iGEM 2013 SOP0021 - Colony PCR with MyTag

iGEM 2013 SOP0005 - Freeze Stock

iGEM 2014 SOP0019 - Miniprep

iGEM 2014 SOP0017 - Fast Digest

iGEM 2014 SOP0015 - Ligation

2. Purpose:

Measurement of GFP under influence of different promoters.

3. Overview

Date (DD-MM-YY)	Person(s) (initials)	Experiments	SOPs
23-06-15	**AC**	**Transformation**	iGEM 2014 SOP0009
24-06-15	**AC**	**Colony PCR**	iGEM 2013 SOP0021
25-06-15	**AC**	**Freeze stock**	iGEM 2013 SOP0005
25-06-15	**AC**	**Miniprep**	iGEM 2014 SOP0019
01-07-15	**AC**	**Fast digest**	iGEM 2014 SOP0017
01-07-15	**AC**	**Ligation**	iGEM 2014 SOP0015
02-07-15	**AC**	**Transformation**	iGEM 2014 SOP0009
03-07-15	**AC**	**Transformation**	iGEM 2014 SOP0009
04-07-15	**AC**	**Freezestock**	iGEM 2013 SOP0005

04-08-15	**AC**	**Colony PCR**	iGEM 2013 SOP0021
Start from scratch			
05-08-15	**AC**	**Transformation**	iGEM 2014 SOP0009
06-08-15	**AC**	**Miniprep**	iGEM 2014 SOP0019
06-08-15	**AC**	**Fast Digest**	iGEM 2014 SOP0017
06-08-15	**AC**	**Ligation**	iGEM 2014 SOP0015
07-08-15	**AC**	**Transformation**	iGEM 2014 SOP0009
09-08-15	**AC**	**Colony PCR**	iGEM 2013 SOP0021
10-08-15	**AC**	**Freeze stock**	iGEM 2013 SOP0005
10-08-15	**AC**	**Miniprep**	iGEM 2014 SOP0019

4. Materials required.

Materials in use

Name	**Components (Concentrations)**	**Manufacturer / Cat. #**	**Room**	**Safety considerations**
EcoRI		**Roche: Fast Digest**		
XbaI		**Roche: Fast Digest**		
SpeI		**Roche: Fast Digest**		
Pst		**Roche: Fast Digest**		
FACS				
PBS				
LB medium				
LB agar medium				

PCR machine				
Equipment for gel-electrophoresis				

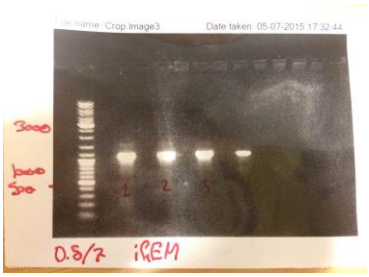
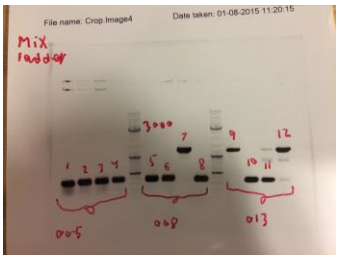
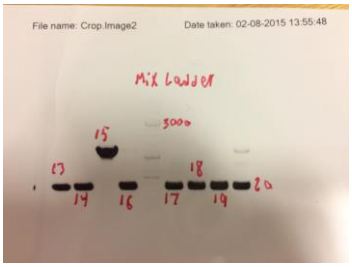
5. Experiment history

Date (DD-MM-YY)	Person(s) (initials)	Alterations to SOPs and remarks to experiments	SOPs
23-06-15	**AC**	Plasmids from plate 1 well 20K, 22K and 22A transformed into MG1655. Plasmid from plate 4 well 21J	iGEM 2014 SOP0009
24-06-15	**AC**	Colony PCR, of 2 cultures per plate	iGEM 2013 SOP0021
25-06-15	**AC**	Freeze stock	iGEM 2013 SOP0005
25-06-15	**AC**	Miniprep	
01-07-15	**AC**	Fast digest, GFP cut with EcoI and Pst. Rest cut with SpeI and Pst.	iGEM 2014 SOP0017
01-07-15	**AC**	Ligation with 0 fmol insert, and 50 fmol insert.	iGEM 2014 SOP0015
02-07-15	**AC**	Transformation into Top10	iGEM 2014 SOP0009
04-07-15	**AC**	Colony PCR (No gel)	iGEM 2013 SOP0021
05-07-15	**CEM**	Gel on the colony PCR from 04-07-15	
06-07-15	**AC**	Miniprep	iGEM 2014 SOP0019
06-07-15	**AC**	Transformation into MG1655	iGEM 2014 SOP0009
08-07-15	**AC**	Transformation into MG1655	iGEM 2014 SOP0009

09-07-15	**AC**	Transformation into MG1655	iGEM 2014 SOP0009
29-07-15	**AC**	Transformation into MG1655	iGEM 2014 SOP0009
30-07-15	**AC**	Miniprep of GFP and plasmids containing different promoters	iGEM 2014 SOP0019
30-07-15	**AC**	Fast Digest of GFP and promoters	iGEM 2014 SOP0017
30-07-15	**AC**	Ligation of plasmids with GFP as insert	iGEM 2014 SOP0015
31-07-15	**AC**	Transformation into Top10	iGEM 2014 SOP0009
01-08-15	**AC**	Colony PCR	iGEM 2013 SOP0021
02-08-15	**AC**	Colony PCR	iGEM 2013 SOP0021
06-08-15	**AC**	Miniprep	iGEM 2014 SOP0019
13-08-15	**AC**	FACS done according to iGEM protocol and worksheet	
19-08-15	**AC**	FACS done according to iGEM protocol and worksheet	
25-08-15	**AC**	FACS done according to iGEM protocol and worksheet	

6. Results

Date (DD-MM-YY)	**Picture**	**Comments**
03-07-15		Transformation inconclusive at this point.

05-07-15		The Gele showed that the plasmid was around 1200 bp long.
07-07-15		Transformation unsuccessful (Wrong plates)
09-07-15		Transformation unsuccessful (Wrong plates)
01-08-215		Colony PCR confirmed Ligation for 2 out of three plasmids
02-08		Colony PCR confirms last plasmid
06-08-2015		Miniprep made from each promoter
		Sequencing
25-08-2015		FACS Measurement
26-08-2015		Two constructs confirmed through sequencing. Construct 1 (J23101+I13504) showed to have a faulty sequence, but did show fluorescence when analyzed through FACS

7. Appendices