iGEM2013 – Microbiology – BMB – SDU	
Project type: Characterization **Project title:** Characterization of device LacI-PLac-rbs-dxs(B.sub)-Linker-GFP **Sub project:**	**Creation date:** 26.08.13 **Written by:** SIS **Performed by:** SIS, MHK

1. SOPs in use

2. Purpose

Characterization of the device LacI-PLac-rbs-dxs(B.sub)-Linker-GFP.

3. Overview

Day	SOPs	Persons	Experiments
1		SIS, MHK	Fluorescence microscopy FACS
2		SIS, PRA	Preparation for FACS
3		SIS, MHK, MH, PRA	FACS
4		PRA	FACS
5		PRA	FACS
6		MH	FACS

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Fluorescence microscope			Class 2 lab	
FACS machine				

5. Other comments

6. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
26.08.13	Fluorescence microscopy FACS	Fluorescence microscopy was performed with GFP filter on a sample from a ONC with IPTG and without IptG.
13.08.28	Preparation for FACS	5 mL LB media was incubated with bacteria and IPTG as indicated in “FACS experiment setup 28.08.13” ON
13.08.29	FACS Preparation for FACS	OD_{600} of ONC was measured. 1 mL ONC was spun with 3500 rpm for 5 min and resuspended in 1 mL 0.9 % NaCl, which was transferred to 2-3 mL 0.9 % NaCl. Half was poured out and further 4 mL was added to get a concentration fitting for the FACS. Only 1-10 was measured. 5 mL LB media was incubated with bacteria (3-4 and 11-18) and IPTG as indicated in “FACS experiment setup 28.08.13” ON

13.08.30	FACS	0.5 mL ONC was spun with 3500 rpm for 5 min and resuspended in 1 mL 0.9 % NaCl, which was transfered to 4 mL 0.9 % NaCl.
13.09.02	Preparation for FACS	5 mL LB media was incubated with bacteria (1-10) and IPTG as indicated in "FACS experiment setup 03.09.13" ON
13.09.03	Preparation for FACS FACS 1-10 FACS 11-18	5 mL LB media was incubated with bacteria (11-18) as indicated in "FACS experiment setup 03.09.13". Cultures were incubated for 4 hours OD_{600} of ONC was measured. 0.5 mL ONC was spun with 3500 rpm for 5 min and resuspended in 1 mL 0.9 % NaCl, which was transferred to 4 mL 0.9 % NaCl. OD_{600} of cultures was measured. 0.5 mL sample was taken before induction with 1 mM IPTG and after 30 min of induction. The samples was spun with 3500 rpm for 5 min and resuspended in 1 mL 0.9 % NaCl.
17.09.13	Preparation for FACS	50 ml LB media was incubated with MG1655 with the following plasmids: pSB1C3-LacI(n)-Plac-Dxs(sub)-GFP pSB1C3-LacI(LVA)-Plac-Dxs(sub)-GFP pSB1C3-Plac-Dxs(sub)-GFP pSB1C3-LacI(LVA)-Plac-Dxs(sub) Triplicates of each culture were made, except pSB1C3-LacI(LVA)-Plac-Dxs(sub). OD600 was measured and samples were induced once they reached exponential phase. Plasmids were either induced with IPTG or not, giving 6 (triplicates*+/-IPTG) samples of each plasmid. Two of the three triplicates of pSB1C3-LacI(n)-Plac-Dxs(sub)-GFP were wrongly induced and a technical triplicate was made from the third. Samples were measured 30 minutes after induction, then 60, then 2 hours and 3 hours (only pSB1C3-LacI(LVA)-Plac-Dxs(sub) and pSB1C3-LacI(n)-Plac-Dxs(sub)-GFP). OD was measured subsequently. Lastly, FACS was performed the next morning.

7. Sample specification

Sample name	Sample content	From	Used for / Saved where

8. Remarks on setup

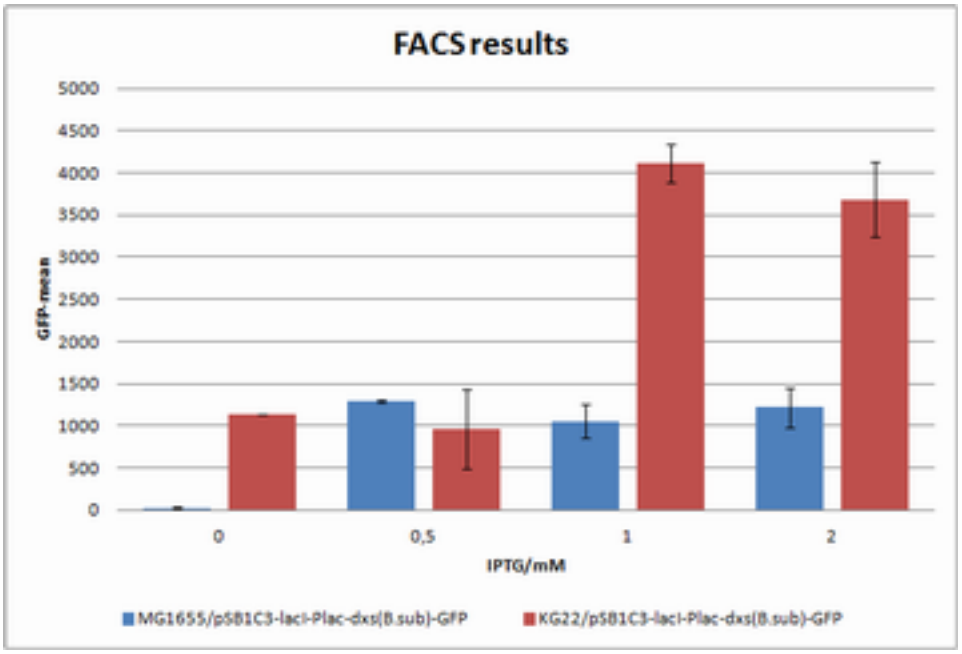
9. Results and conclusions

26.08.13

Fluorescence microscopy results:
The cells grown with IPTG fluoresced more than cells not grown with IPTG in the media, but the cells grown without IPTG did still fluoresced. We do unfortunately not have a picture of the microscopy results, since the picture function of the microscope was out of function.

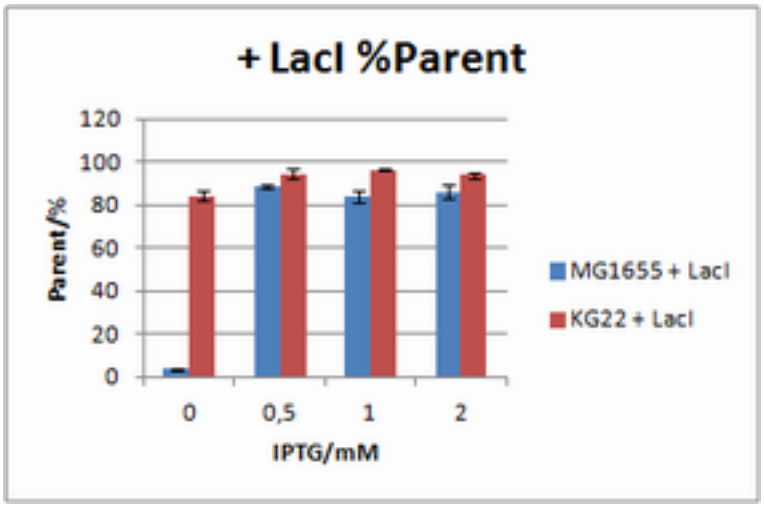
FACS results:
The FACS showed that the culture without IPTG showed fluorescence but more so for the one with IPTG. The WT did not show fluorescence. See document: Batch_Analysis_26082013_LacI.pdf in the same folder as this document.

13.08.28
The mean GFP level in the fluorescent cells illustrated with bars:



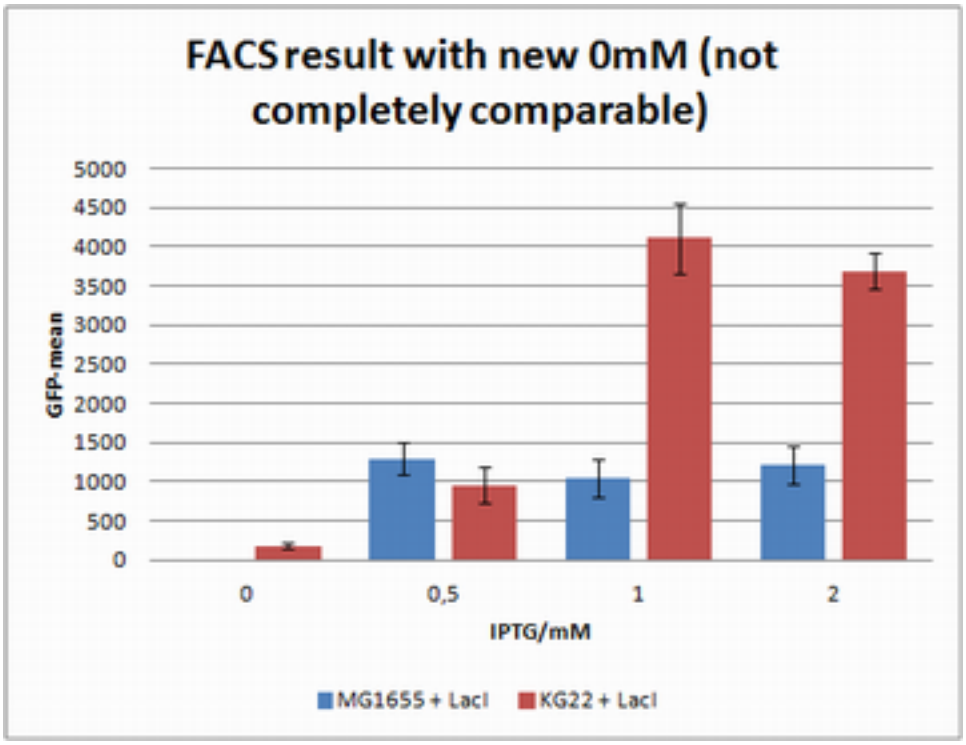
The KG22 strain with 0 mM IPTG had a strange GFP level and those samples was run again

The % of cells fluorescent:

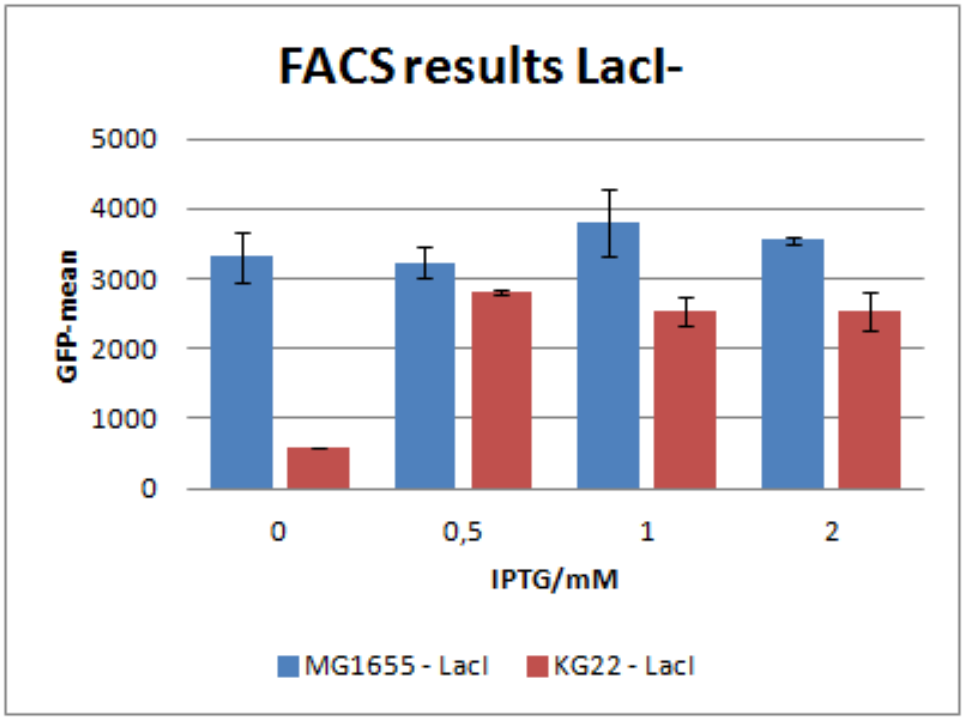


13.08.30

The mean GFP level in the fluorescent cells illustrated with bars (only new data for the 0 mM samples):

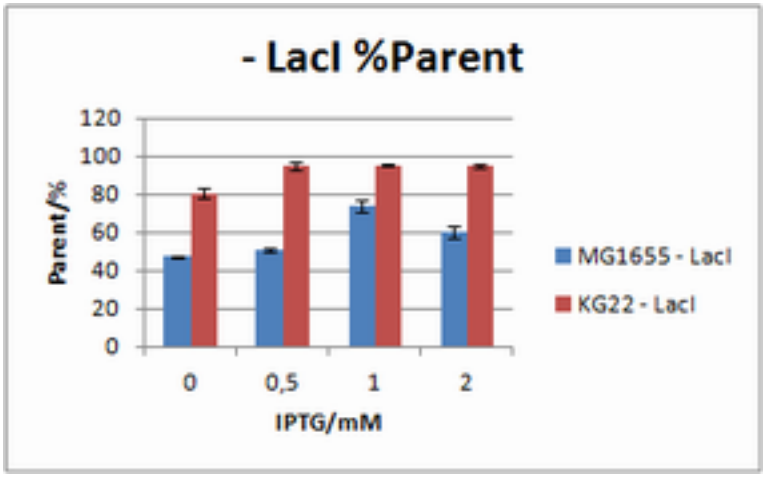


The mean GFP level in the fluorescent cells illustrated with bars:



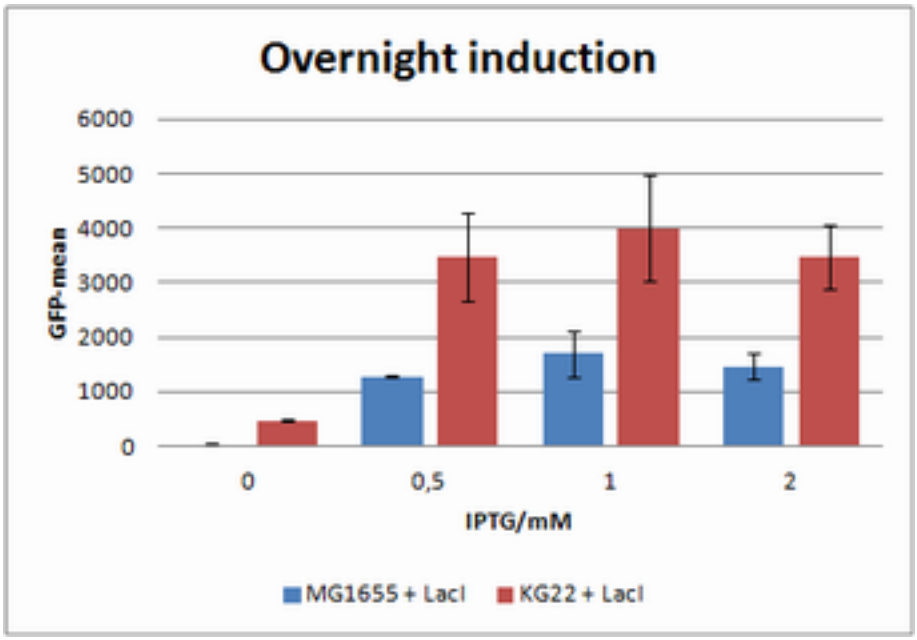
All MG1655 samples had high mean GFP activity and the KG22 strain which overexpresses LacI from the chromosome had increasing mean GFP activity as expected.

The % of cells fluorescent:



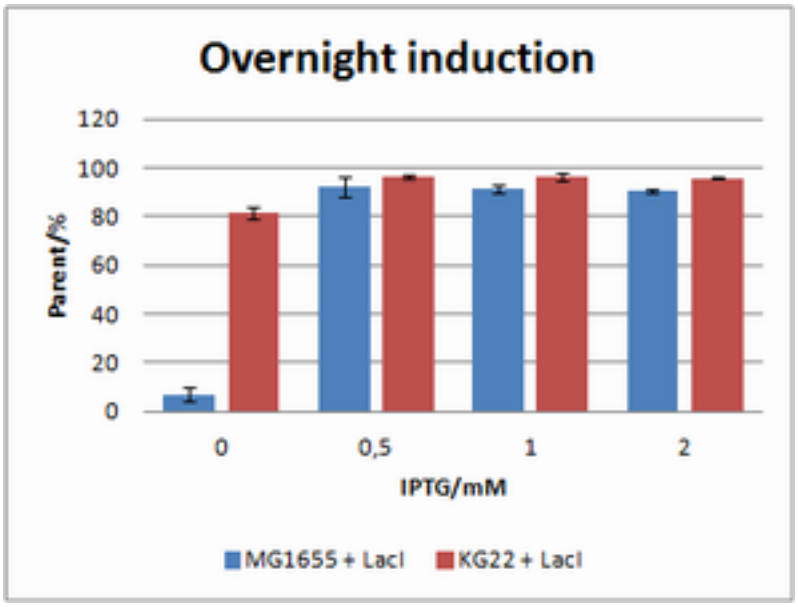
13.09.03

The mean GFP level in the fluorescent cells illustrated with bars (of strains carrying lacI on plasmid grown ON):



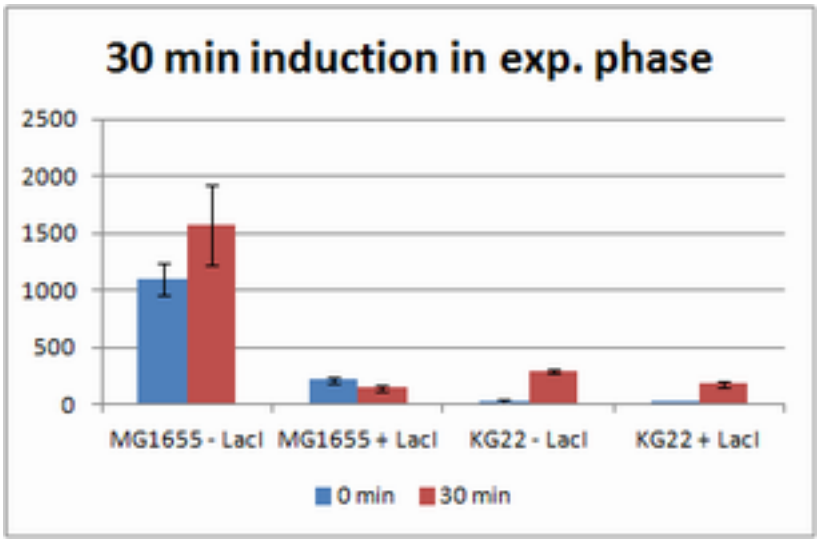
One outlier has been removed in MG1655 + LacI 0.5 mM (samples were measured as: 1445, 1428, and 4712)
It seems that the KG22 strain obtains higher mean levels of GFP than the MG1655 strain when grown ON.

The % of cells fluorescent illustrated with bars (of strains carrying lacI on plasmid grown ON):



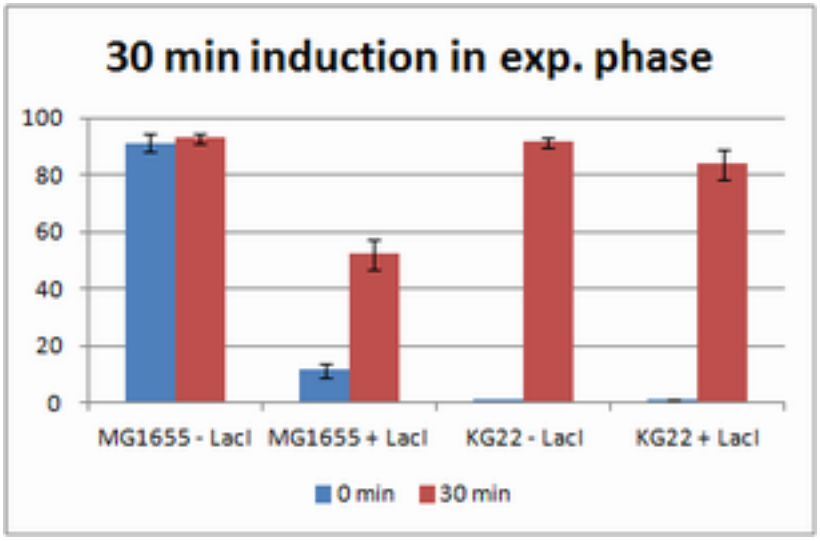
These results reflects the mean GFP levels in the cell

The mean GFP level in the fluorescent cells illustrated with bars (induced with IPTG for 30 min):



The MG1655 strain responds poorly to the IPTG induction, whereas the KG22 strain responds well (when measuring mean GFP in fluorescent cells). The KG22 - LacI has a 1.5 times higher mean GFP activity when induced, but a 5 times higher mean GFP activity when not induced (standard deviation not included in calculation. If included, there might not be a difference between not induced).

The % of cells fluorescent illustrated with bars (induced with IPTG for 30 min):



MG1655 - LacI does not respond to IPTG and almost all cells fluoresce. Half of the MG1655 + LacI cells begin to fluoresce when induced with IPTG, whereas only one tenths of the cells fluoresce when not induced. Equally many of the two KG22 strain's cells fluoresce, and both strains responds well to the IPTG induction.

19.09.13

INSERT RESULTS

10. Appendices