INTER-LAB STUDY WORKSHEET

SECTION 1: PROVENANCE & RELEASE

1.1 Who did the actual work to acquire the measurements?

Chandhuru, Rick, Otto

1.2 What other people should be credited for the measurements?

The iGEM-team and our supervisors.

1.3 On what dates were the protocols run and the measurements taken?

Starting at 19th of September until 28th October.

28th : Running flow cytometer and obtaining data.

27th: Running flow cytometer and obtaining data. Checking on gel[1]

25th: Colony PCR[2]. Inoculation of device 2 (BBa_J23101 and BBa0240) and device 1 (BBa_I20260).

24th: Ligation of BBa_J23101 and BBa0240 worked. Transformation in E. coli.

23rd : Ligation of BBa_J23101 and BBa0240, and BBa_J23115 and BBa_E0240.

22nd: Miniprep & restriction of all 4 Biobricks.

21st: Checking transformation (by running on gel) and inoculation of 5 mL of all 4 Biobricks.

20th: Transformation of competent E. coli with all 4 Biobricks.

Before: tried to transform, but cells were not competent.

1.4 Do persons involved consent to the inclusion of this data in publications derived from the iGEM-interlab study?

Yes

SECTION 2: PROTOCOL

2.1 What protocol did we use to prepare samples for measurement?

- Spun down the cells at 4000 rpm

- Washed the cells twice with PBS

- Resuspended the cells with PBS

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[1] Appendix 1
[2] Appendix 2

- Did the flow cytometer measurement

2.2. What sort of instrument did we use to acquire the measurements?

Model: BD FACSCANTO 2

Manufacturer: BD Biosciences

Blue laser power: 20.40 W

Excitation wavelength: 488 nm

Light filter: FL-1 (530/30)-700

SSC: 400 Volt

FSC: 200 Volt

Parameters:

FSC : 200

SSC: 200

2.3 What protocol did we use to take measurements?

Checking instrument settings and measuring the fluorescence for 10000 events.

2.4 What method is used to determine whether to include or exclude each sample from the data set?

Inoculated few transformants and checked them under the microscope for fluorescence. The colonies that produced green fluorescence were grown overnight and flow cytometer measurements were obtained.

2.5 What exactly were the controls we used?

Non-fluorescent colonies were used as control.

2.6 What quantities were measured? (e.g. red fluorescence, green fluorescence, optical density)

Green fluorescence.

2.7 How much time did it take to acquire each set of measurements?

10 seconds for each sample.

2.8 How much time did it cost to acquire a set of measurements?

Around 1 minute.

2.9 What are the practical limits on the number or rate of measurements taken with this instrument and protocol?

Amount of cells.

SECTION 3: MEASURED QUANTITIES

3.1 What are the units of measurement?

Arbitrary unit.

3.2 What is the equivalent unit expressed as a combination of the seven SI base units?

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3.3 What is the range of possible measured values for this quantity, using your instrument as configured for these measurements? (e.g., a meter stick measures in the range of 0 to 1 meter)

Minimum: 1

Maximum: 10^4

3.4 What are the significant figures for these measurement?

5 significant figures after the comma.

3.5 Is the precision the same across the entire range?

Yes

3.6 When was the instrument last calibrated?

26.09.2014

3.8 How was the instrument calibrated?

Open a silver sachet from the blue/white box named BD FACStm 7-Color Setup Beads, which is in the fridge. Open the tube and add about 1 ml of BD FACStm Setup Bead Diluent B. Vortex the tube for at least 10 seconds and place the tube onto the SIT. Start the program BD FACSCanto Software, by clicking the icon on the left of the desktop, press enter when the software is asking for a password (there's no password required).Click on Cytometer>Setup>Standard Setup… and click on Next button. Click on Next button when "Run setup in Manual mode" is checked. Load the tube with the setup beads onto the SIT and click on OK. Remove the tube when calibration is finished. The setup report is printed automatically. Click on Finish. When you close the program a popup appears with two options i) Run fluidics, shutdown and exit ii) Exit only. Select first option and click on OK if you don't want to run samples on the FACS. Select the second option and click on OK if you do want to run samples on the FACS. (http://2011.igem.org/wiki/images/0/0b/Flowcytometryprotocol.pdf)

SECTION 4: MEASUREMENTS

Please view excel-file.

OTHER:

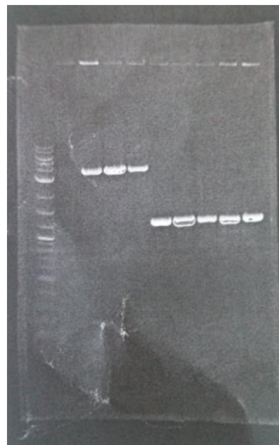
We chose the path of using J23115 as distributed.

APPENDIX 1: DEVICE 1 AND 2

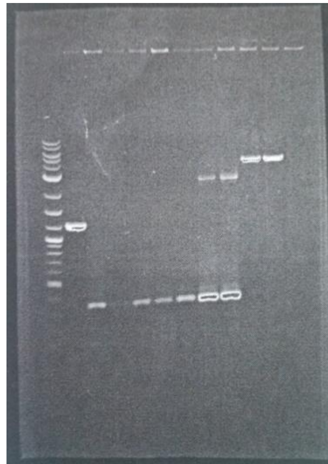
First 6 wells show the DNA isolated from I20260 after double digestion with Pst1 and EcoR1. Last three wells show the DNA isolated from ligated J23101 with E0240.

APPENDIX 2: COLONY PCR (ALL 4 BIOBRICKS)

Last 5 wells are the colony PCR product for BBa_E0240.



From the second well to the 7th well are the colony PCR product for BBa_J23101.



From the 1st to the 5th well are the colony PCR product for BBa_I20260. From the 7th to the 11th well are the colony PCR product for BBa_J23115.