Interlab Study Worksheet

WPI-Worcester

Section I: Provenance & Release

1. Who did the actual work to acquire these measurements?
   Alex Turland and Chloe LaJeunesse

2. What other people should be credited for these measurements?
   Natalie Farny

3. On what dates were the protocols run and the measurements taken? (this will often be a range of dates; make sure you say which data was taken at what times.)

6/23	Initial transformation of all samples
6/25	Ligation of BBa_J23101 + BBa_E0240 and BBa_K823012 + BBa_E0240, BBa_I20260 plate reading
6/27-7/21	Test runs, troubleshooting, sequencing, re-ligations
7/22	Final plate readings (multiple samples) of all 3 sequence-confirmed constructs

4. Do all persons involved consent to the inclusion of this data in publications derived from the iGEM interlab study?
   Yes

Section II: Protocol

1. What protocol did you use to prepare samples for measurement?
   Plate rows from top to bottom: equal number of wells LB, undiluted culture, culture diluted 1:2 with LB, 4 wells culture diluted 1:10 with LB (A test was run to check if GFP from adjacent rows interfered with readings and it was concluded that it did not. Because of this, samples were run directly adjacent to each other on the plate.)

   § What is the model and manufacturer?
   Wallac 1420 Multilabel Counter manufactured by Perkin Elmer

   § How is it configured for your measurements? (e.g., light filters, illumination, amplification)
   Fluorescein (485nm/535nm, 1.0s), 590 nm OD

3. What protocol did you use to take measurements?
   Fluorescein (485nm/535nm, 1.0s), 590 nm OD

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

When using our sequence-confirmed clones, whenever we ran into a sample with values inconsistent with the promoter type (IE a low reading for the high promoter), then the experiment was completely redone (the previous dilutions were scrapped and completely new dilutions were made). Thankfully, whenever this occurred were able to start the experiment over and meet results that were consistent with what we were expecting, pinning the abnormalities as pipetting errors. All samples were included once everything was double-tested.

5. What exactly were the controls that you used?

LB

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

Green Fluorescence

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

32 seconds

8. How much does it cost to acquire a set of measurements?

$0 (plate reader and all supplies previously owned; cost: ~$11,000)

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

It can only take one 96-well plate at a time.

Section III: Measured Quantities

For each type of quantity measured (e.g., fluorescence, optical density), report on the:

1. Units:

§ What are the units of the measurement? (e.g., meters, molecules)

Fluorescence units (photon counts)

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

Fluorescence intensity (p/s/cm2)

2. Precision:

§ 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)

10,000,000

§ What are the significant figures for these measurement?

The machine measures to one whole ‘count’.

§ Is the precision the same across the entire range? If not, how does it differ?
Yes, it is the same through the entire range.

§ How did you determine these answers?
Based on the units of the data we gathered from the machine and looking at the program itself, we were able to determine the significant figures and the range.

3. Accuracy:

§ When was the instrument last calibrated?
May 2014

§ How was the instrument calibrated?
Calibration services for the Victor3 Plate reader are performed by Perkin Elmer. They have not disclosed calibration protocols but their contact info is as follows:
PerkinElmer
940 Winter Street
Waltham, Massachusetts 02451 USA
Telephone : +1 (781) 663-6900

Section IV: Measurements

See Attached File: "Interlab Full Table + Procedure Info.xlsx" for full table and calculations

	Replicate 1		
Identity	Directly Measured	Derived from Measurements	Standard Deviation
BBa_I20260 (Existing Device)	384948.83	699588.98	12600.25
BBa_J23101 + BBa_E0240 (High)	1771649.08	3845141.80	61351.39
BBa_K823012 + BBa_E0240 (Low)	79492.08	163648.14	4080.78

	Replicate 2		
Identity	Directly Measured	Derived from Measurements	Standard Deviation
BBa_I20260 (Existing Device)	359529.33	669203.04	8419.25

BBa_J23101 + BBa_E0240 (High)	1684326.58	3667559.25	110390.34
BBa_K823012 + BBa_E0240 (Low)	74085.33	156215.78	1838.37

	Replicate 3		
Identity	Directly Measured	Derived from Measurements	Standard Deviation
BBa_I20260 (Existing Device)	380186.83	671115.33	16171.68
BBa_J23101 + BBa_E0240 (High)	1653443.58	3637939.68	56038.92
BBa_K823012 + BBa_E0240 (Low)	77894.83	156887.88	5376.45

Additional Information:

Strain: *E.coli* NEB 5-a

Density of cultures are noted in the Full Table under the column titled “OD 590”

Cultures were grown overnight (24 hours) in a 37° shaker.