iGEM 2014 Measurement Interlab Study Worksheet

To participate in this study, please complete the following worksheet and submit to measurement [AT] igem [DOT] org.

Inter-Lab Study Worksheet:

For each assay that you perform, fill out the following worksheet. Answer each question with enough detail to allow another person to replicate your measurements without needing to ask you any questions. This does not necessarily mean you need to describe everything in detail---for example, if you use a standard assay, you just need to give enough information to allow another person to use that assay in the same way that you did.

Section I: Provenance & Release

1. Who did the actual work to acquire these measurements?

The work we report here (performed with biobrick BBa_I20260) has been done by two members of the team (Kristie Tanner and Cristina Vilanova). However, our project includes fluorescence measurements of similar devices (briefly, our constructions involve either a GFP or an RFP under the control of a promoter from Anderson's collection). These measurements were performed by all the members of the team: Kristie Tanner, Pedro Dorado, Paula Villaescusa, Divya Chugani, Alba Frias and Cristina Vilanova. All these results will be published in our wiki page soon ;)

2. What other people should be credited for these measurements? (i.e., who would be an author on any resulting publication. For example, your faculty advisor may have helped design the protocols that you ran.)

Manuel Porcar supervised all the experimental work and helped us with the design of the protocols. He also provided us access to the fluorimeter. Our work would not have been possible without the support of our modelling and Human Practices team (Ernesto Segredo, Marco Fritschi, Lucas Morales and Juan Faus).

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.)

The work reported here was done during the first week of September. The protocols were designed the last week of August. In the case of the other experiments, they have been developed during all the summer.

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

We consent the inclusion of the data regarding biobrick BBa_I20260. We would like to maintain the ownership of the experiments performed with the other biobricks.

Section II: Protocol

1. What protocol did you use to prepare samples for measurement?

A DH5a E. coli strain carrying the construction was striked on LB medium supplemented with kanamycin. A liquid culture was set up in the same and grew in agitation (250 rpm) at 37C until OD600 reached a value around 0,2. Five biological replicates were performed in all the experiments (i.e.: 5 independent cultures of the same E. coli strain were set up).

2. What sort of instrument did you use to acquire measurements?
    - What is the model and manufacturer?
    - How is it configured for your measurements? (e.g., light filters, illumination, amplification)

We used a FP6200 spectrofluorimeter (Jasco, Easton, MD). An excitation wavelength of 501 nm was used (excitation bandwith=5 nm) and fluorescence intensity was recorded at 511 nm (emission bandwith=5 nm). The fluorimeter was configured to operate with high sensitivity in the measurements.

3. What protocol did you use to take measurements?

2 mL aliquots of the culture were transferred to standard plastic fluorimetry cuvettes (Versafluor; Biorad, Irvine, CA). OD was measured with a standard spectrophotometer at 600 nm prior to fluorescence measurement. All fluorescence intensity values were then normalized by OD600. An aliquot of LB medium was used to set the blank in both the spectrophotometer and the fluorimeter.

After measuring, data was processed with Excel. The average and the standard deviation was calculated from the five biological replicates in all cases.

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

We observed very low variability among the biological replicates we prepared, so no samples were excluded from our data.

5. What exactly were the controls that you used?

We used a non-transformed DH5a strain as a control for our measurements.

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

We measured both OD at 600 nm and green fluorescence (excitation wavelength=501; emission wavelength=511).

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

It took less than 20 min since we obtained the aliquots from the culture.

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

The cost is negligible. The price of measuring a bigger set of samples might be limited by the number of fluorimetry cuvettes used.

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

We think that the only limitation is the time between OD measurements and fluorescence measurements. The longer this time is, the less accurate is fluorescence normalization by cell density. However, this was not a problem in our experiment since a low number of samples were processed at a time.

Section III: Measured Quantities

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

2. Units:
    - What are the units of the measurement? (e.g., meters, molecules)
    - What is the equivalent unit expressed as a combination of the seven SI base units? (http://en.wikipedia.org/wiki/SI_base_unit)

Both OD and fluorescence intensity are measured in arbitrary units.

3. 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)
    - What are the significant figures for these measurement? (e.g., on a meter stick, it is common to measure to the nearest millimeter).
    - Is the precision the same across the entire range? If not, how does it differ?
    - How did you determine these answers?

The spectrophotometer used for OD measurements has an accuracy of 0,001. OD values greater than 1 are not accurate.

Regarding the fluorimeter, both the accuracy and the range of measurable values depend on the laser used for excitation. In our conditions, the significant figure was 0,01 arbitrary units of fluorescence.

4. Accuracy:
    - When was the instrument last calibrated?
    - How was the instrument calibrated?

We ignore this information. As stated before, we used sterile LB medium to set the blank, so all our measurements are performed with the same reference.

Section IV: Measurements

1. For each sample, report:
    - the identity of the sample
    - each quantity directly measured
    - each quantity derived from measurements (e.g., fluorescence/OD)

2. For each group of replicates, report:
    - the identity of samples in the set
    - which, if any, of the samples are excluded and why
    - the mean and standard deviation for each quantity measured or derived

The host strain in the experiments reported here was DH5a. We transformed it with biobrick BBa_I20260 and transformation was checked by studying the plasmids obtained by miniprep. We checked plasmid size with a 0.8% agarose gel. As mentioned above, we measured optical density at 600 nm and fluorescence (exc=501 nm; emis=511 nm). We had five biological replicates of each sample (DH5a WT strain and DH5a carrying biobrick BBa_I20260 in pSB3K3 vector) and no sample was excluded. The results are shown in the excel file attached, including the standard deviation for each quantity measured.