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Working

Standard iGEM Cell Measurement Protocol

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[1]iGEM Measurement Committee

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ABSTRACT

This is a protocol from the 2018 iGEM InterLab study which tests various 'standard' parts from the Registry.

EXTERNAL LINK

https://2019.igem.org/Measurement

GUIDELINES

Help with debugging:

- If you have measurements that are off scale ("OVERFLOW"), that data will not be usable. You need to adjust your settings so that the data will be in range and re-run your calibration.
- If your Abs600 measurements for your cell colonies are very close to that of your LB with Chloramphenicol, then your cells have probably not been transformed correctly or grown correctly.
- If your negative and positive control values are very close to each other, that probably means something has gone wrong in your protocol or measurement.

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
1.5 mL Eppendorf tubes				
96 well plate				
Falcon Tube (50 mL)		Fischer Scientific		
Liquid LB medium				
Competent Cells				
Ice & ice bucket				
Chloramphenicol (25 mg/ml in EtOH)	View			

STEPS MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Liquid LB medium				
Chloramphenicol (25 mg/ml in EtOH)	View			

MATERIALS TEXT

Devices (from Distribution Kit, all in pSB1C3 backbone)

Device	Part Number	Plate	Location
Negative control	BBa_R0040	Kit Plate 7	Well 2D
Positive control	BBa_I20270	Kit Plate 7	Well 2B

Test Device 1	BBa_J364000	Kit Plate 7	Well 2F
Test Device 2	BBa_J364001	Kit Plate 7	Well 2H
Test Device 3	BBa_J364002	Kit Plate 7	Well 2J
Test Device 4	BBa_J364007	Kit Plate 7	Well 2L
Test Device 5	BBa_J364008	Kit Plate 7	Well 2N
Test Device 6	BBa_J364009	Kit Plate 7	Well 2P

The 96-well plate should preferably be black with a clear flat bottom.

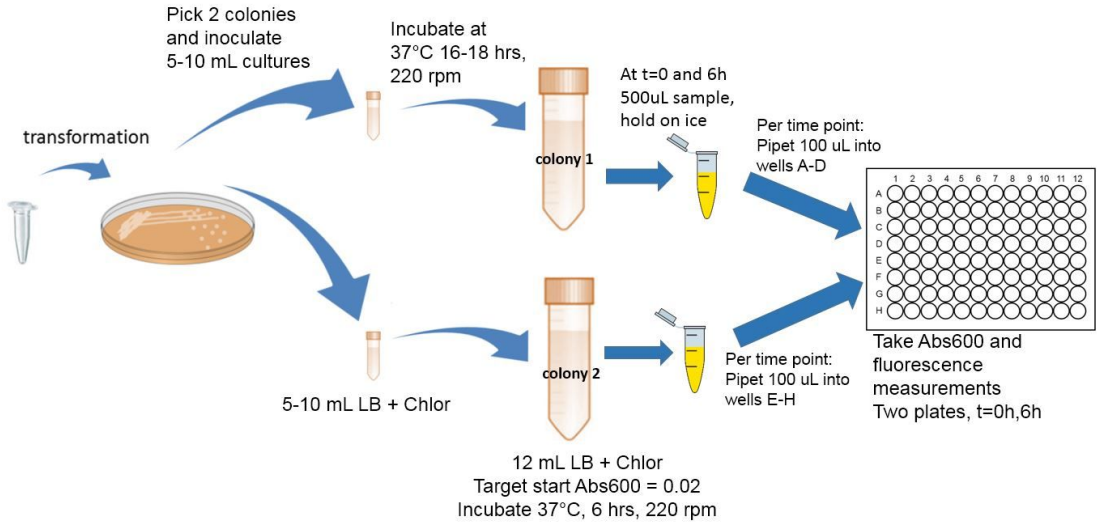
BEFORE STARTING

Prior to performing this protocol you should complete the following calibration protocols:

- OD_{600} Inter-equipment Conversion with LUDOX
- Particle Standard Curve with Microspheres
- Fluorescence Standard Curve with Fluorescein

Completion of the calibrations will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions.
For all of these cell measurements, you must use the same plates and volumes that you used in your previous calibration protocols. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you used in your calibration measurements. If you do not use the same plates, volumes, and settings, the measurements will not be valid.

Read through this entire protocol carefully before you start your experiment and
prepare any materials you may need. See the Guidelines tab on this page for debugging help.



Day 1

1 Transform Escherichia coli DH5α with these following plasmids (all in pSB1C3)

Device	Part Number	Plate	Location
Negative control	BBa_R0040	Kit Plate 7	Well 2D
Positive control	BBa_I20270	Kit Plate 7	Well 2B
Test Device 1	BBa_J364000	Kit Plate 7	Well 2F
Test Device 2	BBa_J364001	Kit Plate 7	Well 2H
Test Device 3	BBa_J364002	Kit Plate 7	Well 2J

Test Device 4	BBa_J364007	Kit Plate 7	Well 2L
Test Device 5	BBa_J364008	Kit Plate 7	Well 2N
Test Device 6	BBa_J364009	Kit Plate 7	Well 2P

Help Debugging Your Transformations:

- We STRONGLY recommend that you use the iGEM protocol to create your competent cells
- Once you have created your competent cells, we STRONGLY recommend that you measure the competency of your cells using the Competent Cell Test Kit
- Finally, we **strongly** recommend that you closely follow the iGEM protocols for resuspending DNA from the kit plates and performing the transformation
- Year after year, we have found that most teams are highly successful when they follow these protocols, even if alternative protocols are used within your lab. If you are having trouble transforming your test devices, please try the protocols above

Day 2

2 Pick 2 colonies from each of the transformation plates and inoculate in 5-10 mL LB medium + Chloramphenicol.

Grow the cells overnight (16-18 hours) at 37 °C and 220 rpm.

Liquid LB medium

Chloramphenicol (25 mg/ml in EtOH)
View

Day 3

3 Make a 1:10 dilution of each overnight culture in LB with Chloramphenicol (0.5 mL of culture into 4.5 mL of LB with Chloramphenicol)

4 Measure the Abs_{600} of these 1:10 diluted cultures

5 Record the data in your notebook

6 Dilute the cultures further to a target Abs_{600} of 0.02 in a final volume of 12 mL LB medium with Chloramphenicol in a 50 mL falcon tube

Falcon tube should be amber, or covered with foil to block light

7 Take 500 µL samples of the diluted cultures at 0 hours into 1.5 mL eppendorf tubes, prior to incubation. At each time point between 0 hours and 6 hours, you will take a sample from each of the 8 devices, two colonies per device, for a total of 16 eppendorf tubes with 500 µL

samples per time point, 32 samples total.

Place the samples on ice.

8 Incubate the remainder of the cultures at 37 °C and 220 rpm for 6 hours

9 Take 500 µL samples of the cultures at 6 hours of incubation into 1.5 mL eppendorftubes. Place samples on ice.

Abs$_{600}$ and Fluorescence Measurement

10 At the end of the experiment, you should have two plates to read. You will have one plate for each time point: 0 and 6 hours. On each plate you will read both fluorescence and absorbance.

Samples should be laid out according to the plate diagram below.

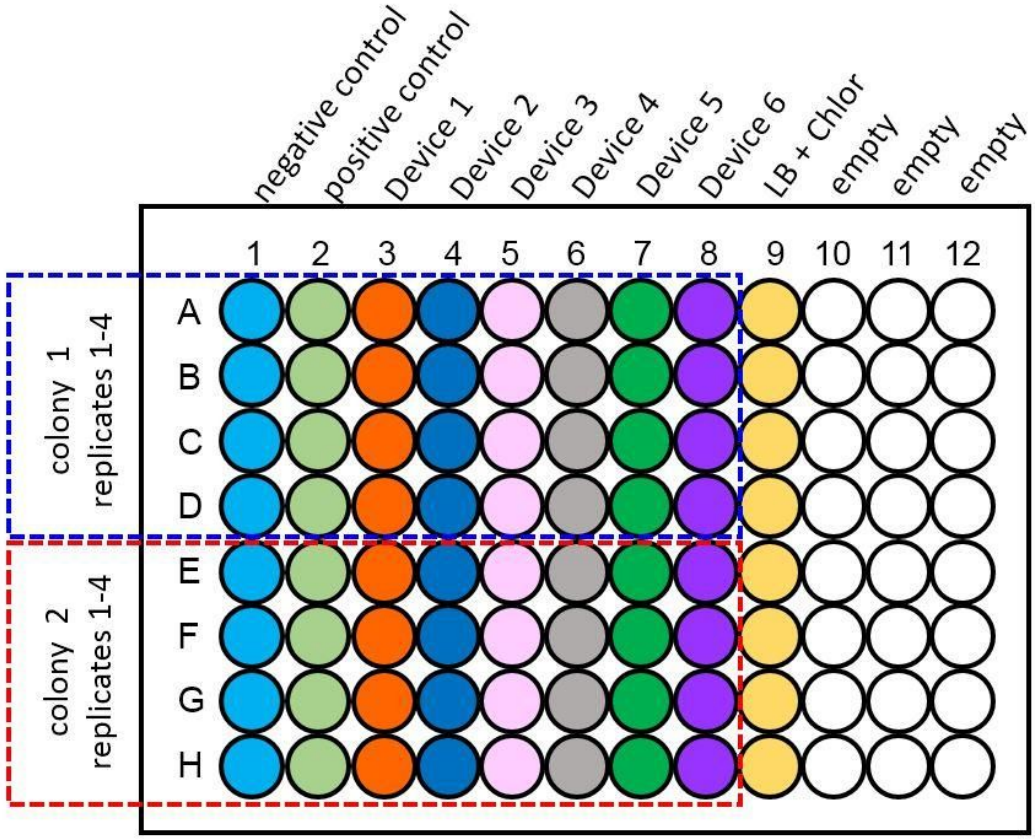


Plate layout for cell measurement calibration protocol

11 Pipette 100 µL of each sample into each well. From 500 µL samples in a 1.5 mL eppendorf tube:

- 4 replicate samples of colony #1 should be pipetted into wells in rows A, B, C and D
- Replicate samples of colony #2 should be pipetted into wells in rows E, F, G and H

12 Be sure to include 8 control wells containing 100 µL each of only LB with Chloramphenicol on each plate in column 9, as shown in the diagram.

Set the instrument settings as those that gave the best results in your calibration curves (no measurements off scale). If necessary you can test more than one of the previously calibrated settings to get the best data (no measurements off scale).

Instrument temperature should be set to room temperature (approximately 20-25 C) if your instrument has variable temperature settings

Congratulations!

13 You have now completed this calibration protocol