**2018 University of Iowa International Genetically Engineered Machine Team**
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Intro:

As the field of synthetic biology emerges as a viable solution to the world's problems, it will experience many of the growing pains other fields have faced. One such challenge is how to communicate the methods and results of ones experiments effectively. The iGEM Interlab Study has worked for the past 5 years to correct this issue.

Last summer, teams took part in the 4th iGEM Interlab Study. In which, teams across the planet contributed data in an effort to reduce variability in GFP measurements by normalizing for optical density. To build upon this, the 5th iGEM Interlab Study attempts to reduce error in fluorescence readings by normalizing measurements to colony forming units (CFU).

Methods:
All procedures conducted can be found in full in the 2018 Interlab Plate Reader Protocol (link document here).

# OD600 Reference Point - Ludox Protocol

1) Added 100 µl LUDOX into wells A1, B1, C1, D1
2) Added 100 µl of dH2O into wells A2, B2, C2, D2
3) Measured absorbance at 600 nm of all samples

| | LUDOX CL-X | H2O |
|---|---|---|
| Replicate 1 | 0.063 | 0.024 |
| Replicate 2 | 0.058 | 0.023 |
| Replicate 3 | 0.056 | 0.024 |
| Replicate 4 | 0.058 | 0.025 |
| Arith. Mean | 0.059 | 0.024 |
| Corrected Abs600 | 0.035 | |
| Reference OD600 | 0.063 | |
| OD600/Abs600 | 1.813 | |

Base on our results, the OD600/Abs600 conversion factor for our spectrophotometer is 1.813.

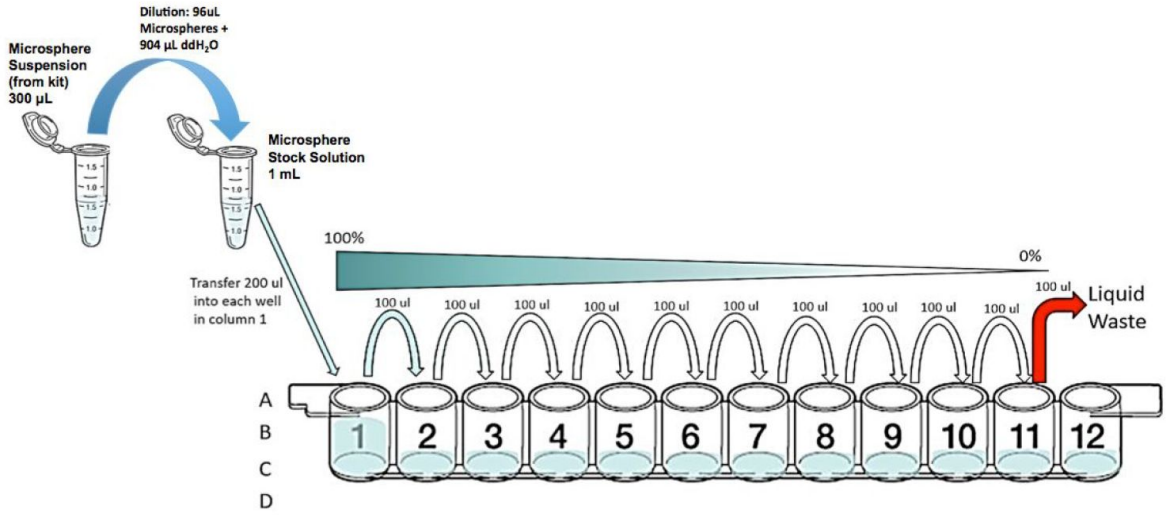
# Particle Standard Curve - Microsphere Protocol

1) Vortex the tube labeled "Silica Bead" (obtained from the iGEM Interlab Kit)
2) Immediately pipetted 96 µL microspheres into a 1.5 mL eppendorf tube
3) Add 904 µL of dH2O to the microspheres
4) Vortexed and set aside as the new silica bead stock

## Serial Dilution of Microspheres

1) Added 100 µL of dH2O into wells A2, B2, C2, D2....A12, B12, C12, D12
2) Vortexed the silica bead stock
3) Added 200 µL of the silica bead stock to A1

4) Transferred 100 µL from well A1 to well A2 and mix by pipetting
5) Repeated step 4 for wells A2 through A11
6) Discard 100 µL from A11
7) Repeated steps 2 through 6 for rows B through D



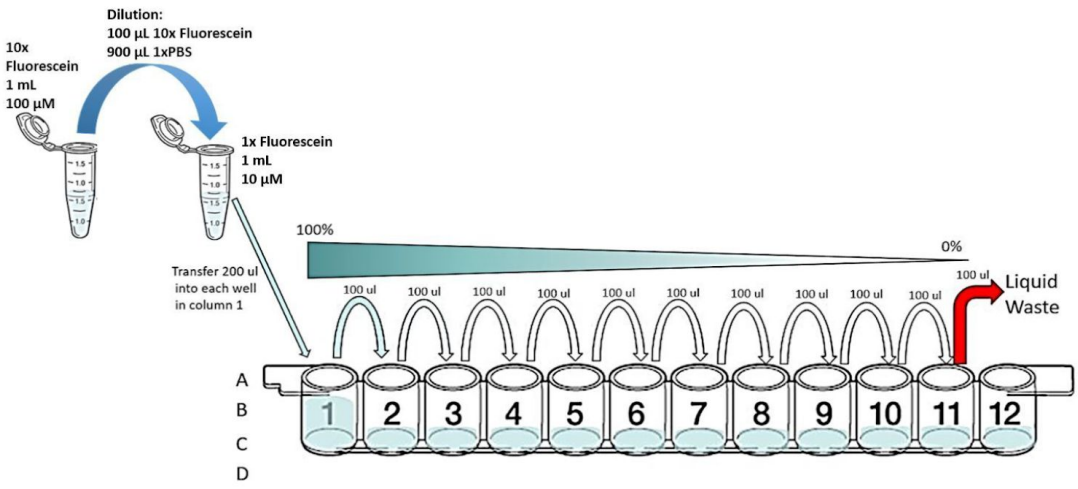

Note: Before being placed into the plate reader, all wells were mixed again by pipetting.

# Fluorescence Standard Curve - Fluorescein Protocol

1) Spun down the fluorescein kit tube (obtained from the iGEM Interlab Kit)
2) Prepared a 10x fluorescein stock solution by resuspending the fluorescein in 1 mL of 1x PBS
3) Created 1x fluorescein stock solution (10 µM) by diluting 100 µL of the 10x fluorescein stock with 900 µL of 1x PBS

## Serial Dilution of Fluorescein

1) Added 100 µL of 1x PBS into wells A2, B2, C2, D2....A12, B12, C12, D12
2) Added 200 µL of 1x fluorescein stock solution into wells A2, B2, C2, D2, E2, F2, G2, H2
3) Transferred 100 µL from well A1 to well A2 and mix by pipetting
4) Repeated step 4 for wells A2 through A11
5) Discard 100 µL from A11
6) Repeated steps 2 through 6 for rows B through D



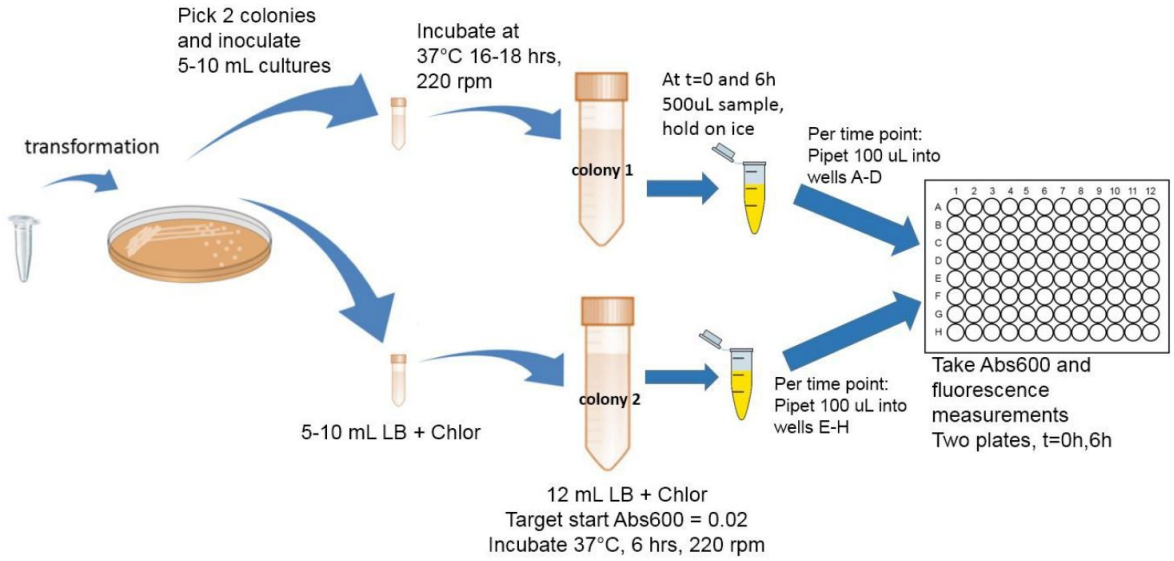

Note: The plate reader used was a Promega Glomax Multi Detection System and the filters used were all sterile and performed under aseptic conditions

# Cell Measurement Protocol

Day 1: The following devices were transformed (link to transformation protocol) into DH5 - alpha competent cells.

| 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 |

**Workflow**

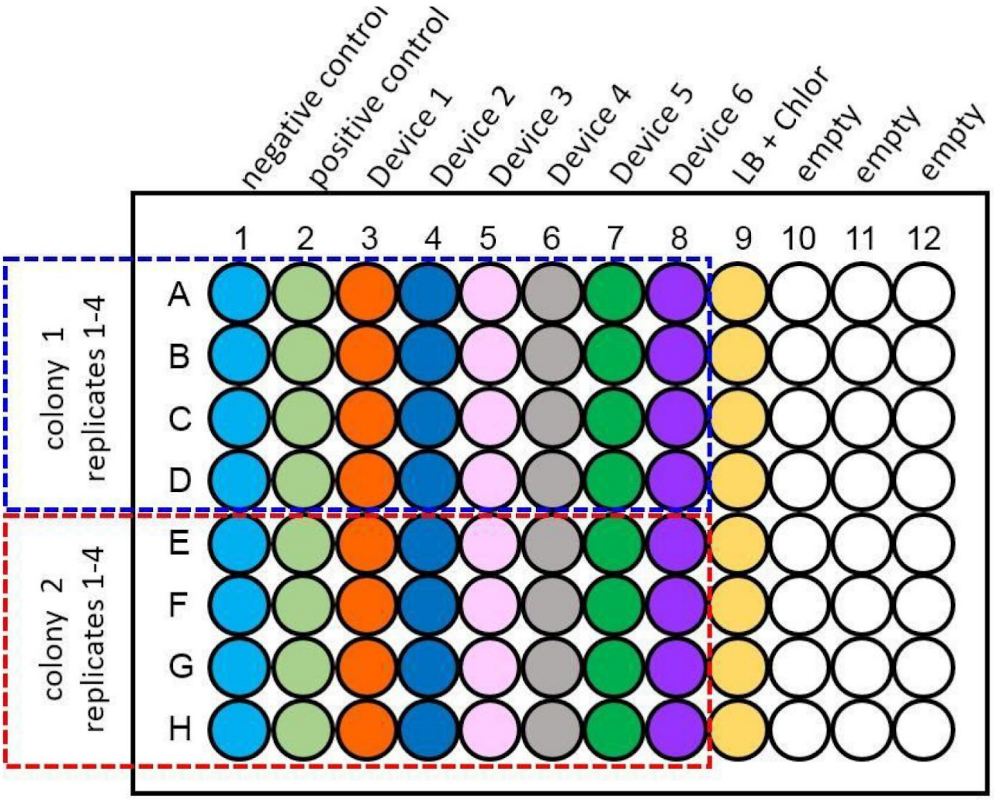



Day 2: Two colonies were picked from each of the transformation plates and inoculated in 5 mL of LB + Chloramphenicol medium. And grown overnight for 18 hours at 35 degrees Celsius and 250 rpm.

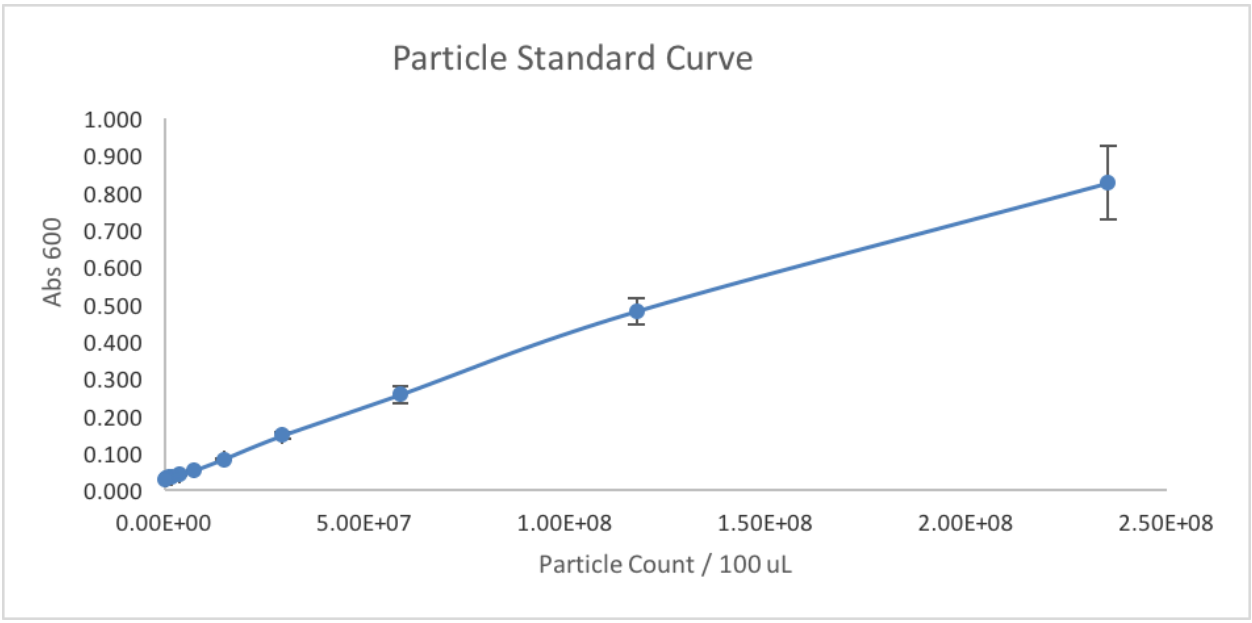
Day 3:

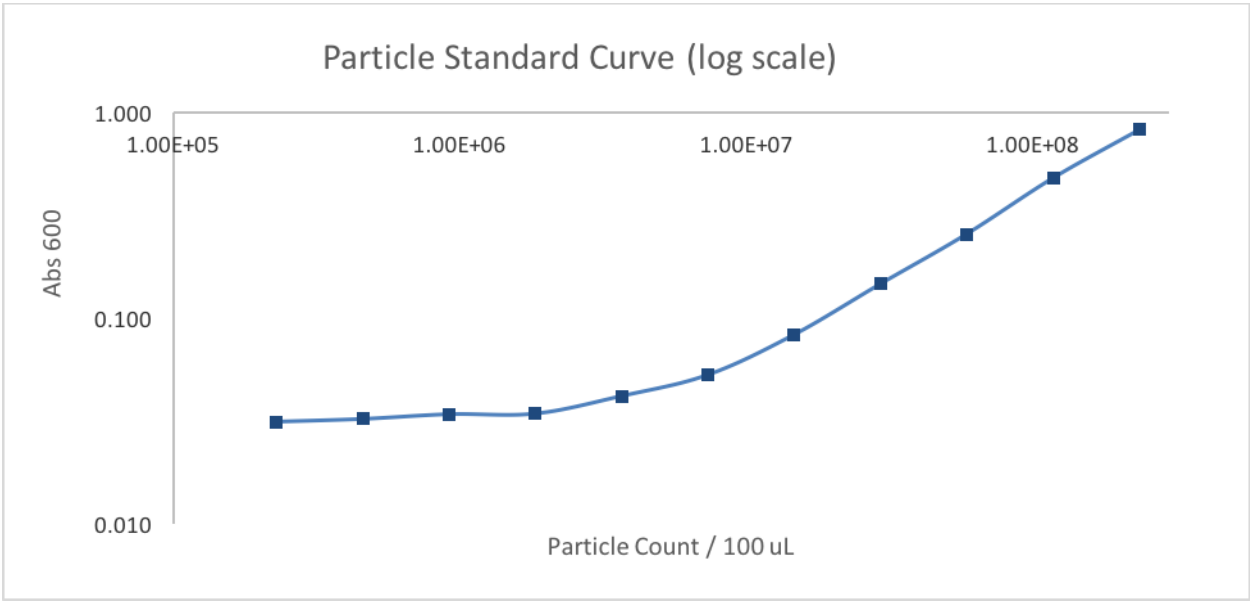
1) Created 1:10 dilution of each overnight culture in LB + Chloramphenicol (0.5mL of culture into 4.5 mL of LB + Chloramphenicol
2) Measured Abs 600 of these 1:10 diluted cultures
3) Diluted the cultures to an Abs 600 of 0.02 12 ml LB + Chloramphenicol in 50 mL falcon tubes that were immediately covered in foil
4) 500 µL of each sample were transferred into individual 1.5 mL tubes as 0 hour samples prior to incubation and placed on ice

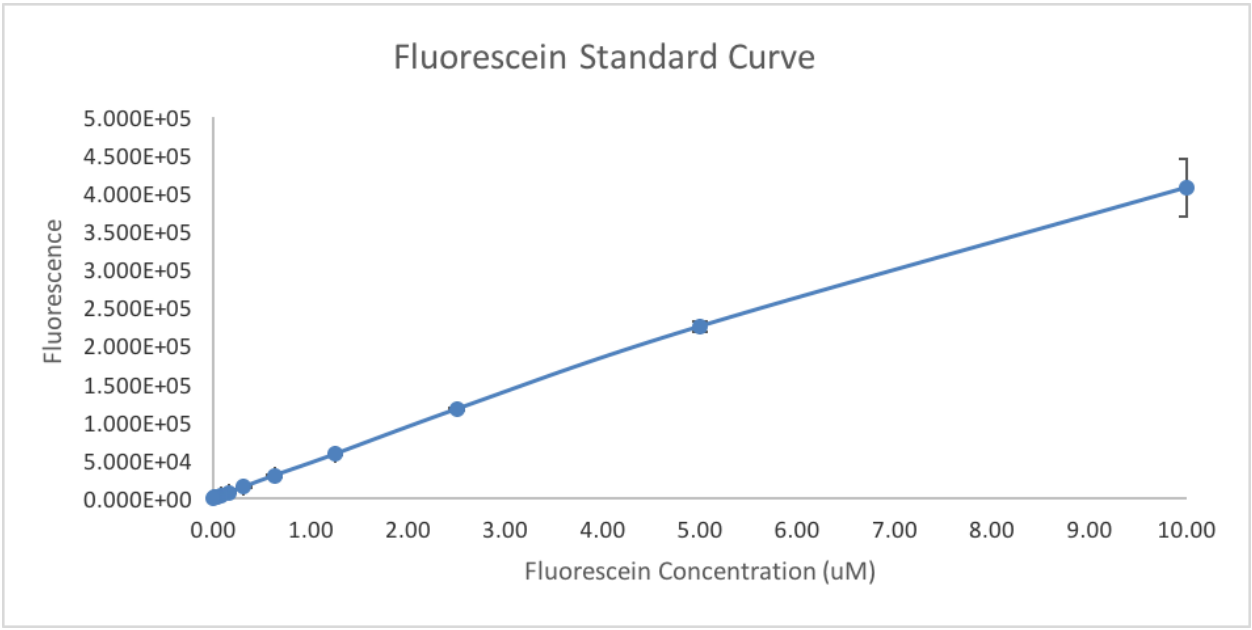
5) Incubated the remainder of the cultures for 6 hours at 37 degrees Celsius at 220 rpm
6) Took 500 µL of each sample and placed them on ice
7) Measured Abs 600 and fluorescence after adding 100 µL of the respective samples as shown in figure …..

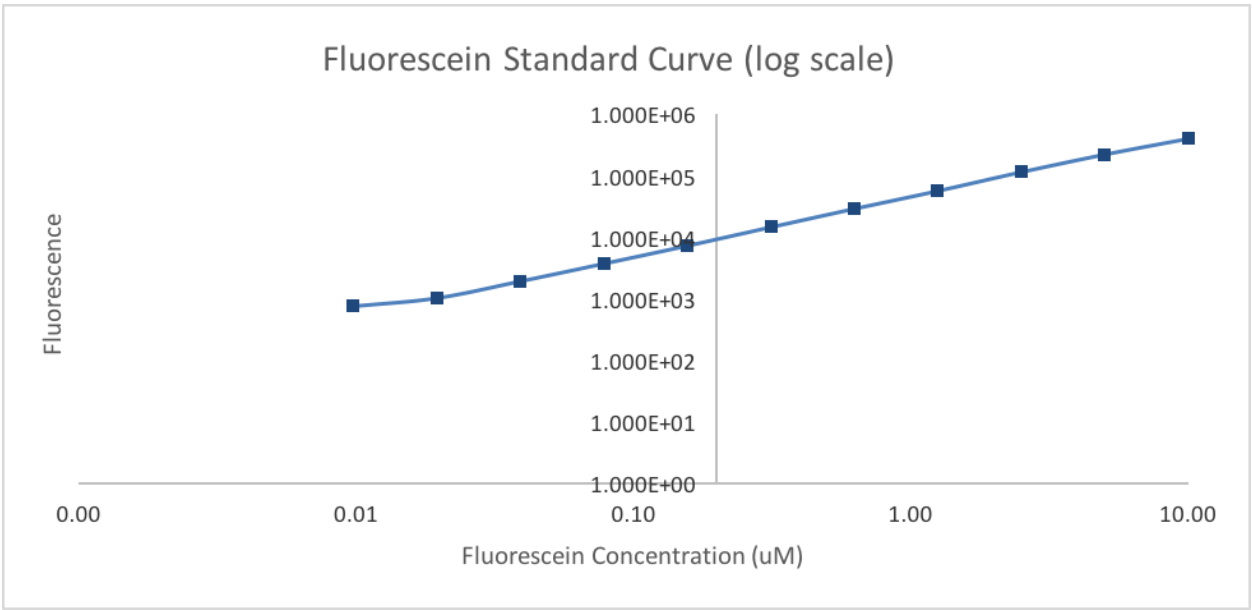



Results:


Particle Standard Curve
Abs 600
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.00E+00
5.00E+07
1.00E+08
1.50E+08
2.00E+08
2.50E+08
Particle Count / 100 uL


Particle Standard Curve (log scale)
Abs 600
1.000
0.100
0.010
1.00E+05
1.00E+06
1.00E+07
1.00E+08
Particle Count / 100 uL




All raw data can be found on our excel spreadsheet (link to our results sheet)