INTERLAB study

The measurement committee of iGEM has been conducting the interlab study for the past three years in order to develop a robust procedure for the measurement of green fluorescent protein. Our team SVCE\_CHENNAI also wanted to be a part of this collective effort taken by the iGEM teams and hence decided to take part in the fourth international interlab measurement study this year. All participating teams were required to measure the fluorescence and OD of E.coli Dh5alpha cells transformed with 8 plasmids which include 6 test devices and 2 controls using a plate reader. We measured the absorbance and fluorescence of our samples using the Biotek Synergy H1 Hybrid Multimode Microplate Reader at 600nm and 460nm/515nm (excitaton peak/emission peak) respectively. The results of our interlab study are shown below.

**OD600 Reference POINT:-** The absorbance of LUDOX - HS40 was measured to obtain a correction factor that would convert the raw absorbance data into standard OD600

|  |  |  |
| --- | --- | --- |
|  | LUDOX-HS40 | H2O |
| Replicate 1 | 0.046 | 0.035 |
| Replicate 2 | 0.046 | 0.036 |
| Replicate 3 | 0.047 | 0.036 |
| Replicate 4 | 0.047 | 0.036 |
| Arith. Mean | 0.0465 | 0.03575 |
| Corrected Abs600 | 0.01075 |  |
| Reference OD600 | 0.0425 |  |
| OD600/Abs600 | 3.953488372 |  |

**FLUORESCEIN STANDARD CURVE :-**

As shown in the table below, we have diluted the sodium fluorescein concentration to 1uM(0.02X) instead of 50 uM(1X). This was because our plate reader is very sensitive and most of the fluorescence readings obtained after serial dilution starting from an initial concentration of 50uM were showing the readings as ‘OVERFLOW’ which did not generate a reliable standard curve.Dilution of the sodium fluorescein concentration to 1uM before performing the serial dilutions generated good fluorescence values and hence a reliable standard curve

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| uM Fluorescein | **1.00** | **0.5** | **0.25** | **0.125** | **0.0625** | **0.03125** | **0.015625** | **0.0078125** | **0.00390625** | **0.001953125** | **0.000976563** | **0** |
| Replicate 1 | OVERFLOW | OVERFLOW | 76670 | 52206 | 22348 | 12142 | 6649 | 3452 | 2235 | 1460 | 1016 | 507 |
| Replicate 2 | OVERFLOW | OVERFLOW | 79785 | 42502 | 21402 | 11002 | 5824 | 3246 | 1929 | 1166 | 845 | 494 |
| Replicate 3 | OVERFLOW | OVERFLOW | 79719 | 40666 | 21415 | 11506 | 5967 | 3041 | 1866 | 1226 | 845 | 483 |
| Replicate 4 | OVERFLOW | OVERFLOW | 81596 | 42899 | 22330 | 12131 | 6268 | 3481 | 2009 | 1266 | 885 | 479 |
| Arith. Mean | #DIV/0! | #DIV/0! | 79442.5 | 44568.25 | 21873.75 | 11695.25 | 6177 | 3305 | 2009.75 | 1279.5 | 897.75 | 490.75 |
| Arith. Std.Dev. | #DIV/0! | #DIV/0! | 2042.7169 | 5183.903444 | 537.3008934 | 549.5072793 | 365.0452027 | 204.7453703 | 161.1652878 | 127.1573828 | 81.05707865 | 12.55322004 |

**CELL MEASUREMENT:-** The E.coliDh5 alpha cells were first transformed with the eight plasmids provided in the interlab measurement kit. This was followed by measurement of fluorescence of the samples after cell growth and sampling.

From the two graphs above it can be inferred that there was an exponential growth in all the devices except Device 1 from hour 0 to hour 6. The decreasing order of growth rate in all devices is:

Device 4 > Device 6 > Device 5 > Device 3 > Device 2 > Device 1

As seen in the two graphs above Device 2 has shown the maximal expression and Device 1, Device 4 and Device 5 has shown moderate expression of GFP respectively. Device 3 and Device 6 did not yield any fluorescence production .The decreasing order of fluorescence in all devices is given below.

Device 2 > Device 1 > Device 4 > Device 5 > Device 3 > Device 6

From the fluorescein/OD600 graphs, we can infer that Device 2 has the maximum fluorescence and hence is the best producer of GFP. The next best producer of GFP is Device 1 which is followed by Device 5 and Device 4. Lowest production is seen in both Device 3 and Device 6.