DIYNAFLUOR: Protocol for Low-Cost 0-10 ng/µL DNA Quantification Assay

Version 0.1 6/12/2024

Introduction

This low-cost DNA quantification assay costs ~$0.13 USD per measurement and has a working range of ~0.3 – 12.5 ng/µL, with an average absolute bias (accuracy) of 0.218 ± 0.149 ng/µL.

For more information, please refer to our Preprint *"DIYNAFLUOR: An Affordable DIY Plug-and-Play Nucleic Acid Fluorometer for eDNA Quantification in Resource Limited Settings"*, which will be made available soon.

Materials

- *SYBR Safe 10,000X Concentrate* (Thermo Fisher Scientific S33102)
- *Lambda DNA 300 ng/uL* (Thermo Fisher Scientific SD0011)
- *LTE Buffer* (10 mM TRIS, 0.1 mM EDTA, pH 8.0) or 1 x TE Buffer (Thermo Fisher Scientific 12090015)
- *1.5 mL Microcentrifuge Tubes (For preparing reagents and samples)*
- *0.5 mL Thin-walled PCR Tubes* (We recommend Axygen PCR-05-C)
- *P1000, P200, P20 Disposable Pipette Tips*
- *Aluminium Foil*

Equipment

Required

- *P1000, P200, P20 Adjustable Pipettes*
- *Fine Point Pen* (For labelling Tubes)

Desirable

- *Tube Racks*
- *Vortex*
- *Mini Centrifuge*

Protocol

1. **Prepare a *10 ng/µL DNA Standard*.**

   We recommend preparing the *10 ng/µL DNA standard* from *300 ng/µL Lambda DNA (Thermo Fisher Scientific)*, however any dsDNA with a known concentration should work.

   For example, for 300 µL of *10 ng/µL Lambda DNA*, add 10 µL of *300 ng/µL Lambda DNA* to 290 µL of *LTE Buffer*.

   Vortex for 2-3 seconds to mix and briefly centrifuge. (Alternatively, gently pipette mix 10-20x with the P200 set to 200 µL. Bring the contents to the bottom of the tube by gently tapping the bottom of the tube on a table).

   Note: The *10 ng/µL DNA standard* can be stored at -20 ^{0}C for use future use, but repetitive freeze thawing should be avoided.

2. **Prepare *200X SYBR Safe Stock Solution* by diluting *SYBR Safe 10,000X Concentrate* in *LTE*.**

   For example, for 500 µL of *200X SYBR Safe Stock Solution*, add 10 µL of *SYBR Safe 10,000X Concentrate* to 490 µL of *LTE Buffer.*

   Vortex for 2-3 seconds to mix and briefly centrifuge. (Alternatively, gently pipette mix 10-20x with the P200 set to 200 µL. Bring the contents to the bottom of the tube by gently tapping the bottom of the tube on a table).

   Keep away from light by wrapping the tube in *Aluminium Foil*.

   The *10 ng/µL DNA Standard* can be stored at -20 ^{0}C for use future use (up to 4 months), but repetitive freeze thawing should be avoided.

3. **Prepare *SYBR Safe Master-Mix* by adding 1 µL of *200X SYBR-Safe Stock Solution* to 199 µL *LTE* for each sample and the two standards.**

   For example, to measure 5 Samples and 2 Standards, Add 7 x 1 µL = 7 µL of *200X SYBR-Safe Stock Solution* to 7 x 199 µL = 1393 µL of *LTE buffer.*

4. **Set up two 0.*5 ml Thin-wall PCR Tubes* for the Standards and one Tube for each Sample. Label the lids of the Tubes.**

   Label the *Standard Tubes* '*1*' and '*2*'. Label the *Sample Tubes* with names that allows them to be easily identified. Do not write labels on the sides of the Tubes.

5. **Prepare the Standards.**

   Add 190 µL of the *SYBR Safe master-mix* to both *Standard Tubes 1 and 2*. Add 10 µL of *LTE* to *Standard Tube 1*. Add 10 µL of the *10 ng/µL DNA Standard* to *Standard Tube 2*.

   Vortex for 2-3 seconds to mix and briefly centrifuge. (Alternatively, gently pipette mix 10-20x with the P200 set to 200 µL. Bring the contents to the bottom of the tube by gently tapping the bottom of the tube on a table).

6. **Prepare the Samples.**

   Add 190 µL of the *SYBR Safe master-mix* to all *Sample Tubes*. Add 10 µL of sample to all *Sample Tubes*.

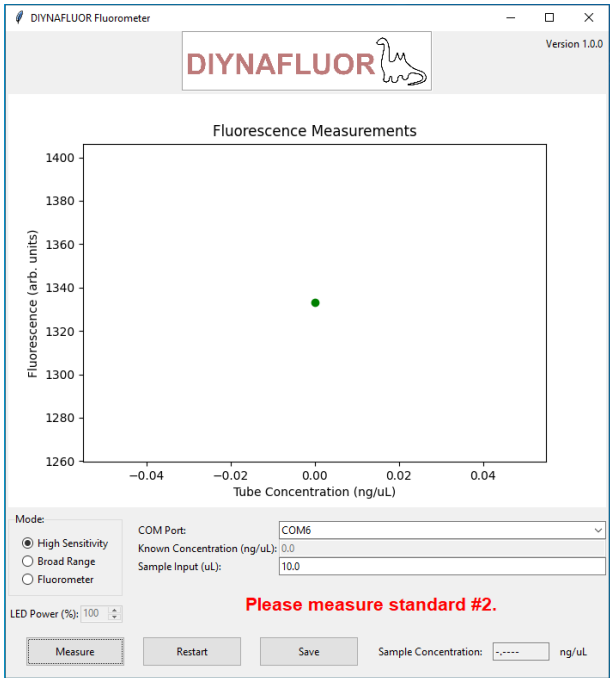
   Vortex for 2-3 seconds to mix and briefly centrifuge. (Alternatively, gently pipette mix 10-20x with the P200 set to 200 µL. Bring the contents to the bottom of the tube by gently tapping the bottom of the tube on a table).

7. **Incubate all Tubes for 2 minutes at room temperature out of direct light.**

8. **Measure *Standard Tube 1*.**

Insert *Standard Tube 1* into the DIYNAFLUOR. Ensure the "*High Sensitivity*" mode is selected, and the *"Sample Input"* is set to "10 µL".

Press "*Measure*".



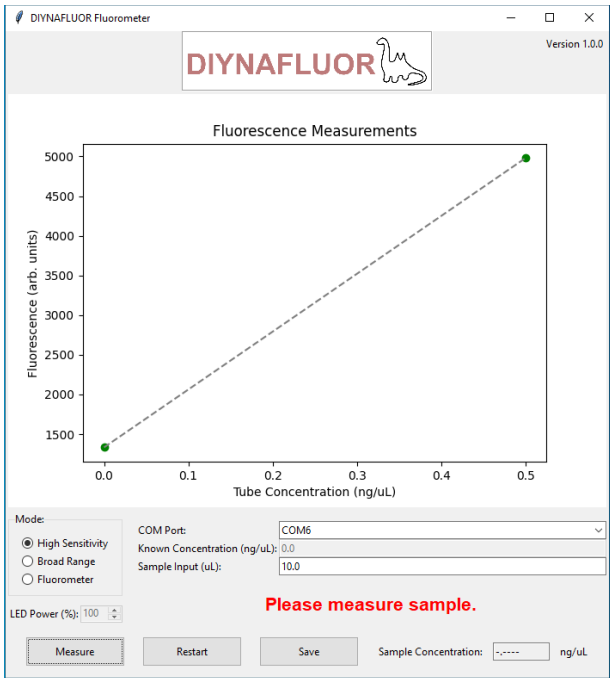
A data point for *Standard Tube 1*'s Fluorescence will be plotted.

Note: Ensure bubbles are not present in Tubes prior to measurements. Spin or gently tap the bottom of the tube on a table to remove them.

9. Measure *Standard Tube 2*.

Insert *Standard Tube 2* into the DIYNAFLUOR.

Press "*Measure*".

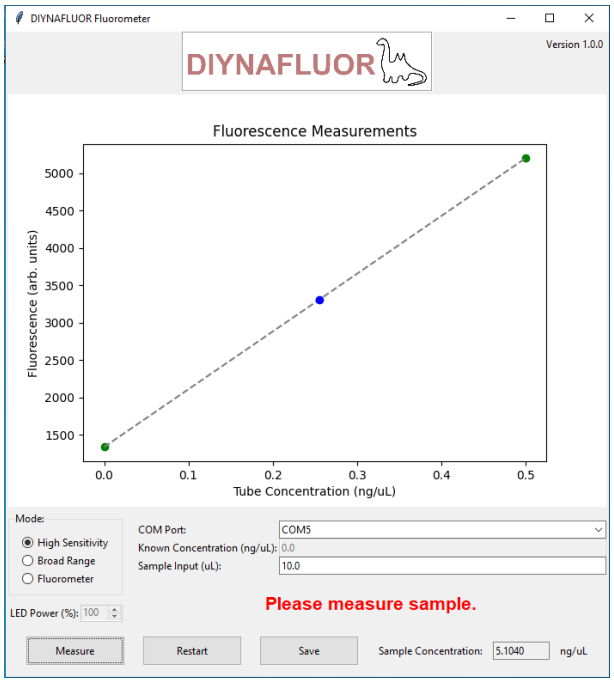


A data point for *Standard Tube 2*'s Fluorescence and a 2-point calibration curve will be plotted.

10. Measure Samples.

Insert a *Sample Tubes* into the DIYNAFLUOR.

Press "*Measure*".



A data point for the measured "*Tube Concentration*" will be plotted on the calibration curve. The "*Sample Concentration*" is the reported in lower right corner of the DIYNAFLUOR GUI. (The "Sample Concentration" is calculated from the dilution factor of Sample added to the *SYBR Safe master-mix*).

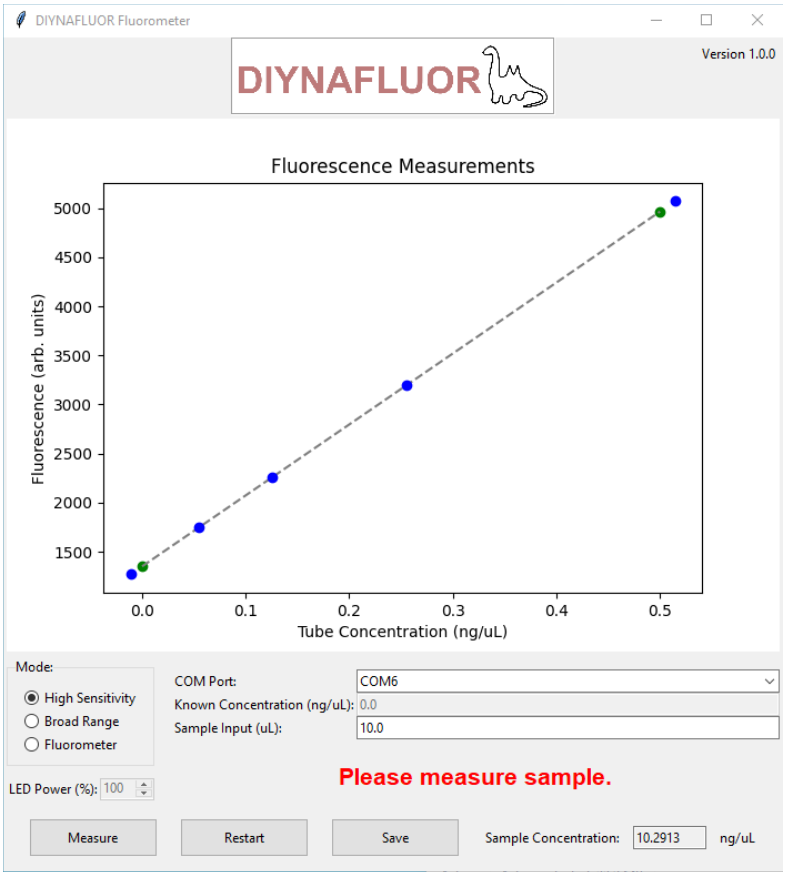
Repeat this process for the remaining samples.

Note: If a Sample's measured Fluorescence is ~25% higher than Standard Tube 2's Fluorescence, the Sample should be diluted and remeasured to fall within the 2-point calibration curve. If a Samples measured Fluorescence is within ~ ±120 units of *Standard Tube 1*'s Fluorescence value, then the Sample has insufficient DNA for measurement.

11. Save the Data.

Press "Save" to export the data as a .csv file.

Example Data: A 2-fold dilutions series of Lambda DNA measured from 0-10 ng/µL.



	A	B	C	D	E
1	Name	Sample Concentration (ng/uL)	Measured Flouresence (arb.)	Tube Concentration (ng/uL)	Sample Input (uL)
2	Standard #0	-	1350.33	0	-
3	Standard #1	-	4966.33	0.5	-
4	Sample #0	-0.224	1269.33	-0.0112	10
5	Sample #1	1.0998	1748	0.055	10
6	Sample #2	2.5092	2257.67	0.1255	10
7	Sample #3	5.106	3196.67	0.2553	10
8	Sample #4	10.2913	5071.67	0.5146	10