Measure femtogram quantities of dsDNA

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- **Nucleic acid quantification was performed on a BMG LABTECH microplate reader in fluorescence mode**
- **Invitrogen's PicoGreen® assay was utilized to quantify dsDNA in 384-well HTS format**
- **Only 10 µL of sample is needed minimizing raw sample and waste**

Introduction

In the modern molecular biology laboratory, DNA samples are a precious commodity, where only small amounts can be sacrificed for quality control analysis, preserving the majority for downstream applications such as Next Generation Sequencing. These samples often contain very small amounts of DNA and so a technique has been developed to measure DNA to femtogram levels using low volumes (10 µL), utilising a high throughput well-plate format.
With the Quant-iT Picogreen reagent Invitrogen provides a solution for measuring low concentrations of DNA. This, together with Cambridge Biosciences AccuBlue enhancer, makes it a highly sensitive fluorescence assay for dsDNA detection.
This application note will investigate the use of very low volume DNA samples in a 384 well plate format on the BMG LABTECH filter-based microplate reader using the Picogreen reagent in combination with Cambridge Biosciences AccuBlue enhancer.

Materials & Methods

All materials were obtained through normal distribution channels from the manufacturers stated.

- Lambda DNA, Invitrogen
- Quant-iT Picogreen dsDNA reagent, Invitrogen
- 1x TE buffer, Promega
- AccuBlue High Sensitivity Enhancer 100x, Cambridge Bioscience
- black 384-well Optiplate-F, PerkinElmer
- Microplate Reader, BMG LABTECH

Consumables such as pipette tips and microcentrifuge tubes were used as required from various sources.

Preparation of the Picogreen working solution
The Picogreen reagent and the AccuBlue Enhancer are diluted in TE buffer to give a final dilution of 1:100 (Picogreen) and 1:10 (AccuBlue Enhancer). For example a 500 µL final volume of working solution contains 5 µL Picogreen reagent and 50 µL AccuBlue enhancer.

Preparation of the dsDNA dilution series
A 2fold serial dilution of the stock lambda DNA solution yielded eight concentrations from 100 pg/µL to 0.78 pg/µL in 1xTE.

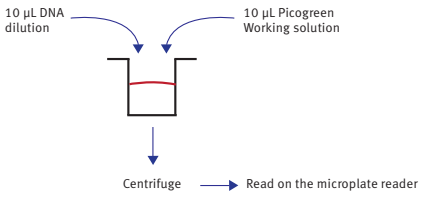

Fig. 1: Easy "Mix + Measure" protocol is used for dsDNA quantification.

Into a 384 well Optiplate, 10 µL of each DNA dilution was added in triplicate, followed by 10 µL of the Picogreen working solution resulting in a final DNA dilution series ranging from 50 pg/µL to 0.39 pg/µL per well. As a blank 10 µL TE buffer was added to 10 µL of Picogreen working solution.
The prepared 384 well plate was then centrifuged briefly at 1000xg to ensure that the solutions were at the base of the well. The plate was then placed into the microplate reader, a focus and gain adjustment was performed and the samples read using the following parameters.

Instrument settings

Excitation filter:	Ex485
Emission filter:	Em520
Gain:	was optimal adjusted
Focal height:	was optimal adjusted
Number of flashes:	10
Shake time:	30 seconds before reading cycle

The data produced was analysed using the BMG LABTECH MARS Data Analysis Software.

Results & Discussion

Figure 2 shows the dsDNA standard curve over the whole concentration range.

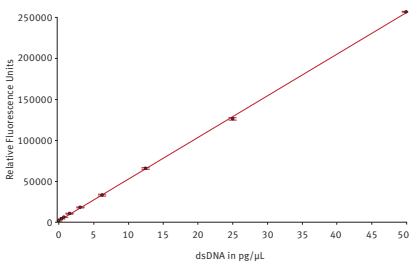

Fig. 2: dsDNA standard curve using DNA concentrations from 0.39-50 pg/µL.

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The standard curve was linear over the whole range from 0.39 pg/µL to 50 pg/µL. Linear regression of the data yielded an R^2 value of 0.9998. Standard error bars between triplicate samples are shown.
After looking in more detail at the lower concentration range (Fig. 3), it can be seen that the calibration curve shows further linearity even into the femtogram per µL level.

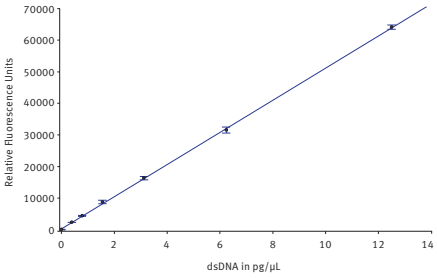

Fig. 3: dsDNA standard curve using DNA concentrations from 0.39-12.5 pg/µL.

Conclusion

The results presented here show that using the microplate reader from BMG LABTECH it is possible to reduce the volume of the sample to 10 µL and run the tests using a 384 well plate format. The method minimises waste, typically less than 5% of sample volume, with a fast, easy and accurate microvolume nuclear acid quantitation. This technique is used routinely in our laboratory.