



A comparison of DNA quantification values obtained by UV spectrophotometry (including NanoDrop) and PicoGreen analysis

Introduction

After completing DNA extraction from a biological sample, it is common practice to quantify the DNA prior to using it in downstream applications. To ensure optimal performance, reactions that utilise nucleic acids typically specify the input quantities of DNA that they require.

There are a range of methods available for the quantification of DNA including absorbance, agarose gel electrophoresis and fluorescent DNA-binding dyes. The traditional method involves measurement of the absorbance of the sample using a UV spectrophotometer. DNA has a maximal absorbance near 260 nm so UV light of this wavelength is passed through the samples. Higher levels of absorbance are indicative of greater concentrations of DNA present within the samples. This method has the advantage that the quality of the DNA can also be assessed; absorbance at 280 nm is also measured to determine the level of protein contamination. The A260 / A280 ratio is indicative of the purity of the DNA samples and values of 1.8 or higher relate to pure DNA samples. A disadvantage of this method is that single-stranded DNA (ssDNA) and RNA also absorb UV light at 260 nm and can therefore interfere with the results and cause overestimation of the double-stranded DNA (dsDNA) concentration. A wide range of UV spectrophotometers are available, varying from traditional instruments that quantify DNA in plates or cuvettes, to instruments such as the NanoDrop (Thermo Scientific) that are designed to quantify DNA from micro-volumes of sample.

PicoGreen® is a fluorescent nucleic acid stain that selectively binds to dsDNA. It has an excitation maximum at 480 nm and emission of fluorescence can be read at 520 nm. PicoGreen® is added

to the DNA samples, signals are read and concentrations determined using a standard curve. As PicoGreen® only binds to double-stranded DNA, it has the advantage that the reported DNA concentration is an accurate estimation of the quantity of DNA that is present within the sample and is not influenced by the presence of ssDNA or RNA. A disadvantage of this method is that it cannot be used to provide an estimate of DNA purity.

DNA extracted in LGC's service laboratories is routinely analysed using traditional UV spectrophotometry as this provides an estimation of both DNA quality and quantity. If required, DNA can be quantified using PicoGreen® in addition to spectrophotometry (subject to additional charge). This study provides a comparison of estimated concentration values obtained using three quantification methods, and offers insight into the repeatability of the three methods.

Method

A set of 44 DNA samples, extracted in LGC's laboratory from human blood samples using our Kleargene chemistry, were quantified using three different methods. Two different UV spectrophotometry instruments were used – a traditional plate reader, the FLUOstar Omega, and the NanoDrop – and the samples were also quantified using PicoGreen®.

1. UV spectrophotometry: FLUOstar Omega plate reader – DNA samples were arrayed into a 96-well plate. Absorbance was measured at 260 nm and 280 nm on an FLUOstar Omega plate reader. The absorbance reads were performed three times. An advantage of this method is that the volume of DNA used for quantification can be returned to the original sample post-analysis.

- UV spectrophotometry: NanoDrop (Thermo Scientific) – DNA samples were quantified individually using the NanoDrop. 1 μL of each sample was dispensed onto the pedestal and absorbance measured. The NanoDrop software calculates a DNA concentration value based on the absorbance values obtained. Each sample was quantified on the NanoDrop three times.
- PicoGreen – a DNA dilution was performed, based on UV spectrophotometry results for

these samples, to ensure that DNA was at an appropriate concentration for the PicoGreen® standard curve. Diluted DNA was arrayed in triplicate into a 384-well plate alongside DNA standard curve samples. An appropriate volume of PicoGreen® was dispensed into each well, and the plate incubated at room temperature, protected from light, for 5 minutes. Fluorescent signal was measured at 520 nm on a FLUOstar Omega plate reader.

Results and Discussion

DNA concentration and purity

Measured DNA concentration of the samples differed significantly between the three methods of quantification ($F_2 = 15$, $P < 0.0001$). Reported DNA concentration was highest when samples were measured using the NanoDrop (mean = 134 $\text{ng} / \mu\text{L}$), and lowest when samples were measured using PicoGreen (mean = 87 $\text{ng} / \mu\text{L}$). Figure 1 illustrates the mean measured DNA concentration for the 44 DNA samples, obtained using three different quantification methods.

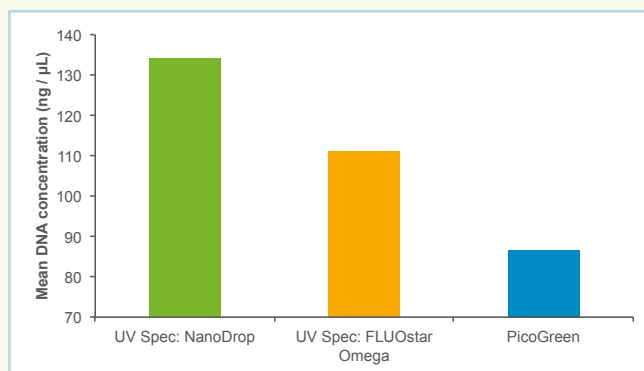


Figure 1. Mean measured DNA concentration for the 44 DNA samples analysed.

Individual paired t-tests were performed to ascertain which measurement methods significantly differed from each other; adjusted P-values of $P < 0.0001$ were obtained for all comparisons, indicating that DNA concentrations obtained with one method differed significantly from those obtained using the other two methods.

Figure 2 illustrates the difference in mean concentration obtained by the three quantification methods for a subset of the 44 DNA samples tested.

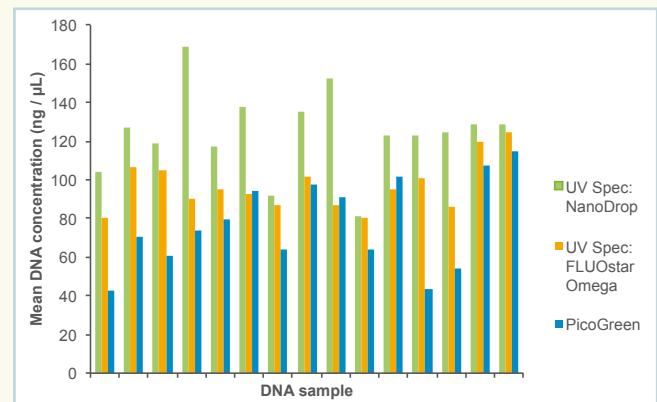


Figure 2. Mean DNA concentration for a subset of 15 of the 44 DNA samples analysed. Green, orange and blue bars illustrate the concentration estimate from NanoDrop, FLUOstar Omega plate reader and PicoGreen® methods respectively. DNA concentrations are consistently estimated as higher using the Nanodrop platform than the FLUOstar Omega or PicoGreen methods.

As PicoGreen® is specific to dsDNA, the lower concentration values determined using this method will be more reliable estimations of actual dsDNA whilst the values obtained by both UV spectrophotometry methods are likely to have been influenced by presence of ssDNA and / or RNA.

The mean A_{260} / A_{280} ratio obtained using the Omega FLUOstar data for the 44 DNA samples was 1.8 which is indicative of pure DNA, and was very consistent across all samples (mean standard deviation = 0.02). These values are similar to the values obtained using the NanoDrop (mean A_{260} / A_{280} ratio = 1.9, mean standard deviation = 0.02).

Repeatability of measurements

As each individual DNA sample was quantified by each of the three methods in triplicate, the repeatability of each method can be calculated. For all three datasets, the standard deviation (SD) for each sample was calculated using the three individual concentration values. The mean SD, calculated using SD values from all samples, was then calculated. The mean standard deviation values for the three datasets are very comparable, as detailed in Table 1, indicating that repeatability of the two methods is very similar. The maximum sample SD (highest SD for an individual sample) is comparable between the FLUOstar Omega and PicoGreen datasets, but is much higher for the NanoDrop dataset. The minimum sample SD values (lowest SD for an individual sample) are comparable between the three datasets.

Standard deviation (SD) ng/ μ L	Quantification Method		
	UV Spec: NanoDrop	UV Spec: FLUOstar Omega plate reader	PicoGreen®
Mean sample SD	9	9	11
Maximum sample SD	67	24	26
Minimum sample SD	1	1	3

Table 1. Comparison of repeatability of three methods for DNA quantification, based on 44 DNA samples. Standard deviation values are comparable between the two methods.

To determine the repeatability of PicoGreen® analysis in our laboratories, a second dilution plate was prepared from the same original DNA samples and the measurement procedure repeated. DNA concentrations calculated for this second dilution plate were compared to those from the original dilution plate. Strong correlation ($R^2 = 0.98$) was observed between the two datasets indicating that calculated DNA concentrations from two dilutions were very similar.

Summary

The NanoDrop, the FLUOstar Omega and PicoGreen® are all reproducible methods for the quantification of DNA samples, although the results obtained using the NanoDrop were more variable, as indicated by the higher maximum sample SD value. DNA concentration values determined by UV spectrophotometry were significantly higher than those determined by PicoGreen®. Of the two UV spectrophotometry-based instruments, the values obtained using the NanoDrop were consistently higher than those obtained using the FLUOstar Omega. The consistently higher values for the UV spectrophotometry methods are attributable to the inability of UV spectrophotometry to distinguish between double-stranded and single-stranded nucleic acids.

Although quantification values from PicoGreen® analysis are a more reliable measure to use in calculations of how much dsDNA to use in downstream applications, UV spectrophotometry analysis is important as it enables the purity of the DNA to be estimated. LGC perform traditional UV spectrophotometry (FLUOstar Omega plate reader) as a standard procedure on DNA extracted in our service laboratories, but offers PicoGreen® analysis as an additional service, should this be required.

Key words: DNA quantification; spectrophotometry, NanoDrop, PicoGreen

www.lgcgroup.com/genomics • genomics@lgcgroup.com

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