

Dye-free quantification of Oragene®/saliva DNA extracts using the Trinean DropSense96® droplet reader and cDrop™ spectral analysis software

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Saliva samples collected with Oragene® self-collection kits from DNA Genotek offer a non-invasive and proven method for accessing high quality and quantity DNA for genomic testing and analysis. Proper quantification of the extracted DNA is a critical step for most downstream applications. Trinean has developed a specific quantification protocol for DNA from Oragene/saliva samples using the DropSense96® droplet reader and the cDrop™ spectral decomposition software. A comparative analysis using a PicoGreen® fluorescent quantification assay shows a comparable specificity for dsDNA quantification. Since the read-out of 96 samples with the DropSense96 is performed in 5 minutes without any prior mixing or incubation, this technology is a viable alternative to fluorescent quantification assays.

Introduction

Currently, quantification using PicoGreen or SYBR® Green fluorescent dyes is the preferred choice to determine the amount of dsDNA present in DNA extracted from saliva. The alternative method, intrinsic UV-absorbance at 260 nm, often leads to overestimation of the DNA content due to the presence of other components that co-purify with DNA (such as RNA). A novel approach by Trinean combines a microfluidic UV/VIS reader with additional spectral decomposition software. This platform generates full UV/VIS spectra for 96 sample droplets within 5 minutes. Analysis using the cDrop software extracts the dsDNA quantity in a dye-free manner by differentiating it from contaminants (such as RNA, proteins, turbid particles, etc.) in the saliva extracts. The purpose of this application note is to evaluate the Trinean method with a cDrop protocol specific for DNA extracted from Oragene/saliva samples using prepIT®•L2P. A comparative

study is performed using the more conventional A₂₆₀ absorbance and fluorescent quantification using PicoGreen dye.

Material and methods

Sample collection

Saliva (2 mL) was collected from 16 healthy adult donors using Oragene self-collection kits according to the instructions packaged within the kit. DNA was extracted from a 500 µL aliquot of each Oragene/saliva sample using prepIT•L2P, an ethanol precipitation-based extraction method, according to the protocol included with the extraction reagent¹. The extracted DNA was dissolved in 100 µL of TE buffer and incubated overnight at room temperature to ensure complete dissolution.

dsDNA quantification

The DNA samples were quantified in quadruplicate using the Quant-iT™ PicoGreen kit (Life Technologies) and the Trinean platform for both classical A₂₆₀ and cDrop-based quantification.

For the PicoGreen assay, a working solution of the dye was made by diluting the stock solution 1/200 in TE buffer. The standard curve was prepared with phage lambda DNA provided in the Quant-iT PicoGreen kit according to the manufacturer's instructions and 100 µL of each standard was dispensed in a 96-well black plate. The extracted saliva DNA samples were diluted 1/10 in TE buffer and 1 µL of each diluted sample was added to 99 µL of TE buffer in 96-well black plates. PicoGreen dye working solution (100 µL) was added to all wells. Thorough mixing was performed by up/down pipetting and 3 minutes on a shaking platform followed by measurement on a BMG FLUOstar reader.

[†] Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

For quantification with the Trinean platform, 2 μL of undiluted samples were dispensed in a microfluidic DropPlate chip (Trinean) and measured directly on the DropSense96 UV/VIS reader. The classical A_{260} -based quantification is obtained immediately using a background correction at 340 nm. The cDrop decomposition analysis was done upon software selection of the appropriate protocol for prepIT•L2P saliva DNA.

Results

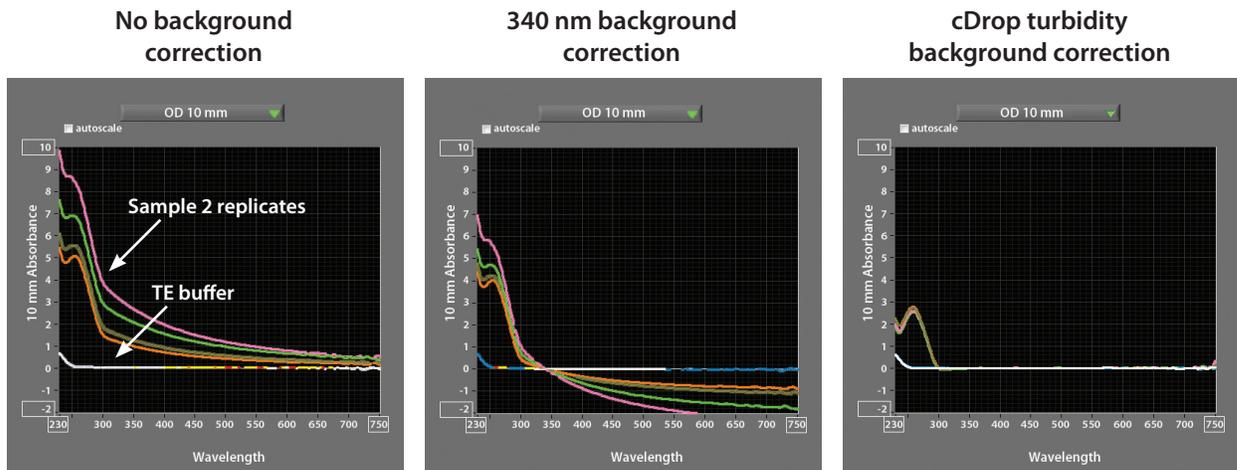
Effect of background correction on the A_{260} quantification

In order to correctly quantify purified DNA samples using the A_{260} absorbance values, a background correction is needed to compensate for turbidity present in the samples. Detailed analysis of the measurements shows that the traditional correction by subtracting the A_{340} (or A_{320}) value does not compensate the turbidity to some extent. However,

it cannot compensate for the typical UV/VIS spectral slope with exponential increase of the turbidity effect towards shorter wavelengths. The background correction used in the cDrop software does compensate for the full spectral slope caused by turbidity by using a fitting algorithm across the whole UV/VIS spectrum. Figure 1 illustrates the effect of both background correction methods on the replicate data of one representative DNA sample. These data show that the cDrop full spectral correction leads to a decrease in replicate variability and A_{260} quantification which is significantly lower than using the A_{340} corrected spectra.

Comparative analysis between PicoGreen assay and cDrop spectral analysis

The cDrop analysis and DNA quantification is based on the selection of an extraction kit-specific protocol. The protocol is built from a specific list of reference spectra derived from the UV/VIS absorbance spectra of all buffers used in the extraction procedure.



	A_{260} (ng/ μL)		
	No background correction	340 nm background correction	cDrop turbidity background correction
Replicate 1	336.92	226.83	127.4
Replicate 2	409.5	266.54	128.9
Replicate 3	272.37	205.07	136.84
Replicate 4	249.09	195.84	138.68

Figure 1: DNA sample 2 was measured in quadruplicate on the DropSense96 reader. The UV/VIS spectral data and A_{260} DNA quantification are shown with and without background correction to compensate for intrinsic sample turbidity. The TE buffer spectra are shown as a non-turbid reference.

When these buffers appeared to have intrinsic UV absorbance, then a specific reference spectrum was included in the reference list next to the reference spectra of dsDNA, total RNA and protein. In this way, a unique reference-based protocol is

created in the cDrop software, allowing accurate dsDNA concentration determination in impure samples. A cDrop analysis of a representative DNA saliva sample is shown in figure 2A.

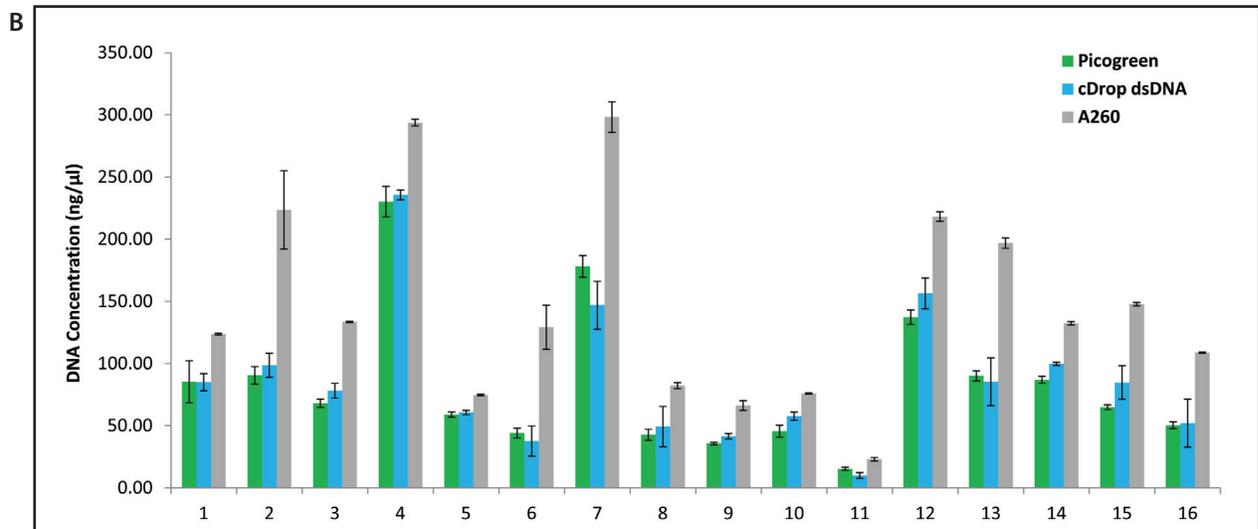
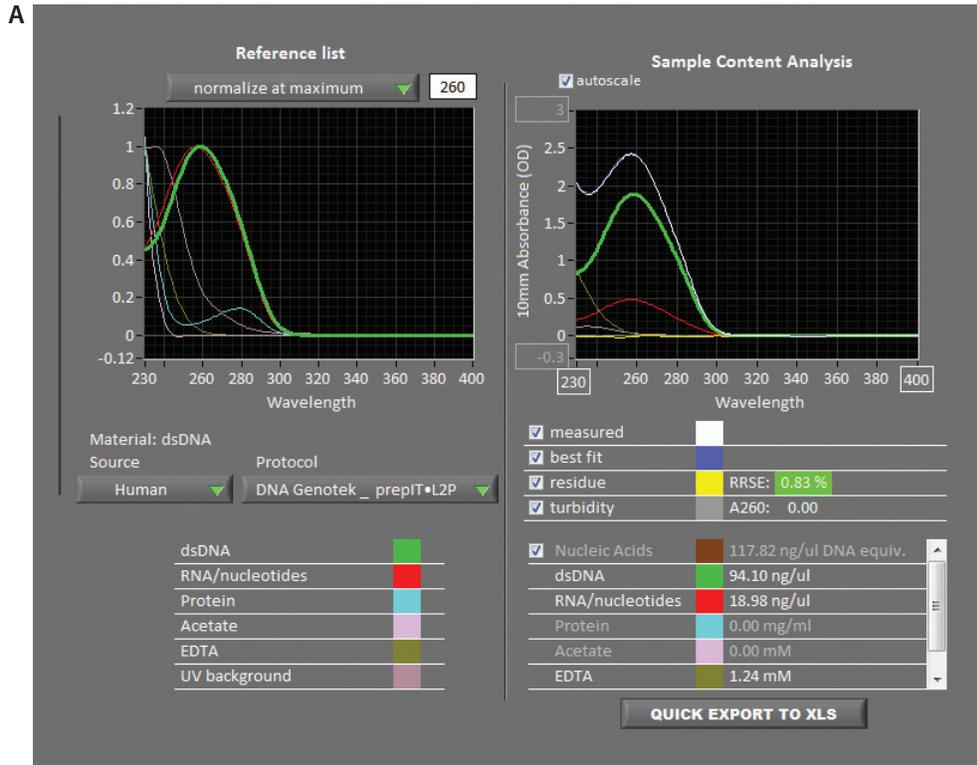


Figure 2: (A) cDrop software screenshot illustrating the reference protocol for the prepiT•L2P extraction kit and the decomposition analysis of a measured DNA sample. (B) Comparison of dsDNA quantification using A_{260} , PicoGreen assay and the cDrop method. The error bars on the graph are the standard deviations of quadruplicate measurements.

