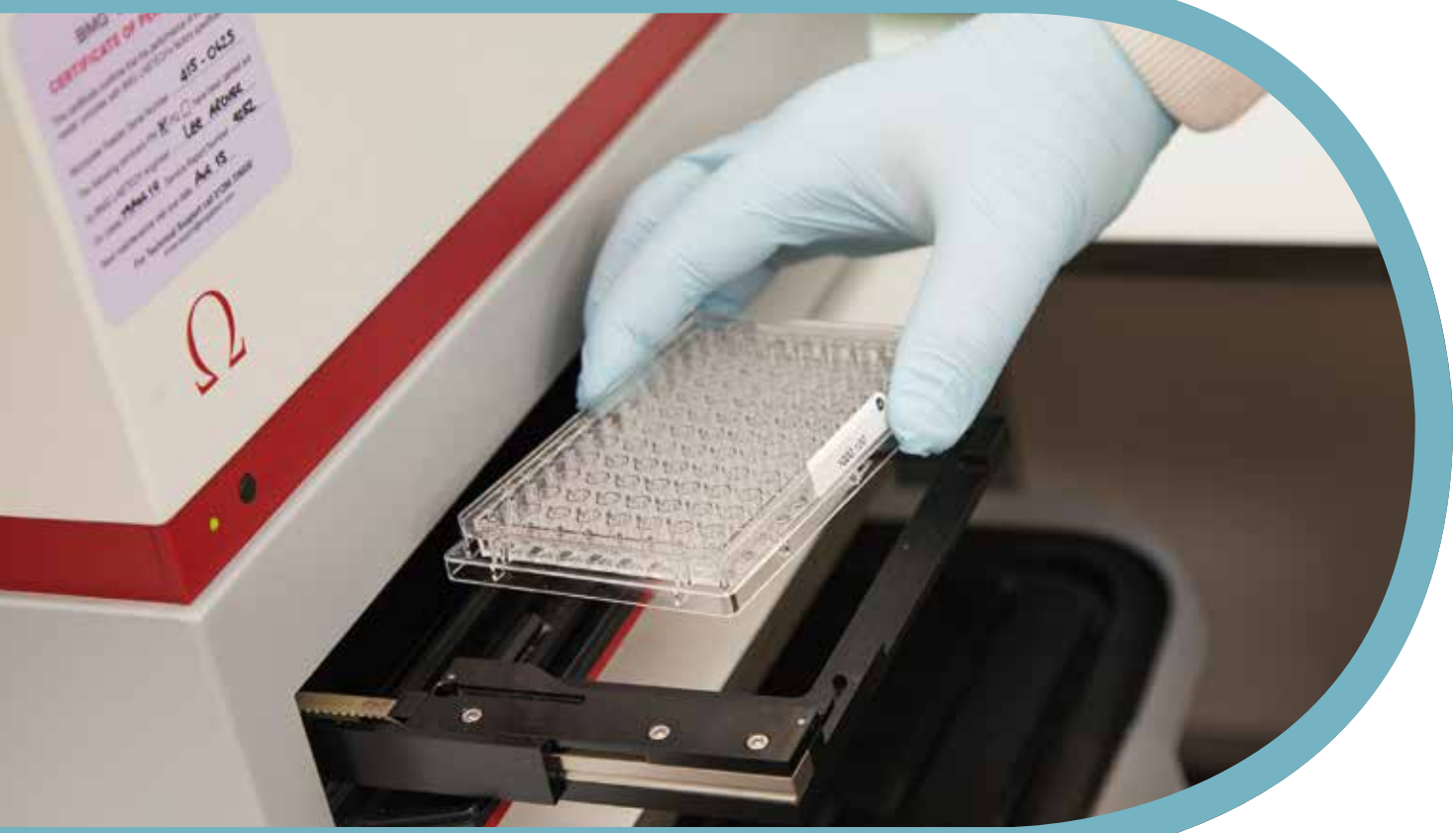
DNA Quantification: Comparison of UV Spectrophotometry and PicoGreen Analysis

Why do we need to quantify DNA?

Following a DNA extraction procedure, it is good practice to quantify the concentration of the DNA sample prior to using it in downstream applications. The input DNA requirements vary between different applications, and accurate quantification is important to ensure that the end result is optimal.

To run KASP genotyping chemistry in our service laboratory, LGC requires 7.5 ng of good quality DNA per sample per SNP (based on human genome size); for detailed information on KASP DNA requirements for service projects including adjustments for genome size, please view our factsheet.



Quantification methods

There are a range of methods available for the quantification of DNA samples. LGC utilise the following two methods:

- UV spectrophotometry (UV spec) – measures absorbance of the sample (at a wavelength of 260 nm). A greater absorbance value relates to greater quantities of nucleic acids. 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® – a fluorescent nucleic acid stain that binds to double-stranded DNA (dsDNA). A higher level fluorescent signal indicates a greater concentration of DNA.

Quantification method	Advantages	Disadvantages	Quantification at LGC	
			Minimum volume requirement	Minimum detectable concentration
UV Spec (Traditional plate reader and NanoDrop)	Estimate of DNA purity is also obtained (A_{260} / A_{280})	Potential overestimation of dsDNA concentration: single-stranded DNA (ssDNA) and RNA also absorb at 260 nm and can interfere with results	Plate reader 50 µL NanoDrop 1 µL	Plate reader 5 µg / mL NanoDrop 2 µg / mL
PicoGreen®	Selectively binds to dsDNA: ssDNA and RNA do not interfere with results	Method cannot be used to provide an estimation of DNA purity	10 µL of diluted sample (typically 1 in 1000 dilution)	2 pg / mL

Laboratory testing and key findings

A set of 44 DNA samples, extracted in LGC's laboratory from human blood samples, were quantified using UV spectrophotometry (both FLUOstar Omega plate reader and NanoDrop) and PicoGreen methods, and the concentrations obtained were compared. See Figure 1 for a comparison of the mean DNA concentrations obtained for all three methods.

- Reported DNA concentration was significantly higher when quantified by UV spec (based on 44 DNA samples, quantified in triplicate for each method)
- The mean UV spec value (NanoDrop) was 35% higher than that obtained by PicoGreen®
- The mean UV spec value (plate reader) was 17% higher than that obtained by PicoGreen®
- Repeatability of each method is comparable (based on average standard deviation values across samples, UV spec NanoDrop SD = 9 ng / µL, UV spec plate reader SD = 9 ng / µL, PicoGreen® SD = 11 ng / µL)

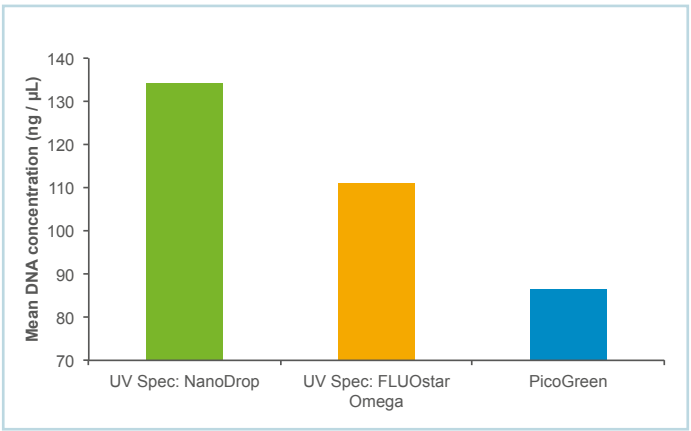


Figure 1. Mean DNA concentration of the 44 DNA samples as determined by both UV spectrophotometry methods and PicoGreen® analysis

For full details of the study, please view our Technical Note (available at www.lgcgroup.com/genomics).