

Verifying the quality and quantity of your ChIP DNA



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Chromatin profiling; comparing the DNA quantification parameters of the Thermo Scientific NanoDrop 2000™ spectrophotometer, Life Technologies Qubit® 2.0 fluorometer and Agilent 2100 Bioanalyzer®.



Chromatrap

Summary

This short guide compares the performance of the NanoDrop 2000™, the Qubit 2.0® fluorometer and the Bioanalyzer 2100® for qualitative and quantitative analysis of double-stranded immunoprecipitated DNA. The advantages and limitations of detection sensitivity, reproducibility and automated analysis of each instrument are discussed. The compatibility of each method with downstream applications including next generation sequencing is also examined.

Introduction

The fields of epigenetics, molecular biology and biochemical research continue to evolve at a rapid pace and the modern lab user must be considerate of the time and cost of performing basic laboratory techniques. One of the most common and often laborious measurements in bioscience labs is that of DNA quantification and characterisation. As such, there are numerous companies selling a wide range of off the-shelf products for DNA quantification, each claiming to have an advantage over their competitor. We set out to provide an overview of some of the most commonly used products for DNA quantification and profiling, a critical step in multiple DNA analysis techniques.

Researchers may wish to quantify DNA for a variety of applications including next generation sequencing (NGS), PCR amplification and plasmid transfection. However, the accuracy of DNA quantification very much depends on the technology being used and whether it is the most appropriate for the sample type being quantified. To obtain the best quality DNA measurements, the sample must be free of contaminants from upstream reactions which may otherwise influence not only the quantification results, but other downstream processes including PCR, NGS and DNA profiling that require a pure sample of DNA. For example, the commonly used phenol-chloroform DNA extraction procedure can significantly impact absorbance measurements if excess phenol is carried over during phase separation. Whilst purification methods are beyond the scope of this guide, it is important to isolate pure DNA for accurate quantification results.

Whilst many users are unaware of the potential inaccuracy of their quantification results, many also choose to turn a blind eye to such information; sticking to what's familiar. However, we believe that it is of the utmost importance to review your quantification techniques in order to reduce the unnecessary consumption of DNA and the wasted time and resources that could result from imprecise quantification.

Thermo Scientific NanoDrop 2000™

Traditionally, DNA has been quantified using spectrophotometry. Most labs will be familiar with the ubiquitous Nanodrop series which measures the amount of ultraviolet radiation absorbed by the bases in solution to estimate sample DNA or RNA concentration. The NanoDrop 2000™ is capable of measuring the sample in less than 5 seconds using only 1 µl of sample. The device is connected to a PC and data is recorded on software that can be exported or printed as a report. Whilst being quick, convenient and simple to use, this method is limited as it is not able to distinguish between RNA, double-stranded DNA, single-stranded DNA, and free nucleotides. Since all of these nucleic acids absorb at 260 nm they will contribute to the total absorbance of the sample, potentially leading to sample overestimation and spurious quantification results.

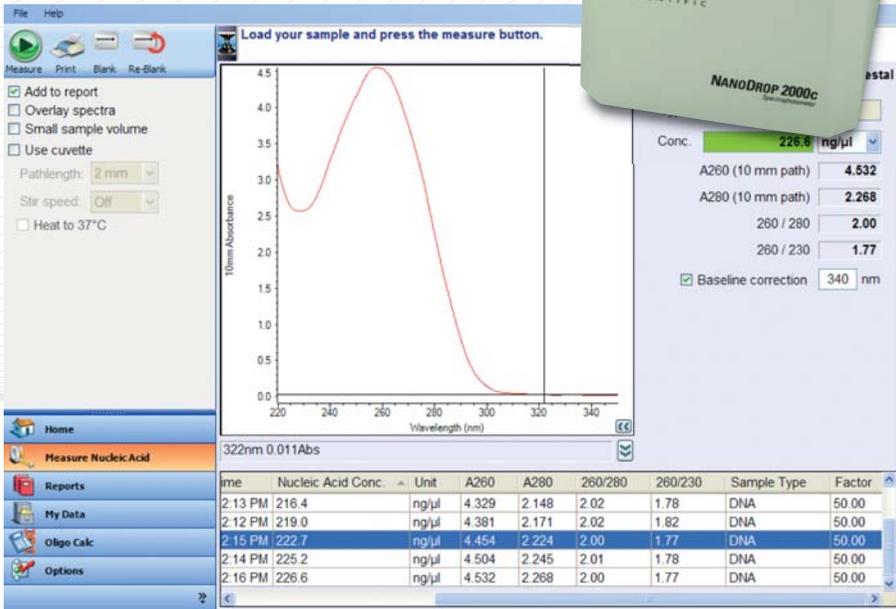
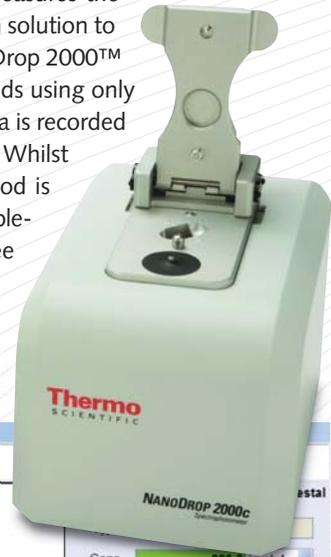


Figure 1 – The NanoDrop 2000™ (upper) and an example of the absorbance profile generated when measuring good quality, “pure” DNA (lower). (Picture courtesy of Thermo Scientific Inc).

Sample quality is commonly assessed by the NanoDrop™ using absorbance measurements at 260 nm. The ratio of absorbance at 260 nm to 280 nm is used as an indication of sample “purity”. A ratio of 1.8 is typically considered “pure” DNA. If the ratio of absorbance is significantly lower, then this suggests the presence of protein, phenol or other contaminants that absorb around 280 nm. Both absorbance measurements can be influenced by the presence of RNA, free nucleotides, sample pH, the wavelength accuracy of the device and the proportion of nucleotides in your sample. For example, measured independently the 260:280 ratio of individual nucleotides ranges from 1.15 to 4.50. Therefore, the resultant 260:280 ratio will be approximately equal to the weighted average of the four nucleotides in your sample. Furthermore, small changes in sample pH can cause a change in the 260:280 ratio. Typically, acidic solutions will reduce the 260:280 ratio, while a basic solution will result in an increase. It is important to ensure that when comparing samples measured using the NanoDrop™ that the pH is the same between undiluted samples and those that have been diluted. For this reason we recommend quantification of immunoprecipitated DNA using a fluorescence-based method for utmost accuracy.

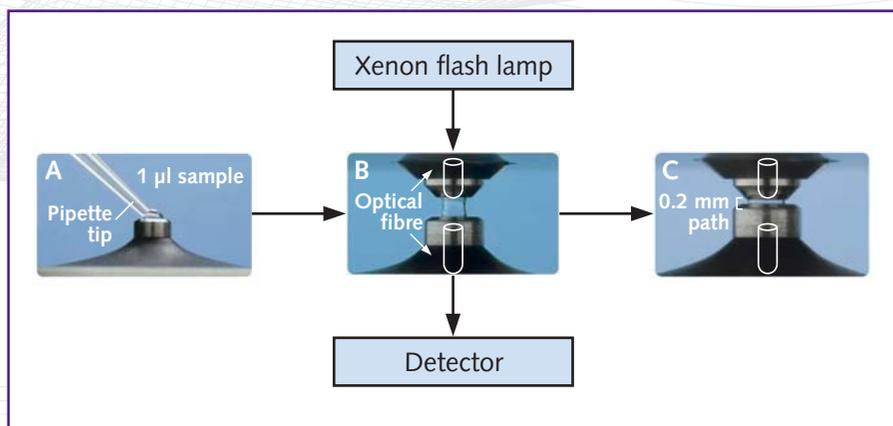


Figure 2 – Fluorescence measurements using the NanoDrop 2000™. Sample is pipetted directly onto the pedestal (A). Once the arm is closed a sample column is formed between the xenon flash lamp and detector (B). The pedestal then moves to automatically adjust for optimal path length and absorbance readings are captured through the optical fibre by the detector (C). (Drawing courtesy of Thermo Scientific Inc).

Life Technologies Qubit 2.0®

Another method employing UV spectroscopy for DNA quantification is that of UV-induced fluorescence using intercalating dyes. Such fluorometric techniques are more accurate and have greater sensitivity than spectrophotometers as the intercalating dye interacts only with double-stranded DNA, and are therefore subject to less interference from superfluous nucleic acids. For example, the Qubit 2.0® (Figure 3) is capable of quantifying DNA in samples with concentrations as low as 10 pg/μl, ideal when quantifying immunoprecipitated DNA beyond the range of UV spectrometers. However, unlike the NanoDrop 2000™, the Qubit 2.0® provides the user solely with information regarding the total concentration of DNA in the sample; it does not provide information about potential contaminants. Furthermore, to quantify samples using the instrument, a dye buffer mixture must be prepared and the sample diluted until it is within the acceptable quantification range. This is a little more time consuming than the NanoDrop 2000™ which has a broad quantification range, but the additional effort is compensated for by the increased accuracy of measurements. Until recently there has not been an alternative technology available for quantification of DNA that reduces time and effort whilst also providing additional data such as qualitative information about the DNA sample.

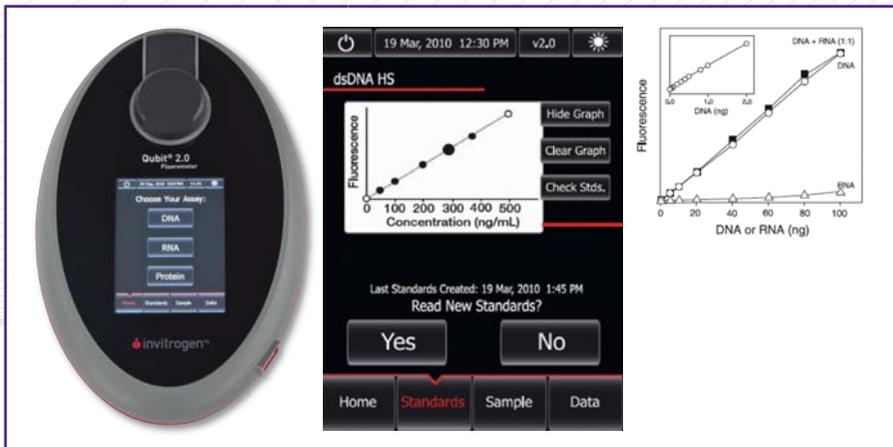


Figure 3 – Life Technologies' Qubit 2.0®. Lightweight and compact, the Qubit 2.0® takes up very little lab space and can be used for the quantification of DNA, RNA and protein. Data can be exported from the device by using a USB flash drive (left). A user-friendly touch-screen interface allows the device to function as a standalone unit without the need for a PC connection (centre). Right, DNA selectivity and sensitivity of the Qubit dsDNA HS assay as performed by the manufacturer. Triplicate 10 μL samples of λ DNA (O), E. coli rRNA (Δ), or a 1:1 mixture of DNA and RNA (■) were assayed in the Qubit dsDNA HS assay. Inset shows sensitivity of assay for DNA. (Copyright Life Technologies Inc.).

Agilent Bioanalyzer 2100[®]

The Bioanalyzer 2100[®] is the first commercially available chip-based nucleic acid analysis system. Using microfluidic technology, 1 μ l of sample is passed through a micro-channel filled with sieving polymer and a fluorescent dye. An electrical voltage is applied which causes the sample to migrate through the channel etched into the chip surface. As the sample moves, DNA fragments are separated by size and detected in real-time, allowing almost instant data analysis and manipulation. A ladder containing fragments of known sizes and concentrations is used to generate a standard curve of migration time versus fragment size. An electropherogram is produced which plots fluorescence intensity versus migration time which can also be displayed as a “virtual gel” using a densitometry plot (Figure 4).



Figure 4 – *The Agilent 2100 Bioanalyzer[®] and high-sensitivity DNA assay components. Beside the instrument, a vortex and chip priming station are used to prepare samples for analysis. The 2100 work area software has several tabs which display data for evaluation of chip runs that can be presented as result tables, gel-like images, histograms, dot plots, or, as above, electropherograms. (Photo copyright Agilent Technologies Inc.).*

Analysis with the Bioanalyzer yields several major advantages over traditional analysis techniques. Primarily, analysis of DNA fragments provides not only information about the concentration of DNA but also the size range of fragments which constitute the nucleic acid sample. An example of the fragmentation profile of chromatin extracted from HEC50 cells using the Chromatrap CHIP-Seq kit is shown in Figure 5.

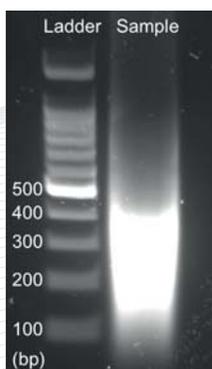
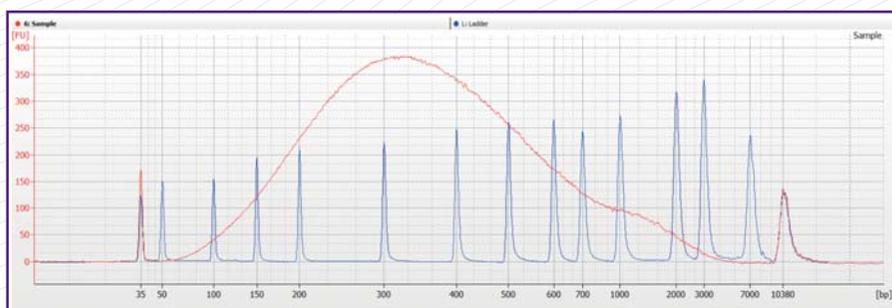


Figure 5 – Sonicated chromatin (red) was analysed using the Bioanalyzer 2100 using the DNA high sensitivity chip (below). Ladder (blue) was overlaid on the measured sample as an internal reference. The corresponding sample as seen on a traditional agarose gel stained with SYBR® Safe (left).



Whilst the Bioanalyzer 2100® does provide qualitative and quantitative information about DNA, it does come at a price. The initial outlay cost of a Bioanalyzer 2100® is approximately 3 times that of the NanoDrop 2000™ and over 10 times the cost of the Qubit 2.0®. Additionally, the cost in the UK of a high sensitivity DNA kit is £312 for 110 samples, equating to £2.84 (\$4.50) per sample analysed. On the other hand, the cost of analysing individual samples using the Qubit dsDNA HS assay and NanoDrop 2000™ are 30p (50c) and effectively zero, respectively. When deciding on which instrument(s) you will choose to use, price is a major factor for many labs. However, the accuracy of quantification should not be substituted for cost. In the long-term, the accurate validation of DNA now will save time and money in the future.

Conclusion

For the accurate quantification of DNA critical for downstream processes, it is highly recommended that a fluorometric method is used. The advantages of the Qubit 2.0® and Bioanalyzer 2100® are clear with respect to sensitivity and the sizing capability of the latter. The NanoDrop 2000® does have the potential to be a cheap and reliable way of determining the crude quantity of DNA or RNA in your sample. But, for applications that require the greatest accuracy when quantifying DNA, such as NGS, it is essential to use fluorescence-based quantification as standard.

Table 1 below compares the quantification parameters of the NanoDrop 2000™, Qubit 2.0® and Bioanalyzer 2100®, the latter two of which use fluorescence-based quantification.

	NanoDrop 2000™	Qubit 2.0® fluorometer	Bioanalyzer 2100®
Quantification method	UV absorbance measurements	Fluorescence-based dye that binds specifically to DNA, RNA or protein	Fluorescence-based dye that binds specifically to DNA, RNA or protein
Specificity for DNA or RNA	Non-discriminatory – cannot distinguish between nucleic acid species	Accurately quantifies DNA and RNA independently. Accurately quantifies dsDNA in the presence of ssDNA	Accurately quantifies DNA and RNA independently
Accuracy at low concentrations	Significantly overestimates concentration	Sufficiently sensitive to accurately quantify 10 pg/µl	Sufficiently sensitive to accurately quantify 5 pg/µl
Sensitivity and range	2-15,000 ng/µl	Effective sample concentration range of 1-500 ng/ml	Listed as 5-500 pg/µl but up to 5 ng/µl for fragmented DNA
Additional data generated	Purity indication from 260:280 nm ratio	None	Qualitative information about fragment sizes and concentration within a specific size range
Standard curve	Not required. Beer-Lambert equation used	Single-point standard curve	Ladder and upper and lower markers included in each run
Unit	Requires PC with software	Standalone	Requires PC with software
Footprint/weight	14 cm x 20 cm / 2.1 kg	13 cm x 21 cm / 0.3 kg	16 cm x 41 cm / 10 kg



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