Fast & accurate DNA quantification with Lunatic

Introduction

Fluorescent dye-based assays are one of the predominant methods for quantifying nucleic acids, but they're bogged down by a lot of steps. If anything goes wrong along the way, the assay needs to be repeated, wasting time and valuable sample. In the typical PicoGreen™ workflow, a DNA standard is serially diluted in the linear range of the fluorescent dye. Then, the standards are diluted in a working solution of the dye to create a standard curve. Finally, the samples are diluted in the working dye solution and the DNA concentration is determined. This process is time-consuming and error-prone, and can also yield inaccurate results if the samples are in an incompatible buffer or saturate the assay.

Lunatic makes nucleic acid quantification easy with a wide dynamic range that can measure concentrations from 1.5–13,750 ng/µL, with no need to fuss with dyes or extra reagents. Maximize your throughput with 96 samples on Lunatic, performing high-speed UV/Vis spectral analysis with just 2ul of sample. The unique microfluidic circuits molded into Lunatic plates ensure that there's no cross-contamination or evaporation. Worried about contaminants in your sample affecting your results? Lunatic's Unmix algorithms allow you to accurately quantify your nucleic acids in the presence of a variety of common impurities and buffer components, so you can move forward with confidence.

This technical note describes how you can use Lunatic to accurately measure DNA concentration using the appropriate application for your workflow.

Streamline your genomic quant

The fluorescent signal from nucleic acid-binding dyes can be affected by impurities like detergents and salts, which can result in mis-reported concentration values. These impurities are commonly introduced during preparation of nucleic acid samples, sometimes by purification kits. Lunatic can handle clean or messy samples alike, enabling quantification of DNA samples at high throughput. With a dynamic range that covers 0.03 to 275 OD and a capacity to measure up to 96 samples, you can run your nucleic acid samples faster. There's no need to prep dyes, run a standard curve, or introduce pipetting error from sample dilutions to reach the linear range of the assay. On top of all that, Lunatic reports purity information along with your DNA quantification, to indicate whether impurities like protein or buffer components are contaminating your sample.

Figure 1: Lunatic: The next-gen UV/Vis reader for DNA quantification. Lunatic uses an SBS-compatible plate format and reads up to 96 samples in 5 minutes.

Lunatic is a UV/Vis reader that can measure the concentration of up to 96 samples in only 5 minutes. Pipet your samples right into the Lunatic plate or integrate it with a liquid handler to automate the whole workflow. An optional 21 CFR Part 11 package is available for the Lunatic system if you need to ensure compliance. The full UV/Vis spectrum is always measured from 230 to 750 nm and the software includes a suite of applications specifically designed for quantifying nucleic acids.

Lunatic applications

Classic A260 for dsDNA measurements

Purified DNA samples with minimal co-absorbing impurities, like proteins or RNA, are ideally suited for UV/Vis quantification. In the classic A260 application on Lunatic, Rayleigh scattering is accounted for across the entire spectrum, providing more accurate quantification than conventional single-wavelength background correction. Applying a wavelength-specific scatter correction is particularly useful for slightly turbid samples, which can arise from bead carryover after purification. After baseline correction, the absorbance value at 260 nm and the dsDNA concentration factor of 50 are used to calculate dsDNA concentration, c, as follows:

$OD260 \times 10 \times 50 = c$

A260/A280 and A260/A230 ratios are also calculated, which provides quality information. Lunatic gets your dsDNA concentration and purity measurements spot-on.

Method: A stock solution of calf thymus DNA was prepared in Tris-EDTA buffer (TE buffer), pH 8.0 at 900 ng/µL, then diluted to samples of approximately 100 ng/µL. 500 nm NIST traceable size standard beads were added to the DNA solution to a final concentration 1,000-fold diluted from stock. A sample of pure dsDNA was also prepared without beads. 2 µL of sample was loaded in triplicate into a High Lunatic plate using TE buffer as a blank. The samples were measured using the "dsDNA (Turbidity)" application on Lunatic. The data of the bead-spiked sample was recalculated using the "dsDNA (Single point)" application to compare concentration results with the conventional single-wavelength background correction method.

Results: The on-screen results for the A260 dsDNA application of a pure dsDNA sample (Figure 2) and a sample spiked with beads (Figure 3A) show the baseline-corrected UV/Vis spectrum (black line) after subtraction of the turbidity profile (gray line). After recalculation, Lunatic displays the UV/Vis spectrum (black line) of the bead-spiked sample after subtracting the absorbance at 340 nm with the dsDNA (Single point) application (Figure 3B). Comparison of the DNA concentration results indicates a higher reported concentration from the dsDNA (Single point) application. An average A260/A280 ratio of approximately 1.8 and A260/A230 ratio greater than 2 were also determined for each application, indicating a pure DNA sample.

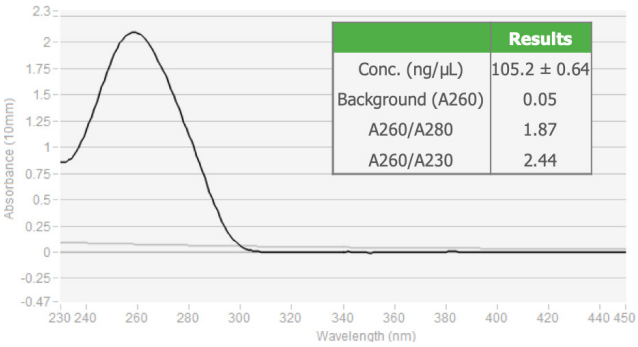
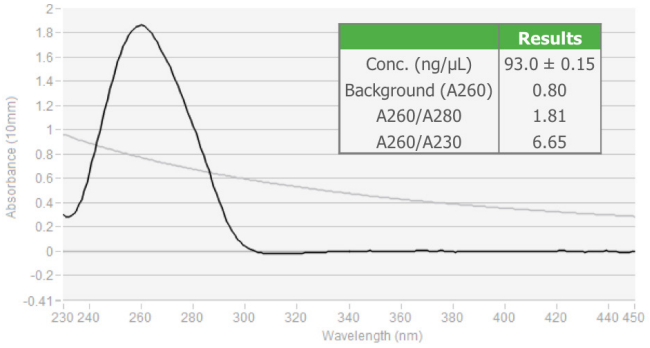


Figure 2: Representative absorbance spectrum of calf thymus DNA in 1X TE buffer. The inset table displays the average concentration ± SD of 3 replicates, background at 260 nm, A260/A280 and A260/A230 ratios.

Check sample purity with Unmix applications

DNA extractions can contain a complex mixture of DNA, RNA, protein, buffer components, and even cell debris. Each component has its own absorption characteristics and these impurities often confound quantification by UV/Vis spectrophotometry. An A260/A280 ratio of approximately 1.8 and an A260/A230 ratio greater than 2 indicate "pure" DNA. In addition to absorbance ratios, Lunatic provides a complementary method to determine DNA purity. Unmix applications quantify DNA concentration with proprietary algorithms. The spectral features in the raw absorption spectrum are analyzed and the contribution from DNA is determined in the presence of co-absorbing impurities. The "DNA (Tissue/blood)" Unmix application on Lunatic is designed for DNA extracted from tissue or blood and measures DNA concentration in samples which may contain RNA. The "Nucleic Acids (DNA equiv.)" application works for DNA from any source and measures total nucleic acid concentration in the presence of protein. Both of these Unmix applications handle buffer components including phenol and thiocyanate salts, commonly found in samples after phenol-chloroform, DNAzol™ or TRIzol™ extractions.

A



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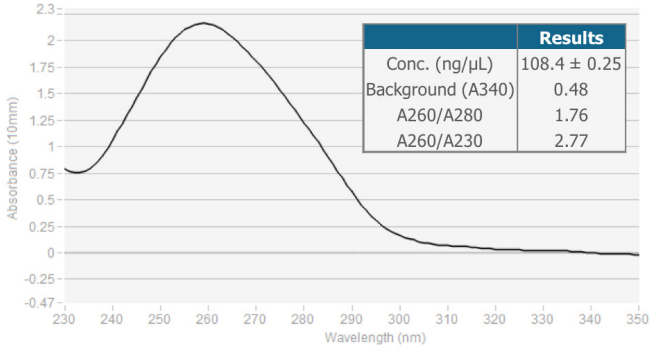

Figure 3: Representative absorbance spectra of calf thymus DNA in 1X TE buffer with 1000-fold diluted 500 nm NIST standard polystyrene beads using the A260 dsDNA application **(A)** or the dsDNA (Single point) application **(B)** on Lunatic. The inset tables display the average concentration ± SD of 3 replicates, background at 260 nm, A260/A280 and A260/A230 ratios.

Method: Calf thymus DNA was diluted to approximately 100 ng/µL in TE buffer, pH 8.0 and combined with approximately 50 ng/µL calf liver RNA or 0.1% bovine serum albumin (BSA). 2 µL of each sample was loaded in quadruplicate into a High Lunatic plate using water as a blank. The samples containing RNA were measured using the "DNA (Tissue/blood)" application on Lunatic. Samples with BSA were measured with the "Nucleic Acids (DNA equiv.)" application.

Results: The on-screen results from the DNA (Tissue/blood) application of a sample containing DNA and RNA show the raw UV/Vis spectrum (black line) and the deconvoluted spectra of the sample components (Figure 4). The DNA spectrum (green line) is separated from the impurity spectrum (blue line). In this case, the Unmix algorithm was able to identify RNA as an impurity and report its concentration. The residue (yellow line) indicates the quality of fit of the deconvolution algorithm to the measured spectrum.

The on-screen results from the Nucleic Acids (DNA equiv.) application of a sample containing DNA and protein show the raw UV/Vis spectrum (black line) and the deconvoluted spectra of the sample components (Figure 5). The nucleic acids spectrum (purple line) is separated from the impurity spectrum (blue line) and nucleic acid concentration is reported as DNA equivalents. This application is suited for nucleic acid samples that may have contaminating protein, phenol, or guanidine buffer salts present. The impurity

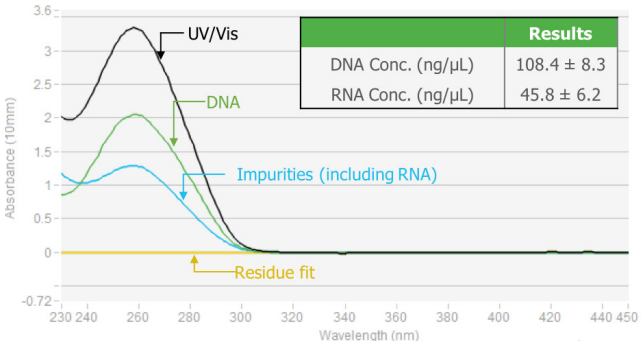

Figure 4: Deconvoluted absorbance spectra of calf thymus DNA and calf liver RNA in TE buffer using the DNA (Tissue/blood) application on Lunatic. The inset table lists the average DNA and RNA concentrations ± SD of 4 replicates.

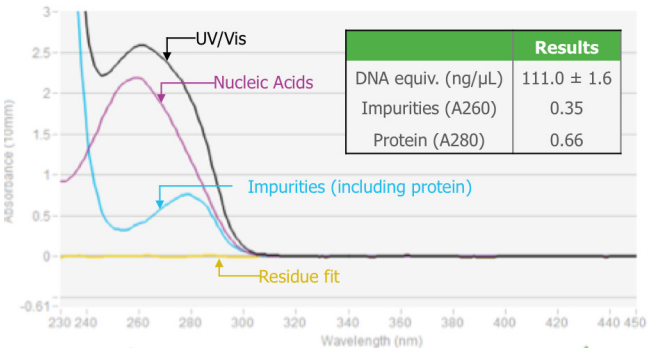

Figure 5: Deconvoluted absorbance spectra of calf thymus DNA and 0.1% BSA in TE buffer using the Nucleic Acids (DNA equiv.) application on Lunatic. The inset table lists the average nucleic acid concentration ± SD of 4 replicates. The absorbance of impurities is reported at 260 nm and 280 nm as determined by the Unmix algorithm.

contributions at 260 and 280 nm are displayed with the 280 nm component attributed to protein contamination (inset table).

Lunatic spots what dyes miss

Qubit™ and PicoGreen are fluorescent nucleic acid dyes commonly used for quantification, with high sensitivity and specificity for dsDNA. However, these dyes have some drawbacks. Impurities like salts or detergents in the dsDNA sample can increase or decrease the fluorescent signal, depending on the component. Concentrations as low as 100 mM NaCl interfere with binding between dsDNA and the PicoGreen dye, significantly reducing fluorescent signal.[1,2] This can lead to under-reporting of DNA concentrations. If a sample contains a low concentration of nucleic acids, a higher sample volume needs to be used in the fluorescence assay to ensure the measurement is within the linear range. Fluorescent dye assays do not have purity metrics, like the A260/A280 and A260/A230 ratios provided by UV/Vis assays, so there is no way to know if a sample will adversely impact fluorescence-based quantification. Using just 2 µL of sample, Lunatic accurately quantifies nucleic acid samples in the presence of a wide range of impurities. Purity information with absorbance ratios and sample deconvolution with Unmix algorithms can help qualify samples just prior to fluorescence-based quantification.

Method: Calf thymus DNA was diluted to approximately 100 ng/µL in TE buffer, pH 8.0 from a stock solution and combined with none or one of the following impurities to the indicated final concentration: 50 ng/µL calf liver RNA, 400 mM NaCl or 2.75 mM EDTA. 2 µL of each sample was loaded in quadruplicate into a High Lunatic plate using water as a blank and measured with the "DNA (Tissue/blood)" application on Lunatic. 2 µL of each sample was also measured in quadruplicate with the Qubit dsDNA BR Assay Kit and 1 µL of each sample was measured in quadruplicate with the Quant-iT™ PicoGreen dsDNA Assay Kit. Both fluorescent assays were performed with a Qubit fluorometer.

Results: DNA concentration values were measured by the "DNA (Tissue/blood)" application on Lunatic and compared to Qubit and PicoGreen quantification methods (Figure 6). Concentration values correspond closely to the theoretical value (red line) in samples comprised of 100 ng/µL DNA in the presence or absence of excipients other than NaCl. Qubit and PicoGreen significantly under-reported the DNA concentration in a sample containing NaCl while the measurement by Lunatic was more accurate.

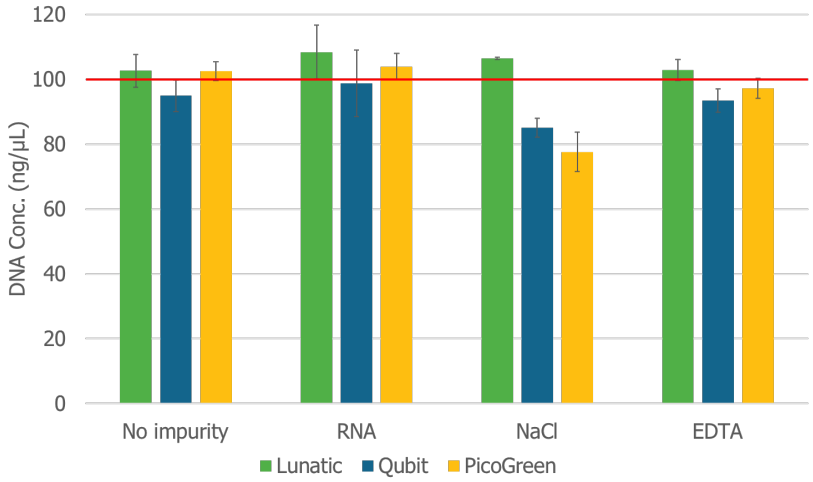

Figure 6: Comparison of dsDNA samples spiked with impurities measured by UV/Vis with Lunatic and fluorescence by Qubit and PicoGreen assays. Average values of 4 replicate measurements are shown, error bars denote the SD. The red line at 100 ng/µL indicates the theoretical DNA concentration in each sample.

Summary

With the capability to measure dirty or pure samples in a fast, high-throughput, and reproducible manner, Lunatic is a game-changer for genomics. No need to waste time prepping fluorescent dyes or adjusting samples to make sure you're in the linear range. Never again fret over incompatible impurities, mis-calibrating a standard or introducing pipetting error. Lunatic uses just 2 µL and quantifies your nucleic acid samples with a large dynamic range spanning 0.03 to 275 OD while measuring sample purity. The unique Lunatic plates protect your samples, which means no cross-contamination or evaporation. Measure up to 96 samples in only 5 minutes. Lunatic is complete with built-in applications that make it easy to measure DNA concentration from any source.

References

1. Characterization of PicoGreen interaction with dsDNA and the origin of its fluorescence enhancement upon binding, Al Dragan, et al., Biophysical Journal, 2010; 99(9):3010–9.

2. Characterization of PicoGreen reagent and development of a fluorescence- based solution assay for double-stranded DNA quantitation, VL Singer, et al., Analytical Biochemistry, 1997; 249(2):228–38.

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