

Comparing Different DNA and RNA Quantification Methods for Biological Samples with Low Nucleic Acid Abundance

Z. Haj-Ahmad², B. Lam, PhD¹ and Y. Haj-Ahmad, Ph.D¹

¹Norgen Biotek Corporation, Thorold, Ontario, Canada

²Centre for Biotechnology, Brock University, St. Catharines, Ontario, Canada

INTRODUCTION

After the purification of nucleic acids (RNA or DNA) from biological samples, it is common to perform a quantification or quality assessment of the isolated RNA or DNA. Downstream applications of nucleic acids such as expression analysis using PCR, Microarrays or Next Generation Sequencing (NGS) often require particular quantities and purities for optimal performance.

Though there are several methods for quantifying nucleic acids, most techniques rely on measurements of absorbance (optical density). In spectrophotometric analysis, absorbance readings are performed at 260 nm (A260). However, traditional spectrophotometry may only be used to quantify nucleic acids down to 10-50 ng. Microvolume spectrophotometers, such as the Thermo Scientific NanoDrop, have a limit of detection in the 1-10 ng range. To increase the sensitivity of quantification, dyes are often used. Fluorescent dyes, such as RiboGreen or PicoGreen, exhibit little fluorescence and possess a negligible absorbance signature in their free form. When bound to nucleic acids, the dyes fluoresce with an intensity that can be measured to determine nucleic acid content, with a limit of detection at about 1 ng. Additional technologies, such as the Agilent Bioanalyzer's Small RNA assay may detect quantities in the 50 pg range. However, this may not completely overcome the difficulties in quantifying RNA or DNA from liquid biopsies where the expected yield could be in the lower pg or sub-pg per μ L range.

For low input samples such as exosomes, plasma, and urine, which have RNA/DNA yields that are well below the detection limit of many of the aforementioned methods, Norgen has developed two PCR-based kits for quantification: the Low Abundance DNA Quantification Kit (Cat# 57200) and the Low Abundance RNA Quantification Kit (Cat# 58900). These kits can quantify RNA or DNA of a wide spectrum of concentrations, including the pg per μ L and sub-pg per μ L range. The kits consist of a proprietary primer mix which, when used with the provided 2x PCR Master Mix, can amplify human RNA and DNA from a variety of inputs (such as different liquid biopsies). The unknown DNA is accurately quantified by using a standard curve constructed from the provided RNA or DNA

Standard. Here we demonstrated the accurate, sensitive quantification of DNA and RNA with Norgen's kits, using a blind test with numerous experimenters. Norgen's novel quantification kits for RNA and DNA provide better quantification of RNA or DNA of low abundance as compared to other quantification methods, in particular from samples known to have low nucleic acid quantity such as plasma/serum and urine.

MATERIALS AND METHODS

Test DNA/RNA Purification

HeLa DNA was prepared using Norgen's Cell and Tissue DNA Isolation Kit (Cat# 53100). Plasma DNA was prepared using Norgen's Plasma/Serum Circulating DNA Maxi Kit (Slurry Format) (Cat# 51300). HeLa RNA was prepared using Norgen's Total RNA Purification Kit (Cat# 17200). Plasma RNA was prepared using Norgen's Plasma/Serum Circulating RNA Mini Kit (Cat#55000).

Test DNA/RNA Quantification

Three experimenters were given blind samples of purified DNA and RNA (with pre-determined concentration) and quantified the DNA and RNA using either Norgen's Low Abundance RNA Quantification Kit (Cat# 58900), Norgen's Low Abundance DNA Quantification Kit (Cat# 57200), the PicoGreen/RiboGreen assay, or NanoDrop.

RESULTS AND DISCUSSION

Dynamic Range of RNA and DNA Quantification Methods

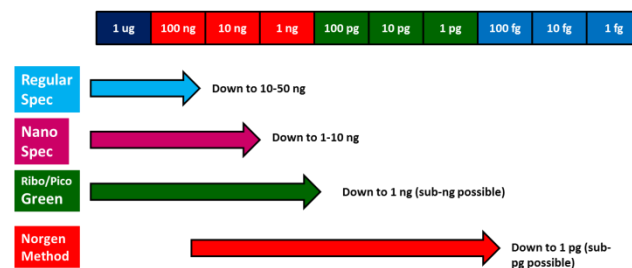


Figure 1. Sensitivity of DNA and RNA Quantification using Norgen's Low Abundance DNA Quantification Kit and Low Abundance RNA Quantification Kit Compared to Other Methods. A diagram representing the dynamic range of different RNA or DNA quantification methods is presented here. Norgen's Low Abundance RNA Quantification Kit and Low Abundance DNA Quantification Kit can quantify purified RNA or DNA from low abundance samples in the pg and sub-pg range.

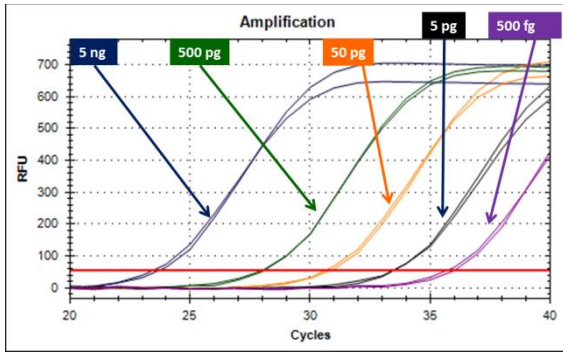


Figure 2. Sensitivity of DNA Quantification in the Picogram Range Using the Low Abundance DNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of a DNA standard dilution series. Norgen's Low Abundance DNA Quantification Kit can quantify purified DNA from low abundance samples such as liquid biopsies (plasma or urine). As little as 500 fg of DNA could be quantified using Norgen's kit.

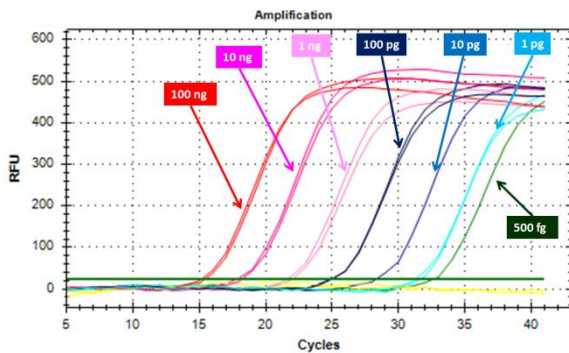


Figure 3. Sensitivity of RNA Quantification in the Picogram Range using the Low Abundance RNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of an RNA standard dilution series. Norgen's Low Abundance RNA Quantification Kit can quantify purified RNA from low abundance samples such as liquid biopsies (plasma or urine). As little as 500 fg of RNA could be quantified.

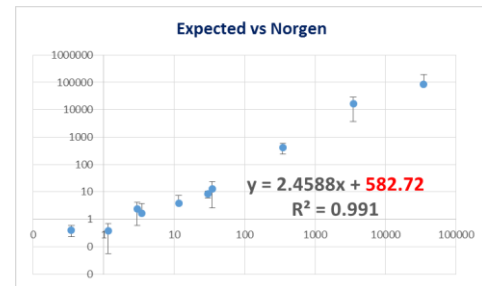
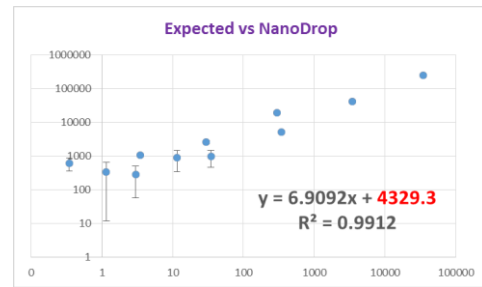
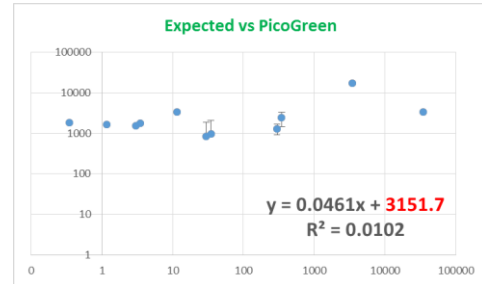


Figure 4. Comparison of Expected DNA Amount and Norgen's Low Abundance DNA Quantification Kit, NanoDrop Method, and PicoGreen Method (All Experimenters Combined). Blind DNA samples were provided to three experimenters to quantify using Norgen's kit, NanoDrop and PicoGreen. The expected results and the observed results were plotted. The most accurate method should yield a slope closer to 1 and a y-intercept closer to 0. Among the three methods, Norgen's kit yielded the closest slope and y-intercept of 2.46 and 582.77, respectively. Therefore, Norgen's kit achieved the most accuracy in DNA quantification. Graph axis unit is in pg/ μ L.

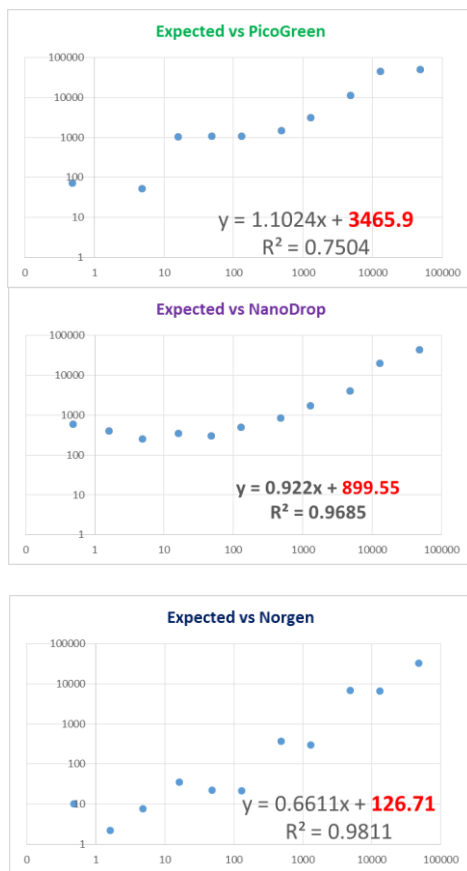


Figure 5. Comparison of Expected RNA Amount and Norgen’s Low Abundance RNA Quantification Kit, NanoDrop Method, and PicoGreen Method (All Experimenters Combined). Blind DNA samples were provided to three experimenters to quantify using Norgen’s kit, NanoDrop and RiboGreen. The expected results and the observed results were plotted. The most accurate method should yield a slope closer to 1 and a y-intercept closer to 0: among the three methods, Norgen’s kit yielded the closest slope and y-intercept of 2.46 and 582.77, respectively. Therefore, Norgen’s kit achieved the most accuracy in RNA quantitation. Graph axis unit is in pg/ μ L.

CONCLUSIONS

1. Norgen’s Low Abundance DNA Quantification Kit and Norgen’s Low Abundance RNA Quantification Kit allow for accurate, sensitive quantitation of nucleic acid samples.
2. Norgen’s Low Abundance DNA Quantification Kit and Norgen’s Low Abundance RNA Quantification Kit outperform the NanoDrop and PicoGreen methods of quantitation, achieving more accurate detection at low nucleic acid concentrations