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Project Title: A Comparison of Quantitative Efficiencies of the Applied BioSystems 7500 Sequence Detection System (Real-Time PCR) versus NanoDrop Instrumentation

Abstract

Accurate DNA quantitation is important in the analysis of forensic DNA evidence to properly amplify and develop a profile and is currently achieved using RT-PCR. The Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer is a more cost-effective method for quantifying DNA. The ABI 7500 SDS RT-PCR instrument has been found to be very sensitive and requires minimal sample, but there is a high cost associated with the running of each sample. The extraction kit alone costs approximately \$3.20 for each reaction. The Nanodrop is established for its ability to measure the absorbance of small volumes, having a wide detection range, providing quick measurement, all with low cost. This study is a comparison of the precision of the NanoDrop One to the Applied Biosystems 7500 Sequence Detection System (SDS). Following IRB approval, dilution series ranging from 0.625 ng of total DNA to 10 ng of total DNA were prepared using extracted buccal samples. Replicates of the dilution samples were measured on both instruments for a total of 100 samples. The results showed that the RT-PCR method is more precise and thus better suited for low level forensic samples. The average deviation of the measured concentrations of each sample for each dilution concentration ranged from 0.13 to 0.23 ng of DNA for the NanoDrop results. The RT-PCR results showed an average standard deviation range for the same samples of 0.008 to 1.9 ng of DNA. The NanoDrop was determined to have a more consistent variation in its measurements for all the dilution concentrations, while the precision of the RT-PCR method increased as the amount of DNA decreased. As the nature of forensic evidence is minimal in quality, concentration, and quantity, confidence in quantification is critical. It is possible that the NanoDrop is sufficient for the quantification of DNA in exemplars since there is inherently more DNA usually present, but further research would need to be performed to assess the accuracy of the NanoDrop with lower level evidentiary DNA samples.

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Bio

Raeghan is a senior in the Forensic Science program at the University of New Haven. In addition to being in the Honors Program, she is a sister and Academic Chair of Chi Kappa Rho. Her post-graduation plans are to pursue a Master's degree in Cellular and Molecular Biology.

