

Applied Biosystems 7500 Real-Time PCR System Forensic Validation with the Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit Assays

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Real-Time PCR DNA quantification is performed prior to STR analysis as a means of providing information as to the quantity and nature of forensic DNA samples. In 2003, Real-Time PCR was introduced to the Human Identity community through the validation study of the Quantifiler™ Human DNA Quantification kit and the Quantifiler™ Y Human Male DNA Quantification kit used with the ABI PRISM® 7000 Sequence Detection System v1.0. This validation introduces the newly released Applied Biosystems 7500 Real-Time PCR System with v1.2.3 software with the Quantifiler™ Kits. Validation experiments that were carried out according to the Scientific Working Group on DNA Analysis Methods (SWGDM) guidelines focused on instrument performance parameters, i.e. precision, accuracy, reproducibility, sensitivity and background, relevant to the intended use of the Quantifiler™ kit assays. Standard curve replicates were analyzed on each of three 7000 and three 7500 instruments. The cycle threshold (CT) values, R2 values, and slopes were compared statistically to determine precision and accuracy which established 95% confidence intervals for each platform. To demonstrate reproducibility and sensitivity, replicate DNA samples were quantitated in six separate Real-Time PCR runs and compared statistically between each platform. To test background detection on the instruments, 95 no-template controls were amplified along with one positive control for each of the Quantifiler kits on all of the instruments. The data generated in the validation studies demonstrated that the 7500 Real-Time PCR System provided accurate results when used in conjunction with the Quantifiler™ kits for the analysis of genomic DNA samples and produced results which were statistically similar to the results produced on the previously validated ABI PRISM® 7000 Sequence Detection System.