WORKFLOW OPTIMIZATION USING APPLIED BIOSYSTEMS® NEW QUANTIFILER® TRIO IN CONJUCTION WITH THE DEVELOPMENT AND USE OF AN EXTERNAL STANDARD CURVE

Megan M. Boll, M.S.F.S; Kristen L. Smith, M.S.F.S.; Christian G. Westring, Ph.D., NMS Labs

Continued increases in sample submissions to DNA crime labs has necessitated the development of progressively faster methods that involve less hands-on analyst time, and yield informative results accurately and reliably. DNA quantitation is one step in the process that can have a large impact on sample to result time when used either as a screening technique, or as a reliable source for the appropriate downstream testing. Current methods and quantitation kits being utilized have become increasingly more accurate and have been found to generate more reproducible results than previously utilized techniques. Methods such as real-time quantitative PCR have been improved to provide additional information beyond an estimation of total DNA in a sample. They can now accurately target both total human DNA and human male-specific DNA in a sample, in addition to simultaneously targeting areas that can identify potential quality concerns. Applied Biosystems® has recently developed a new quantitation assay, Quantifiler® Trio. This assay simultaneously provides a qualitative and quantitative assessment of total human and male-specific DNA through the use of both small and large autosomal target loci, as well as a locus on the Y-chromosome.

Although these advancements have a positive impact on the outcome of DNA analysis, there still remains a considerable amount of time devoted to the set-up of the assay and reagent consumption, especially with the preparation of standards. A standard of known DNA concentration is serially diluted to provide a range of DNA concentrations against which the unknown samples are measured. Currently, this standard curve is prepared and run with each assay performed. This process may result in significant variation in the parameters of a standard curve, which can lead to subsequent error in determining the DNA concentration of a sample. A slight error in a quantitation result can further lead to less than optimal STR genotyping results.

In this study, multiple quantitation assays were performed using full and half-scale quantitation reactions. A series of dilutions were prepared from extracted single source DNA to assess the correlation between a low or undetermined quantitative value and the success of the STR profile generated. The accuracy seen in the full and half-scale reactions were compared. To determine the assay's reliability in detecting male DNA concentration when present in an abundance of female DNA, male:female mixture samples were prepared from multiple known contributors at different ratios and quantitated.

A set of samples was taken from aged-dried blood to simulate degraded samples to evaluate the degradation prediction feature of the Quantifiler® Trio quantification kit. To save on future preparation time, an external standard curve was generated by using the run specific standard curves from each assay. These curves were used to perform a linear regression on the cycle threshold values. Resulting quantitation values generated with the use of an external standard curve were compared to the initial quantitation values generated using the run-specific standard curve.

Based on the quantitation values obtained from lower level samples and the resulting DNA profiles, Quantifiler® Trio was found to be accurate at determining the DNA concentration. This allows for a higher success rate in determining the outcome of the subsequent DNA profiling of

a sample. This level of accuracy is especially necessary when using Trio as a screening tool. The half-scale reaction samples were found to be more accurate and precise to their target value in comparison to the full-scale reaction. For the remainder of the study, half-scale reactions were utilized. Quantitation results generated from replicates with a DNA concentration as low as 0.782 pg/µL were accurately determined. Quantifiler® Trio was found to be reliable at quantitating a small amount of male DNA in the presence of a much higher female contributor. A successful male quantitation value was seen at a ratio of 1:1000 male:female DNA. The ability of the kit to identify samples that are possibly degraded was found to be a useful and reliable tool as well. An external standard curve was successfully validated by using ten previous run-specific standard curves. There was no significant difference in the quantitation values obtained for samples run with an assay-specific standard curve and those with an external standard curve. By implementing both Quantifiler® Trio and an external standard curve, a laboratory can greatly decrease the cost and amount of time needed for quantitation, while also increasing the successfulness of DNA typing.