NANOGRAMS OR COPY NUMBER: DETERMINING DNA TEMPLATE AMOUNT FOR THE POWERSEQ™ CRM NESTED SYSTEM

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Through collaboration with the Battelle Memorial Institute, the Ohio Attorney General's Office Bureau of Criminal Investigation (BCI) validated massively parallel sequencing (MPS) based mitochondrial DNA (mtDNA) testing for Missing Person's casework. New methods and technology can create difficult workflow choices for a laboratory which may have long term effects on casework procedures. Validating MPS-based mtDNA testing required determining if amplification template DNA amount would be calculated based on ng/µl of genomic DNA or copy number of mtDNA.

When initially validating the PowerSeq™ CRM Nested System, a MPS-based control region mtDNA kit, the Kavlick Assay[a] was utilized. The Kavlick assay is a hybrid absolute/relative qPCR method which yields information on the quality and amount of mtDNA present in a DNA sample. Use of this method allows for sample dilutions to be based on mtDNA copy number instead of genomic DNA input. While the assay proved particularly useful for the validation, the requirement of ordering numerous reagents from multiple vendors and preparing large amounts of dilutions placed a new burden on quality control. In an effort to decrease quality assurance testing, the PowerSeq™ CRM Nested kit was also tested with the use of Quantifiler™ Trio based input amounts.

Two sets of serial dilutions were tested; one based on mtDNA copy number and the other based on total genomic DNA. The PowerSeq™ CRM Nested kit was determined to be robust enough to accommodate samples with a wide range of inputs. The data shows that at the lowest Quantifiler™ Trio values, full haplotypes were obtained. Additionally, a very broad range of mtDNA copy number inputs produced full and accurate haplotypes. This study shows that the PowerSeq™ CRM Nested performs equally well whether the amplification input is calculated in mtDNA copy number or total human DNA amount. As a result, the amplification input amount will be calculated based off of Quantifiler™ Trio data for routine casework applications. This will aid seamless integration of the MPS workflow with laboratory practices currently in place at Ohio BCI.

[a]Kavlick, Mark F. *Development of a triplex mtDNA qPCR assay to assess quantification, degradation, inhibition, and amplification target copy numbers*. Mitochondrion, Volume 46, 2019, Pages 41-50.