B6 A Comparison of InnoQuant® and Quantifiler® Trio as Quantification/Degradation Methods for Predicting Next Generation Sequencing (NGS) Success

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Learning Overview: After attending this presentation, attendees will better understand the relationship between DNA quantification/degradation for forensic-type samples and NGS library yield as it relates to NGS success in obtaining a full profile of Short Tandem Repeat (STR) loci.

Impact on the Forensic Science Community: The results in this presentation will impact the forensic science community by providing information from DNA quantification/degradation that will streamline analysis by helping the analyst to decide to pursue STR or mitochondrial DNA (mtDNA) analysis.

DNA quantification kits can not only provide information on the amount of DNA in a sample, but also on the state of its degradation. Studies have been reported on the accuracy and reproducibility of the InnoQuant® and Quantifiler® Trio quantification methods in comparison with other commercially available DNA quantification kits capable of degradation estimation.[1,2] The Degradation Index (DI) is a ratio of a short and long fluorescent fragment with degraded DNA giving ratios >1. However, there has not been a report on an evaluation of the DI in conjunction with NGS library preparation success rates. This research on DNA quantification will give biologists a better assessment for how challenging samples will perform when subjected to library preparation, the first step for NGS processing. In addition, knowing the DI helps the analyst predict the success of obtaining a full STR profile by conventional Capillary Electrophoresis (CE), but it is not known if this is true for NGS as well.[3] Analysts can use this information to decide whether to pursue mitochondrial DNA (mtDNA) sequencing, which would be more successful in providing information on the source of the sample than STR analysis.

In this work, DNA from samples of blood (5µL homogenized whole blood; treated with either sonication or Ultraviolet (UV) irradiation in an attempt to artificially degrade the DNA; n, 24), bone (50mg homogenized powder; n, 12), and hairs (1cm fragments cut from root end; n, 12) from three donors each was extracted following standard procedures and the DNA was subjected to quantification and degradation analysis in duplicate using the InnoQuant® (IQ) and Quantifiler® Trio (QT) quantification kits. Library preparation, amplification, and indexing was performed using the MiSeq™ FGx Forensic Genomics System with 1ng DNA and Primer Mix B multiplex. After purification, libraries were quantified using the PowerSeq™ Quant MS System. From 90 libraries, 30 were selected to be normalized, pooled, and sequenced on the MiSeq™ FGx instrument with the MiSeq™ ForenSeq™ Sequencing Kit. Sequencing data was analyzed using the ForenSeq™ Universal Analysis Software (UAS) to produce autosomal STR profiles using the manufacturer's default settings (analytical threshold, 4.5% and interpretation threshold, 7.6%).

No correlation was observed between the library yield and the level of the DI in the ranges obtained for both kits. Blood DI values ranged from 0.72–1.8 (IQ) and 0.80–1.7 (QT); bone DI ranged from 2.5–15.9 (IQ) and 1.8–6.8 (QT); and hair DI ranged from 0.76–4.74 (IQ) and 0.76–4.57 (QT). Linear regression analysis was performed to determine whether a relationship existed between the reported DI for both kits and NGS library yields. Results showed weak linear relationships for all sample types from both kits (R^2: 0.0487 [IQ] and 0.1175 [QT] for blood; 0.2167 [IQ] and 0.0926 [QT] for bone; and 0.2176 [IQ] and 0.0495 [QT] for hair).

The amount of DNA used to prepare the library strongly influenced both the library yields and NGS success. Average library yields ranged from 145,000pM (1.0ng), 4,700pM (67–90pg), 3,000pM (1–28pg) and 2,400pM (<1pg). The sequencing success was judged by the percentage of STR loci (n, 28) reported by the NGS analysis software. For example, 99–100% of the STR loci were reported when 1.0ng was used to prepare the libraries from blood and 82% from bone. However, with decreased input, fewer loci were reported (80pg bone: 35–96%; 30pg hair: 71–93%; 8pg hair: 4%).

In summary, this study demonstrated that NGS STR genotyping success appears to be influenced more by the amount of DNA used to prepare the library than by the degradation state of the DNA. The two quantification kits behaved similarly with respect to the DI.

Reference(s):

1. Goecker, Z.C., Swiontek S.E., Lakhtakia A., Roy R. Comparison of Quantifiler Trio and InnoQuant human DNA quanification kits for detection of DNA degradation in developed and aged fingerprints. *Forensic Sci. Int.* 2016;263:132-138.
2. Holmes A.S., Houston R., Elwick K., Gangitano S., Hughes-Stamm S. Evaluation of fur commercial quantitative real-time quantification kits with inhibited and degraded samples. *Int. J. Legal Med.* 2018:132;691-701.
3. LeFebvre A.K., Ayers S., Gahn L., Sinha H., Brown H., Murphy G., et al. Improving STR profile success rates for property crime specimens using InnoQuant Human DNA Quantitation & Degradation Assessment Kit. *J. Forensic Investigation* 2017:5;1-9.

NGS-STRs, DNA Quantitation, DNA Degradation

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