B75 Whole Genome Amplification as a Potential Means for Sample Immortalization

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After attending this presentation, attendees will better understand the evaluation of Whole Genome Amplification (WGA) for DNA sample archiving.

This presentation will impact the forensic science community by highlighting the pros and cons of using WGA to generate abundant quantities of DNA from samples that are currently in limited amounts. Specifically, this study was designed for a database of samples that have been collected as part of the National Institute of Justice-funded research, with ancestry and phenotypic information of the donors. The *immortalization* of these samples will allow sharing them with other researchers to improve ancestry and phenotype prediction models.

The availability of high-quality DNA from the aforementioned samples is limited, as only three cheek swabs were collected per individual. A potential solution to this problem is the use of WGA to increase the concentration of DNA extract. To determine if WGA is a viable solution for maintaining reference samples of limited quantity, the most important parameter is whether WGA can yield a complete and balanced representation of the markers to be genotyped, be it Short-Tandem Repeats (STRs) or Single Nucleotide Polymorphisms (SNPs).

In this study, the REPLI-g® Mini Kit (a strand displacement-based amplification incorporating ϕ29 polymerase) was used to amplify DNA extracted from buccal swabs. Five samples were amplified in triplicate with an input of 2ng while 23 samples were amplified singly with an input of 10ng (the amount recommended by the manufacturer). The amplified product was quantitated using multiple methods: Quantifiler® Human; Quantifiler® Trio; and, an in-house TaqMan® assay targeting a single copy region on Chromosome 5. Quantitation results varied significantly with the three methods, suggesting that the WGA process amplifies the targets of the three TaqMan® assays with varying efficiency. Based on Quantifiler® Human results, WGA yielded an average of 250ng of DNA from a 10ng input (ranging from 28ng to 900ng), whereas with a 2ng input, the reaction yielded an average of 54ng (ranging from 4ng to 208ng). Two samples with an input of 10ng and one sample with an input of 2ng failed to amplify.

The WGA product was then amplified with AmpflSTR® Identifiler® Plus using 0.3ng of input DNA (based on Quantifiler® Human results) in a 5µL reaction volume (conditions optimized for reference samples at George Washington University (GWU)). The WGA product was also genotyped at 50 SNP loci, using SNaPshot® assays developed at GWU with 2ng of input DNA (based on Quantifiler® Human results). Off-scale peak heights and split peaks were obtained from the Identifiler® Plus amplification products, suggesting a possible Quantifiler® Human underestimation of the WGA yield in respect to the STR regions targeted in the assay. This issue is being further investigated.

For WGA-DNA input levels of both 10ng and 2ng, one allele dropped out per profile on average. All profiles showed locus-to-locus imbalance. The average peak height ratio between sister alleles in the 10ng samples was higher than in the 2ng samples. The 2ng triplicates yielded a full consensus profile although each replicate contained one instance of drop out. Of the tested SNPs, 49/50 were successfully genotyped; the failed SNP also failed in the original (non-WGA) DNA extract, possibly due to PCR primer efficiency issues. All peaks were present and distinguishable from artifacts/noise and genotypes were consistent with those obtained from the original DNA extracts.

According to the results obtained, WGA is a promising solution for sample archiving, although caution must be exercised. In this experiment, SNPs appeared more robust than STRs. Further studies evaluating a larger panel of markers with Next Generation Sequencing (NGS) technology, and evaluating higher levels of input DNA, will better assess the potential of WGA for sample immortalization.

As technology rapidly progresses, it is likely that in the foreseeable future new and comprehensive methods for high-level multiplexing of multiple marker types will be available to the forensic community. Thus, future availability of these well-characterized samples will be increasingly important in the development of methods and interpretation guidelines for ancestry and phenotype prediction.

WGA, SNPs, Sample Archiving