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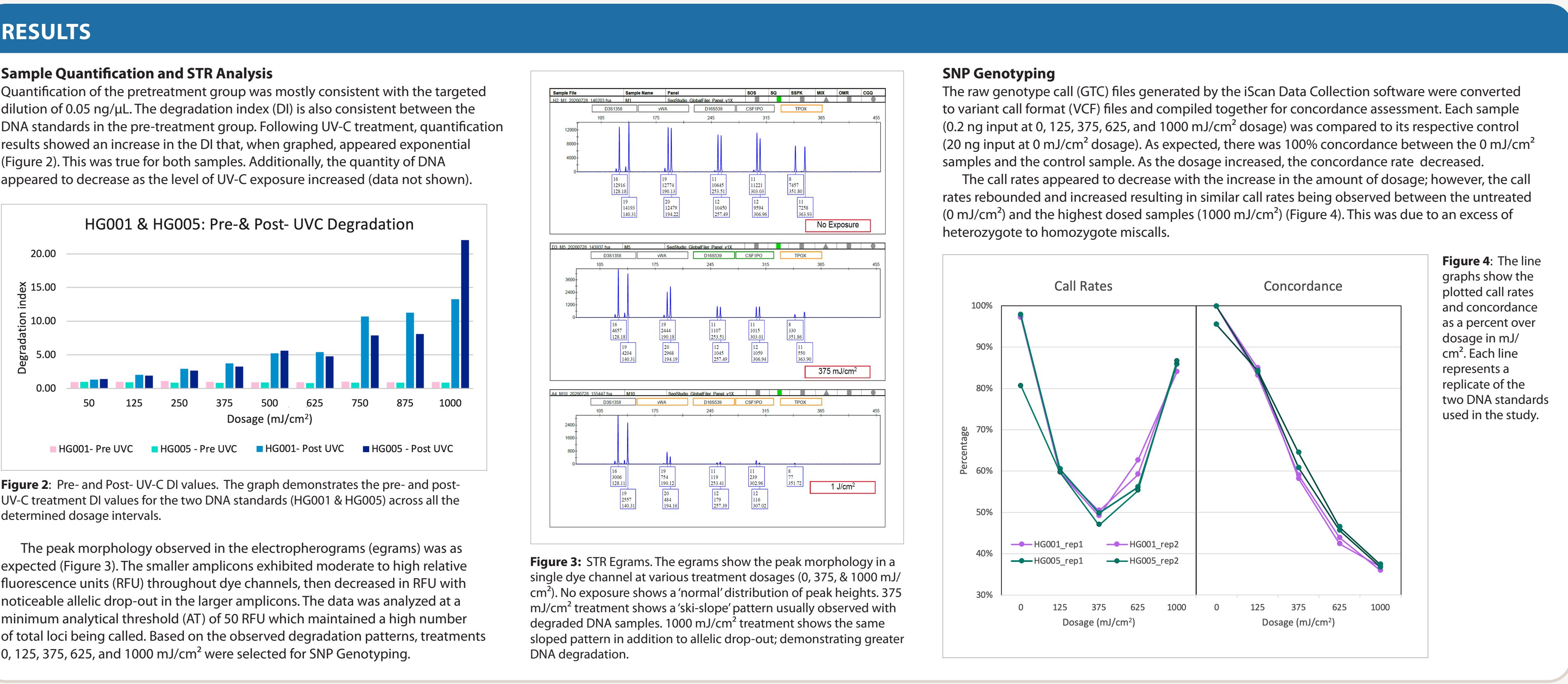
ABSTRACT

The Infinium® assay workflow is a genome-wide microarray genotyping assay that utilizes the BeadChip platform.¹ This accurate and flexible microarray technology allows for the ability to interrogate a large number of single nucleotide polymorphisms (SNP) through unlimited loci multiplexing.^{2,3,4} Previous work to optimize sensitivity of the assay demonstrated a total DNA input <1.0 ng successfully generated high quality genotyping data.

DNA degradation is a common factor affecting forensic DNA samples that are not stored properly, that have been stored for extended periods of time or that have been exposed to the elements (i.e., sunlight or heat). Current short tandem repeat (STR) typing kits can handle DNA degradation providing, in most cases, partial DNA profiles with moderately degraded DNA. For single nucleotide polymorphism (SNP) genotyping analysis, the quality of the data is critical and loss of SNPs due to degradation will affect the ability to accurately search genealogical databases (GEDmatch, FamilyTreeDNA®, etc.). In addition to a larger goal of assessing the Illumina Global Screening Array (GSA), a whole-genome SNP genotyping method, assessment of the array's ability to handle degraded DNA is of main interest and is the focus for this project.

For this study, genomic DNA was experimentally degraded using ultraviolet C (UV-C) light at defined intervals up to 1.0 J/cm². Samples were quantified pre- and post-treatments and typed using GlobalFiler™ to confirm degradation. The STR profiles coupled with the Degradation Index (DI) from the quant method demonstrated that as the dosage increased, so too did the amount of degradation (quantified via visual pattern in the profile and increase in DI). Five (5) samples expressing specific degradation patterns based on STR results were selected for genome-wide SNP genotyping, in duplicate, on the iScan® to evaluate the performance of the Infinium GSA in its ability to accurately type low copy number (LCN) degraded DNA sample types.

Analysis of the SNP data showed similar trends in most aspects. Concordance of the degraded samples did trend down as the dosage amount increased. Concordance was performed between treated samples and a control sample run alongside. One metric used to evaluate the performance of the assay is the call rate, which is a percentage of the total number of SNPs genotyped over the total number of SNP targets in the assay. When evaluating the samples using this metric, the trend was unexpectedly parabolic. As the dosage level increased, the curve trended down then re-bounded and began increasing, resulting in the 0.0 mJ/cm² and the 1.0 J/cm² samples having very similar call rates.



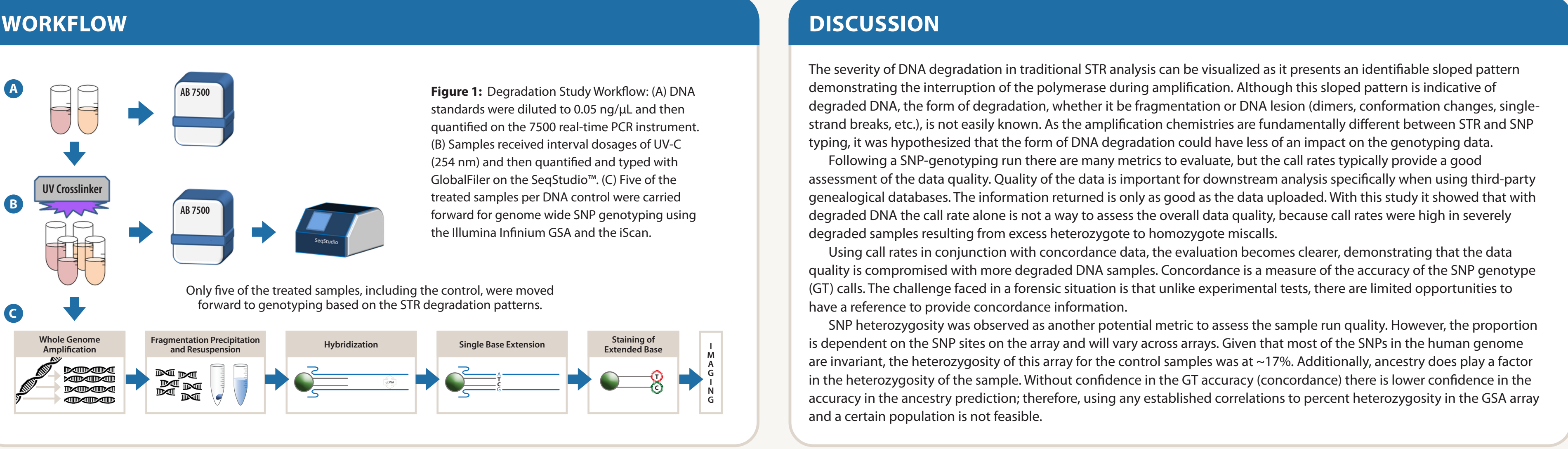
METHODS

Two Coriell standard DNA samples (NIST: HG001, HG005) were prepared with PCR grade water in 0.25 – 1.5 μL tubes. Samples were normalized to 0.05 ng/μL in a volume of 20 μL, of which 2 μL was used for pre-UV quantification. Once prepared, samples were covered in foil to initiate control treatment of UV-C exposure. Samples were stored at 4°C until UV-C treatments. Samples were removed from 4°C, the foil was removed, and the dosage applied.

Each of the prepared samples were quantified prior to and then following treatment using the Applied Biosystems 7500 real-time PCR system with the Qiagen Investigator Quantiplex Pro quantification kit. The samples were initially diluted to a concentration below the recommended target of 1 ng/μL for the GlobalFiler kit.

Genomic DNA (15 μL) was amplified with GlobalFiler with a 1 μL aliquot used for STR typing on the SeqStudio and 4 μL for the SNP-genotyping. Electrophoresis was conducted as a confirmation that UV-C treatments caused degradation to the DNA. STR data was analyzed using GeneMapper® ID-X v1.6 analysis software. Treated samples that displayed degradation in STR profile interpretation were genotyped in duplicate.

Additionally, one sample from each standard was taken forward with no treatment at 20 ng/μL as a reference for concordance. Genotyping took place using the Infinium HTS assay workflow using the GSA kit and scanned on the Illumina iScan. The data generated was analyzed in GenomeStudio (GS). Concordance and call rate analysis was performed using BCFtools and R.



CONCLUSIONS

A greater number of SNP sites that are called allows for more comparable sites when doing downstream analyses (ancestry, phenotype and kinship inferences). Call rates are the number of called sites over the total number of sites available. A call rate of >95% tends to produce high quality and accurate data. However, the data generated here were unexpectedly atypical given the known elements of the experiment. Samples known to have been severely degraded were observed to produce GT data with call rates relatively similar to those of pristine samples. The concordance data validated the trend that was expected, showing that the GT calls were less concordant as the samples became more degraded. Reliance on this analysis is problematic as with forensic cases, most often the DNA evidence is from an unknown individual.

One of the variables that was controlled for in this experiment was the manner of degradation. With the multiple ways a DNA sample can become degraded, controlling the manner for this study was considered an important element in order to understand if there were any potential effects the assay chemistry had on the data outcome. The particular manner of degradation in this experiment could be a possible explanation for the unexpected trend in the call rates.

Further evaluation is warranted, but in the absence of additional metrics, independent of a reference, the call rate alone is not dependable to assess overall sample quality if the quantification data suggests sample degradation given low DNA quantity (0.05 ng/μL).

PATH FORWARD

As an immediate priority, establishing a Degradation Index (DI) threshold under our current quant method will provide a preliminary and sufficient screening method to ensure that samples that are going to generate data unacceptable for SNP genotyping are not carried forward without communicating the potential outcome limitations.

In addition to a DI threshold, more experiments looking at other specific forms of degradation could provide useful information on the appropriate analysis, as well as seeing if the unexpected observations with the call rates are reproducible. From these studies, methods can be evaluated to repair DNA degradation in order to achieve better SNP results.

Ultimately, the continuation of what has been accomplished and any future studies will be performed on the NextSeq™ 2000. Whole genome sequencing can provide a greater depth and breadth of assessment of the SNPs specific to the GSA array and, in its own right, provide a new data set to evaluate in order to establish additional service opportunities when it comes to forensic genetic genealogy.

REFERENCES

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ACKNOWLEDGEMENTS and CONTACT INFORMATION

This work is supported by Signature Science, LLC through the Center for Advanced Genomics.



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