A Comprehensive Massively Parallel Sequencing Workflow for Severely Degraded Nuclear DNA

Using the Dental Forensic Extraction Kit (DFK[MR]), InnoQuant® HY DNA Quantfication Kit and the MiSeq FGx™ Forensic Genomics System to efficiently obtain conclusive results from challenging samples.

Introduction and Methods

Degraded DNA poses a critical challenge for forensic DNA laboratories and is often encountered in crime scene samples, cold case evidence, and unidentified remains associated with missing persons cases or mass disaster tragedies.

Severely degraded samples, such as teeth and bones, are traditionally reserved for mitochondrial DNA processing due to inadequate results achieved with standard nuclear DNA testing protocols. Recent improvements in upstream extraction methods and quantification chemistries, coupled with massively parallel sequencing (MPS) technology, collectively increase the likelihood of obtaining informative nuclear DNA profiles from challenging samples.

The data described herein demonstrate the superior performance of a comprehensive workflow on degraded DNA samples. This workflow includes the following:

- Dental Forensic Kit (DFK) – a novel dental tissue recovery method developed at Universidad de los Andes, Santiago, Chile (Figure 1) [1,2,3,4,5]
- InnoQuant HY DNA Quantification Kit (InnoGenomics) – a highly sensitive real-time qPCR assay for simultaneous assessment of human and male DNA quantity, quality, and integrity (Table 1) [6,7]
- MiSeq FGx Forensic Genomics System (Verogen) – a DNA-to-answer MPS solution designed specifically for forensic DNA applications (Figure 2) [8,9,10,11,12]

The primary advantages for each method include:

- Dental Forensic Kit (DFK) –
  - Faster, more efficient extraction with higher yield
  - Decreased contamination risk by omitting the traditional grinding step
  - Preserves the tooth to allow for future re-extraction or dental examinations
- InnoQuant HY DNA Quantification Kit –
  - Extremely sensitive and reproducible DNA quantitation down to < 0.001 ng/µL using high copy number retrotransposable element (RE) targets
  - Improved genotyping success rate through accurate degradation index, PCR inhibitor detection, and true negative screening
- MiSeq FGx Forensic Genomics System –
  - Simultaneous amplification of up to 230 STR and SNP markers, plus Amelogenin, with 200 amplicons between 63 - 180 bp in size [10]
  - Highly discriminating profiles can be consistently obtained with ≤ 16 pg total DNA input [9]
  - Ability to simultaneously generate phenotype and biogeographical ancestry estimation [9,10,11]

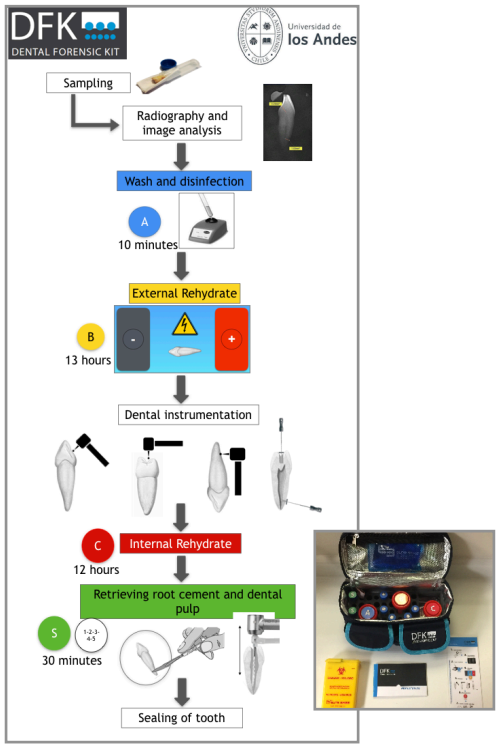

Figure 1: DFK method overview (Universidad de los Andes, Santiago, Chile) – The DFK method improves DNA recovery from dental remains with a unique and efficient dental tissue extraction method. This versatile method may also be applied to bone samples. The process begins with radiograph imaging of the tooth, followed by washing and external rehydration. A specialized dental instrument then perforates the tooth or bone to allow for internal rehydration while minimizing damage to the sample. Both root cement and dental pulp may be obtained for subsequent DNA extraction using the Quick Extract™ FFPE DNA Extraction Kit (Lucigen®). The tooth is then sealed and retained, allowing for future testing if necessary. [1,2,3,4,5]

Degradation Study Experimental Design

To evaluate the combined performance of InnoQuant HY quantification and MPS analysis with the MiSeq FGx System, an initial degradation study was performed utilizing DNA originating from an anonymous male blood bank donor. Degradation levels from moderate to severe were attained via sonication at 50°C ranging from zero to 16 hours. DNA extracts were quantified with InnoQuant HY to obtain short and long target concentrations (ng/µL) and the corresponding degradation index (DI = [Short/Long]) for each sample. Sequencing libraries were prepared from the quantified DNA using the ForenSeq DNA Signature Prep Kit, utilizing a maximum DNA input volume of 5 µL, followed by sequencing on the MiSeq FGx Instrument and data analysis using the ForenSeq Universal Analysis Software.

Following the initial degradation study with artificially degraded DNA, several teeth and a jaw bone specimen with postmortem intervals (PMI) ranging from seven days to 45 years were processed. In a collaborative experiment, Universidad de los Andes, Santiago, Chile performed the DFK extraction method to obtain root cement, dental pulp and jaw bone DNA. These samples were then quantified with InnoQuant HY before processing with the MiSeq FGx Forensic Genomics System.

Table 1: InnoQuant HY Kit Configuration

Target	Genomic Location	Amplicon Length	Reporter Dye
Short	Yb8 autosomal RE	80	HEX
Long	SVA autosomal RE	207	Cy5
Male	Y chromosome	79	FAM
IPC	Synthetic sequence	172	TAMRA

Table 1: InnoQuant HY Kit Configuration - InnoQuant HY is a real-time qPCR system containing four DNA targets – two different sized RE autosomal targets, male specific targets, and an internal positive control (IPC) target. The use of high copy number Alu and SVA REs (>1000 copies per genome) as the two autosomal targets significantly enhances both sensitivity and reproducibility of DNA quantitation and degradation detection in forensic samples. [6,7]

Figure 2: The MiSeq FGx Forensic Genomics System - The MiSeq FGx System consists of the ForenSeq DNA Signature Prep Kit, the MiSeq FGx Instrument, and the ForenSeq Universal Analysis Software. The ForenSeq DNA Signature Prep Kit utilizes PCR amplification and magnetic bead-based chemistries to prepare targeted DNA sequencing libraries for the MiSeq FGx instrument. MPS data are generated for up to 230 genetic markers plus Amelogenin per sample. Upon completion of sequencing, the ForenSeq Universal Analysis Software generates semi-automated allele calls and displays genotype data for analyst review. In addition to short tandem repeats (autosomal, Y chromosome and X chromosome STRs), single nucleotide polymorphism (SNP) markers are targeted, including identity-informative SNPs (iSNPs), biogeographical ancestry-informative SNPs (aiSNPs), and phenotypic-informative SNPs (piSNPs). [8,9,10,11,12]

Results

The progressively degraded blood samples resulted in short target concentrations ranging from 0.87 to 0.17 ng/µL and long target concentrations from 0.87 to 0.0004 ng/µL. The degradation indexes (DI) ranged from 1 (no degradation detected) to 460 (severely degraded). The samples were amplified with ForenSeq according to the InnoQuant HY long target quantities, resulting in total DNA inputs ranging from 1.7 to 0.002 ng.

Figure 3 shows the total number of STR alleles called and iSNP loci typed for samples with increasing levels of degradation. The total number of autosomal, Y- and X- STR alleles called for each sample ranged from 85 (100%) with a DI of 1 at 1 ng total DNA input to 23 alleles called (27%) with a 460 DI and 2 pg of total DNA input. Total iSNP loci typed ranged from 94 to 70 (100% - 74%).

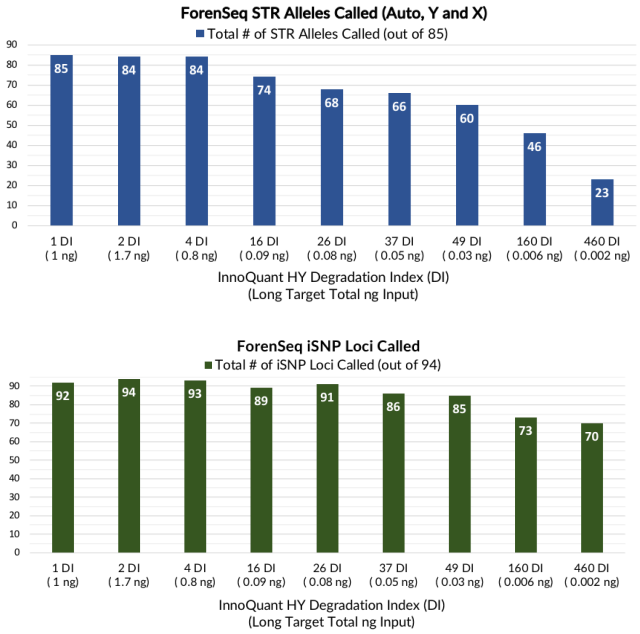

Figure 3: Total number of STR alleles and iSNP loci - Total number of STR alleles detected (blue) and total number of iSNP loci called (green) for artificially degraded blood samples with DI ranging from 1 (not degraded) to 460 (severely degraded).

In addition to STR and iSNP data, aiSNP and piSNP data provide phenotype and biogeographical ancestry estimation in the ForenSeq Universal Analysis Software. Hair and eye color phenotype estimations, which require 100% piSNP locus call rates, were successfully generated for the first four samples down to 90 pg total input and a DI of 16 [11]. Hair color estimations for the four samples originating from the same anonymous blood donor indicated 74% red, 22% blond, 4% brown and 0% black. Eye color estimations were 96 – 97% blue, 2 – 3% intermediate and 1 – 2% brown. Biogeographical ancestry estimation was consistently depicted as the European population group for all nine samples (Figure 4).

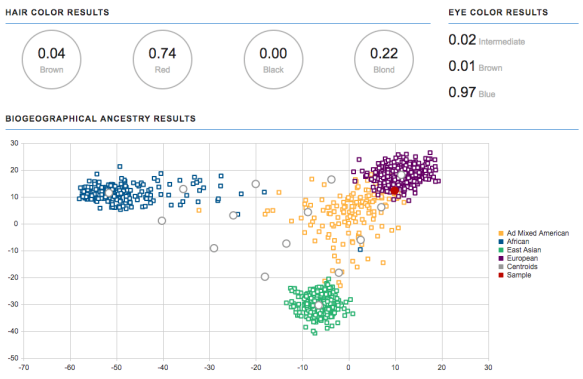

Figure 4: Phenotype and biogeographical ancestry estimation - Hair color, eye color and biogeographical ancestry estimation in the ForenSeq Universal Analysis software for degraded blood with 90 pg input and DI of 16 (sample indicated with red dot).

Data from the dental remains study indicate that the root cement or dental pulp recovered using the DFK method yielded DNA of sufficient quality and quantity for the majority of the teeth samples. Several extracts with PMIs ranging from seven days to approximately six months were not significantly degraded. As shown in Figures 5 and 6, teeth extracts with DI ranging from 0.7 to 7.8 and long target total DNA inputs from 0.05 to 5.7 ng resulted in 46 to 49 ForenSeq autosomal STR allele calls and 58 to 94 iSNP loci typed. By comparison, the same teeth extracts previously resulted in 25 to 31 autosomal STR allele calls with Identifiler® Plus amplification using the maximum 10 µL DNA input run on the Applied Biosystems® 3100 CE instrument.

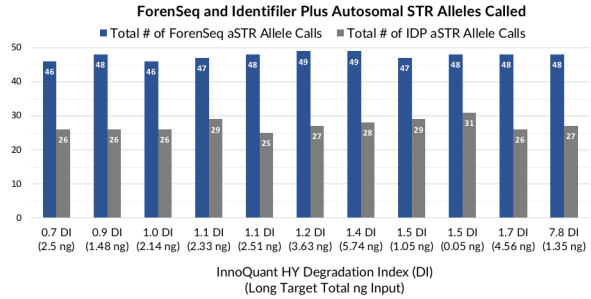

Figure 5: Total number of ForenSeq (blue) and Identifiler Plus (grey) autosomal STR alleles detected in teeth extracts with DI ranging from 0.7 to 7.8.

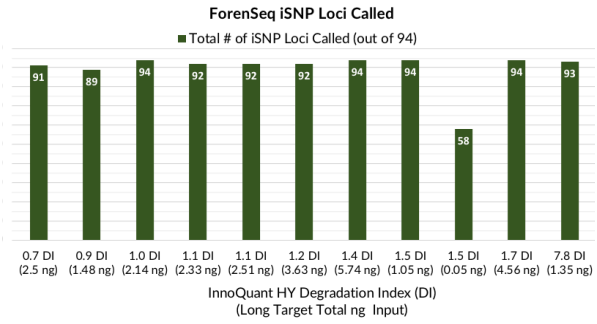

Figure 6: Total number of ForenSeq iSNP loci detected in teeth extracts with DI ranging from 0.7 to 7.8.

The more challenging samples also produced highly discriminating STR and SNP results, despite more pronounced degradation, extremely low levels of DNA input and indication of inhibition as detected by the InnoQuant HY IPC.

One of the more challenging teeth samples with a DI of 76 and total long target input of 0.001 ng resulted in 78 total STR alleles and 85 iSNP loci typed (Figure 7). Additionally, a 45-year PMI jaw bone extract was successfully typed with 48 STR alleles and 59 iSNP loci called at a long target input of 0.01 ng and a DI of 4.7 (Figure 7). This 45-year PMI bone sample had previously yielded zero STR alleles with the Identifiler Plus kit run on the 3100 instrument.

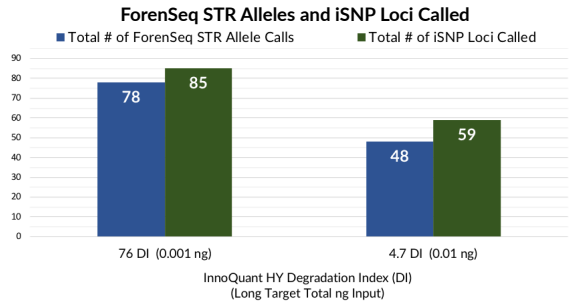

Figure 7: Total number of ForenSeq STR alleles and iSNP loci detected in extremely low quantity/quality teeth and bone extracts.

Conclusions

The combined DFK extraction method, InnoQuant HY quantification and MiSeq FGx Forensic Genomics System provides a robust workflow capable of achieving successful nuclear DNA results from challenging, low-level and degraded samples. Sufficient DNA was recovered during DFK extraction and InnoQuant HY provided accurate quantification data to enable optimal amplification with the ForenSeq DNA Signature Prep Kit. The application of this workflow to real-life, challenging dental remains generated a significant amount of usable data for identification.

Learn More

To learn more about the DFK extraction method, visit: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2014188345&redirectedID=true

To learn more about the InnoQuant HY Human and Male DNA Quantification & Degradation Assessment Kit, visit: http://innogenomics.com/

To learn more about the MiSeq FGx Forensic Genomics System, visit: http://www.verogen.com/

References

1. Universidad de los Andes, Santiago, Chile DFK Patent: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2014188345&redirectedID=true
2. http://www.lucigen.com/home.php?cat=282
3. P. Carrasco, C. Brizuela, I. Rodriguez, S. Muñoz, M. Godoy, C. Inostroza, Histological transformations of the dental pulp as possible indicator of post mortem interval: a pilot study. https://doi.org/10.1016/j.forsciint.2017.09.001
4. P. Carrasco, C. Inostroza, M. Godoy M, G. Gatti, Innovative method and kit DFK(MR) of sample preparation from dental human remains for human genetic identityForensic Science International Genetics (fsigen 2018-7), Submitted January 12, 2018.
5. Application of NGS in the analysis of human DNA from dental remains with new Forensic Kit DFK(MR) Unpublished results (manuscript in preparation)
6. http://innogenomics.com/
7. A. Loftus, G. Murphy, H. Brown, A. Montgomery, J. Tabak, J., Baus, et al., Development and validation of InnoQuant® HY, a system for quantitation and quality assessment of total human and male DNA using high copy targets, https://doi.org/10.1016/j.fsigen.2017.04.009
8. http://www.verogen.com/
9. A. Jager, M. Alvarez, C. Davis, E. Guzman, Y. Han, L. Way, et al., Developmental validation of the MiSeq FGx Forensic Genomics System for targeted next generation sequencing in forensic DNA casework and database laboratories Forensic Sci. Int. Genet. 28 (2017) 52-70. https://doi.org/10.1016/j.fsigen.2017.01.011
10. ForenSeq DNA Signature Prep Reference Guide http://www.verogen.com/
11. ForenSeq Universal Analysis Software Guide v1.2 http://www.verogen.com/
12. MiSeq FGx Instrument Reference Guide http://www.verogen.com/

Verogen • 1.833.837.6436 toll-free (North America) • +1.858.285.4101 tel • info@verogen.com • www.verogen.com

VEROGEN