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]
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

<table>
<thead>
<tr>
<th>Target</th>
<th>Genomic Location</th>
<th>Amplicon Length</th>
<th>Reporter Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short</td>
<td>Yb8 autosomal RE</td>
<td>80</td>
<td>HEX</td>
</tr>
<tr>
<td>Long</td>
<td>SVA autosomal RE</td>
<td>207</td>
<td>Cy5</td>
</tr>
<tr>
<td>Male</td>
<td>Y chromosome</td>
<td>79</td>
<td>FAM</td>
</tr>
<tr>
<td>IPC</td>
<td>Synthetic sequence</td>
<td>172</td>
<td>TAMRA</td>
</tr>
</tbody>
</table>

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]

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%).

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).
Applica'on Note: Forensic Genomics

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 Identifier Plus kit run on the 3100 instrument.

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](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/](http://innogenomics.com/)

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

References


5. Application of NGS in the analysis of human DNA from dental remains with new Forensic Kit DFK(MR) Unpublished results (manuscript in preparation)


