Application Note

Optimizing purification of DNA from challenging bone samples using feedback from the Investigator® Quantiplex® Pro Kit

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Introduction

Human identification is commonly based on the analysis of short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs). The multiplex assays used for analyzing STRs are highly sensitive and require that samples contain a sufficient amount of DNA of high enough quality for a successful STR profile to be created. DNA quantification with quality assessment is therefore an important step prior to STR analysis, as it can help reduce rework and resource waste. This is especially important for challenging samples that are difficult to extract, such as bone samples.

The quantitative real-time PCR-based technology in the Investigator Quantiplex Pro Kit provides a straightforward and fast approach for assessing DNA quantity and quality prior to STR analysis. This kit can quantify human genomic DNA in a sample, provide a measure of the integrity of DNA and reveal the presence of inhibitors. The Quantiplex Pro Kit can concurrently quantify male DNA, if any, in the sample; this is particularly useful in sexual assault cases. Further, the kit contains a system to measure DNA degradation that can reveal the degradation status of the DNA.

In this study, we sought to design an optimized protocol for the recovery of high-quality human DNA from bone samples for downstream STR analysis. First, we extracted genomic DNA from bone using the QIAquick® PCR Purification Kit in the presence of added detergent and proteinase K to enhance demineralization. Next, we evaluated the relative efficacy of the augmented bone extraction treatments by comparing the overall concentration of the purified DNA, the purity of the extracted DNA and its suitability for downstream PCR amplification using the Investigator Quantiplex Pro Kit.

Materials and methods

Sample preparation

Samples were removed from specimens of human bone with a Dremel® drill and cutting wheel attachment. Surfaces were sanded with aluminium oxide sanding stones and then manually washed three times in molecular biology grade water with agitation, then three times in 70% ethanol with agitation. Samples were allowed to dry for 1–2 hours in a laminar flow cabinet then pulverized to fine powder ▷

in a MC1 container for a laboratory blender. Bone powder was weighed and target quantities (0.1 g–0.5 g) were added to a 15 ml conical tube for further treatment.

Effect on DNA recovery of adding detergent to a demineralization buffer

A buffer was prepared with 0.5 M EDTA in molecular biology grade water and proteinase K was added for a final concentration of 20 mg/µl. Two sets of three samples (0.25 g) were processed in triplicate (from L tibia, R femur, L femur) in a 15:1 ratio of demineralization buffer to bone (15 ml/1 g of sample). One sample set was processed without added detergent. Detergent N-lauryl sarcosyl (1 %) was added to the incubation buffer of a duplicate set of samples. Samples were vortexed to mix thoroughly. Tubes were sealed with parafilm and incubated overnight (18–24 hours) at 56°C with agitation.

Optimizing proteinase K concentration and input sample mass

A buffer was prepared (0.5 M EDTA, 1% N-lauryl sarcosyl) in molecular biology grade water. Proteinase K was added to the buffer for final concentrations of either 10 mg/µl or 20 mg/µl. Bone powder inputs of 0.1 g, 0.25 g, and 0.5 g were tested with three samples processed in triplicate for each experimental group in a 15:1 ratio of each demineralization buffer to bone. Samples were vortexed to mix thoroughly. Tubes were sealed with parafilm and incubated overnight (18–24 hours) at 56°C with agitation.

Concentration and purification of nucleic acids

Concentration of bone lysates was performed according to manufacturer's instructions in an Amicon® Ultra-15 30kDa centrifugal filter unit with a target range for retentate of 150–300 µl. DNA from lysates was purified by binding to a silica membrane column using the QIAquick PCR Purification Kit following manufacturer's instructions. DNA was eluted in 30 µl of buffer and stored until quantitation at 2–8°C, or at –20°C. An extraction blank was processed for each experimental group.

Analysis of nucleic acids

Eluates were processed with the Investigator Quantiplex Pro Kit according to manufacturer's instructions. For each extracted sample, values were recorded for concentration of human genomic DNA, concentration of human male DNA and concentration of the human degradation target. The specific PCR products were detected on an Applied Biosystems® 7500 Real-Time PCR System. The human quantification target region was detected using the FAM™ dye channel. The target region for male DNA quantification, selected for high sensitivity in the presence of mixed female/male DNA samples, was detected using the ATTO 647N dye channel. The degradation target was detected in the ATTO 550 dye channel. The degradation target allows for a precise assessment of the degradation status of the DNA and calculation of a sample Degradation Threshold. The Degradation Index is set to 10 as a default in the analysis software. If a sample has a Degradation Threshold above the specified index, then the DNA is most likely degraded. A higher threshold indicates greater degradation. The Investigator Quantiplex Pro Kit also contains an internal amplification control detected in the JOE™ dye channel that indicates successful amplification and identifies the presence of PCR inhibitors.

PCR amplification

Samples were normalized to 1 ng per PCR, amplified with the GlobalFiler™ PCR Amplification Kit, typed with the SeqStudio™ Genetic Analyzer System instrument, and analyzed with GeneMapper™ ID-X software v1.6. All procedures were carried out according to manufacturer's instructions.

Results

Effect on DNA recovery of adding detergent to a demineralization buffer

The presence of detergent (1% N-lauryl sarcosyl) in the demineralization buffer enhanced the recovery of total DNA from bone samples (Figure 1A). In addition, the addition of detergent produced DNA with a lower Degradation Threshold than for the standard demineralization buffer. For example, for sample 575509, the Degradation Threshold values with detergent and without detergent were, respectively, 63.74 and 86.06 (Figure 1B), indicating a greater degree of DNA degradation in the latter. For all samples of DNA recovered from demineralized bone, however, there was a clear decline in signal for larger loci and alleles, suggesting that further optimization is required (Figure 2).

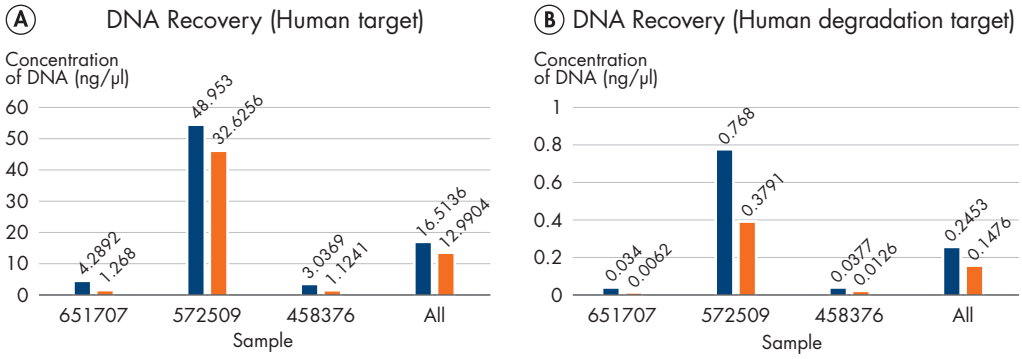

Figure 1. DNA recovery from bone was enhanced by including detergent in the demineralization buffer. A Mean recovery of human target DNA extracted with and without N-lauryl sarcosyl detergent in the buffer. **B** Mean recovery of human degradation target DNA in the same samples measured after extraction with and without N-lauryl sarcosyl in the buffer. Quantitation was performed using the QIAGEN Investigator Quantiplex Pro Kit and Applied Biosystems 7500 Real-Time PCR System Instrument.

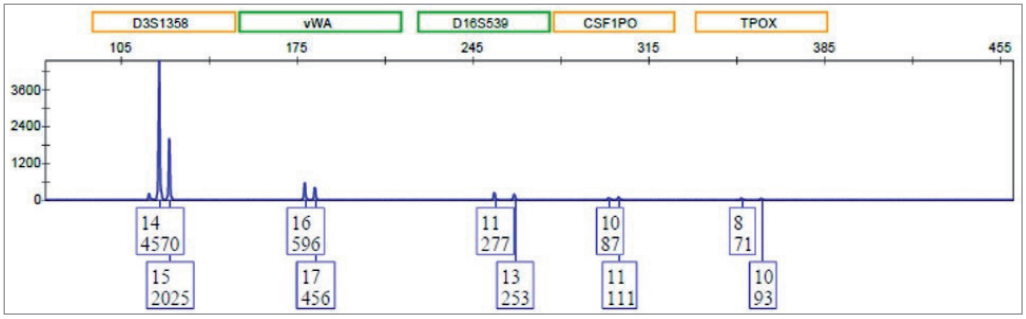

Figure 2. Degradation of DNA with added detergent is shown by a decline in signal for larger loci and alleles. Representative electropherogram result of FAM™ dye channel from sample 615707A2 (demineralized with 1 % detergent; Degradation Threshold = 126.48). Sample was normalized to 1 ng per PCR, amplified with the GlobalFiler PCR Amplification Kit, typed with the SeqStudio Genetic Analyzer System instrument, and analyzed with GeneMapper ID-X software v1.6.

Optimizing proteinase K concentration and input sample mass

The addition of proteinase K at a final concentration of 20 mg/µl to the demineralization buffer resulted in greater recovery of DNA from bone samples than when a final concentration of 10 mg/µl was used (Figure 3A). The difference in concentrations of recovered DNA was significant (p = 0.0214) when bone powder samples of 0.1 g were processed. An increased level of recovery was also seen for 0.25 g of input bone sample, but the difference was not statistically significant.

The advantage of greater DNA recovery with 20 mg/µl of proteinase K in the demineralization buffer was offset by the consistently higher Degradation Threshold found for DNA recovered with this treatment (Figure 3B), indicating a greater degree of DNA degradation than when a final concentration of 10 mg/µl was used. The Degradation Threshold of recovered DNA from 0.25 g samples treated with 20 mg/µl proteinase K (90.1143) was significantly different (p = 0.0029) from the value when 10 mg/µl of proteinase K was used (36.472).

To investigate optimum sample mass input, a standard demineralization buffer (1) was used (0.5 M EDTA, 1% N-lauryl sarcosyl, 20 mg/µl proteinase K) and replicate bone samples of 0.1 g, 0.25 g and 0.5 g were processed for determination of total DNA recovered (total human target and human male target). On average, between 14 ng and 470.6 ng of DNA was recovered per gram of extracted bone powder and sufficient DNA for PCR input was recovered from all samples (>1 ng/µl of human target DNA). Recovery was consistently improved from 0.1 g to 0.5 g, with recovery of male DNA showing a sustained linear increase (Figure 4). Input with 0.25 g was seen as an optimal balance between efficiency and recovery of total human DNA.

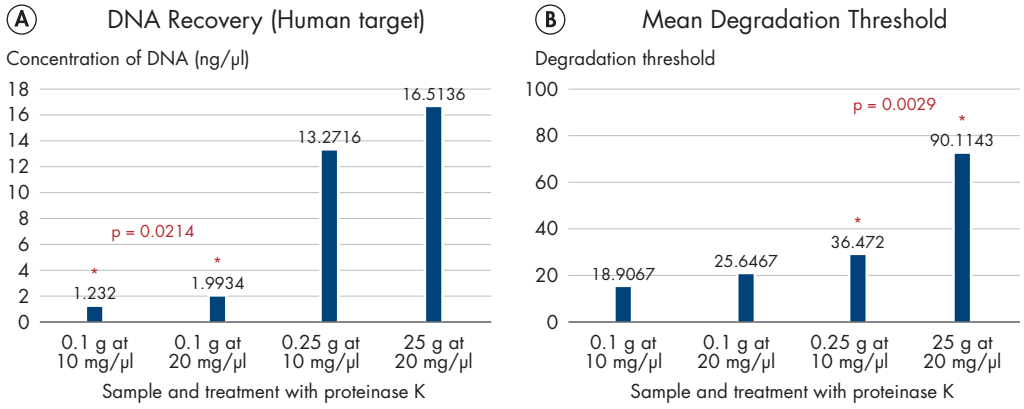

Figure 3. A higher concentration of proteinase K in the demineralization buffer increased total DNA recovery from bone but quality of the recovered DNA was reduced. A Mean recovery of human target DNA from 0. 1 g or 0.25 g of bone sample after extraction with 10 mg/µl or 20 mg/µl of proteinase K in the buffer. **B** Mean DNA degradation thresholds in the same samples measured after extraction with 10 mg/µl or 20 mg/µl of proteinase K in the buffer. Quantitation and analysis were performed using the QIAGEN Investigator Quantiplex Pro Kit and Applied Biosystems 7500 Real-Time PCR System Instrument.

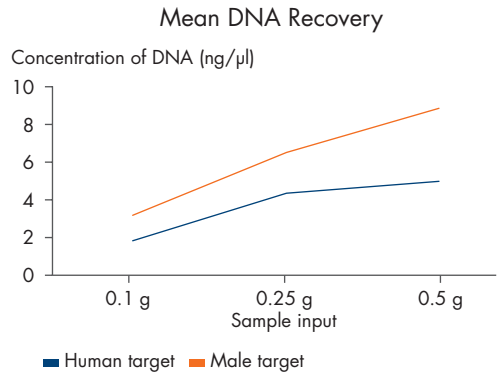

Figure 4. Mean DNA recovery at human DNA and male DNA targets varied according to input mass of bone powder (0.1 g, 0.25 g, 0.5 g). Quantitation was performed using the QIAGEN Investigator Quantiplex Pro Kit and Applied Biosystems 7500 Real-Time PCR System Instrument.

Discussion and conclusions

In this work, we tested the efficacy of various demineralization treatments on the extraction and purification of DNA from bone samples for downstream STR analysis. We used the Investigator Quantiplex Pro Kit to comprehensively evaluate the extracted DNA, allowing us to accurately determine the impact of the various extraction methods and reagents on the quantity and quality of the DNA recovered.

We found that omission of N-lauryl sarcosyl detergent from a standard demineralization buffer (1) adversely affected the amount of total DNA recovered from bone samples. In addition, higher Degradation Threshold values suggested that DNA recovered in buffer without detergent was likely to be more degraded than DNA recovered in buffer with 1% N-lauryl sarcosyl.

In addition, we found that demineralization of bone samples in a standard buffer with N-lauryl sarcosyl and 20 mg/µl proteinase K resulted in good recovery of total DNA from bone samples, but the yield advantage was offset by a decline in DNA quality. Lowering the concentration of proteinase K in the demineralization buffer from 20 mg/µl to 10 mg/µl decreased the total amount of DNA recovered but the quality of extracted DNA was improved. For total human target DNA, an amount of 0.25 g of starting material returned an optimal balance between efficiency and recovery. These points may become important considerations when choosing a demineralization treatment to prepare bone samples for downstream applications where high-quality template is critical to success.

Summary

Using quantitative real-time PCR, the Investigator Quantiplex Pro Kit can quantify human genomic DNA in a sample and concurrently quantify male DNA (if any), confirm that the sample contains enough DNA for downstream analysis and indicate if the sample contains inhibitors. A calculated Degradation Threshold can inform on the integrity of DNA in the same sample.

References

1. Loreille, O.M., et al. (2007). High efficiency DNA extraction from bone by total demineralization. Forensic Science International: Genetics. 1: 191-195.

Ordering Information

Product	Contents	Cat. no.
Investigator Quantiplex Pro Kit	For use on Applied Biosystems 7500 Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
QIAquick PCR Purification Kit (50)	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104

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