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HID in action

An Efficient Method of Extracting DNA from Bone Remains from the Spanish Civil War— A Comparative Study of Two Methods: PrepFiler BTA™ and DNAzol® Methods

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Introduction

In recent years Spain began the process of recovering the bodies of people who went missing during the Spanish Civil War. This project has been named "Recovery of Historical Memory." The first 24 bodies which were lying in the "Pozo de las Brujas", (Aruca-Gran Canaria) were recovered in December 2008. These remains had been thrown inside a well on March 19, 1937. This well was approximately 50 metres deep and had never produced water (Figure 1).



Figure 1. The excavation site "Pozo de las Brujas".

The DNA identification work was conducted by two laboratories: Department of Genetics (University of La Laguna) and our laboratory, Forensic Genetics Service (University of Las Palmas de Gran Canaria). To obtain the DNA, both laboratories used the same extraction protocol involving DNAzol®/QIAquick® (Qiagen) reagents¹. The mtDNA control region sequence of the 24 bodies was obtained and we were able to identify 14 unique haplotypes together with five couples who shared haplotypes. However, when comparing these sequences with those obtained from samples of suspected relatives, it was not possible to obtain conclusive identifications because of the limited number of appropriate samples available from relatives for comparison purposes.

Given the difficulties we encountered in establishing the identity using mtDNA, we decided to analyze the nuclear DNA using the new AmpF/STR® NGM™ PCR Amplification Kit from Life Technologies. This approach seemed to be promising due to the increased ability of the NGM™ Kit to recover information from severely degraded samples, and the kit's remarkably high tolerance to inhibitors. Moving on to the quantification, using RT-PCR of the DNA extracted, we were able to obtain values between 0.02 ng and 1.6 ng total DNA. However, the amplification and analysis of STRs resulted in only seven partial profiles.

At this time we became aware of a new optimized lysis buffer for difficult forensic samples, PrepFiler BTA™ kit (Bone, Teeth, Adhesive), which is used in combination with the Applied Biosystems® PrepFiler™ Forensic DNA Extraction kit. This extraction method was applied to 20 of the 24 remains detailed above. The results were very satisfactory. Starting with only 50 mg of pulverized bone (10 times less than the previous

method), we obtained values between 0.05 to 5 ng of total DNA, and the STR analysis of these 20 individuals allowed us to obtain 13 complete profiles and five partial profiles.

Clearly better results were obtained using the PrepFiler BTA™ method, but to make a comparison between both methods, it was necessary to contrast the results based on the same sample from an individual and the same amount of powder. The goals we set for this comparative trial were to compare the DNA extraction efficiency of both methods and compare the quality of extracted DNA, contrasting the results obtained in the quantification by RT-PCR and STR profiles.

Materials and Methods

Prevention of Contamination

All extractions were performed in a dedicated laboratory physically separated from the main genetics department. The laboratory is constantly irradiated with UV lamps and frequently cleaned with bleach. All sample manipulations were performed in a laminar flow cabinet, with dedicated pipettes and sterile filter tips. Solutions were commercially acquired when possible; otherwise they were autoclaved and UV-treated. Lab coats, face shields, hats, and sterile gloves were used at all times. To monitor contamination during extraction, an extraction blank was processed together with the sample. PCR contamination was monitored using a negative control per reaction.

Samples

The samples used were pulverized femur bone samples about 71 years old. We selected five individuals and from each took four aliquots of 50 mg powder. Two aliquots were extracted independently and in parallel by the standard method of the extraction laboratory (DNAzol® reagent) and the other two by the PrepFiler BTA™ method. The pulverized samples were obtained from the less damaged areas of bone. A layer of approximately 1 mm was removed from the surface by sanding with a drilling machine in order to reduce contamination from previous handling. The sample was then ground to a fine powder with the drilling machine.

Extraction

DNAzol® Method:

Lysis: 800 µL of DNAzol® reagent was added to 50 mg of bone powder and left in agitation (900 rpm) at room temperature for two and a half days.

DNA Purification: The lysate was purified using the QIAquick® PCR Purification Protocol (Qiagen). The DNA elution volume was 100 µL.

PrepFiler BTA™ Method:

Lysis: 220 µL of PrepFiler BTA™ lysis buffer plus 3 µL of DTT (1M) and 7 µL of Proteinase K (20mg/mL) were added to 50 mg of bone powder and left stirring at 1,100 rpm and 56° C for 18 hours.

DNA Purification: The DNA purification was performed following the Standard Protocol of the PrepFiler™ Extraction kit. The DNA elution volume was 50 µL.

Real-Time PCR Quantification

The number of molecules used as a template for PCR amplification was determined by real-time PCR quantification using TaqMan® reagent-based chemistry in an Applied Biosystems® 7500 Real-Time PCR System. Primers, probes, thermal cycling, and analysis conditions were as described by Hudlow – 2008². The quantification results were analyzed using SDS software v 1.4.

This real-time PCR assay consists of four probes that quantify non-degraded DNA (human and male), degraded DNA and the IPC (Internal PCR Control).

Concentration of DNA

DNA samples were concentrated using the Amicon Ultra 0.5 mL 100K centrifugal filter and by following their specifications for DNA/RNA concentration. For each method, the two DNA samples extracted from each individual were concentrated together.

Amplification

The extracted DNA was amplified with the AmpFℓSTR® NGM™ PCR Amplification Kit (Applied Biosystems) in a GeneAmp® 9700 Thermal Cycler.

Electrophoresis and Analysis

PCR products were electrophoresed on an Applied Biosystems® 3130xl Genetic Analyzer. The STR profiles were analyzed using GeneMapper® ID Software v 3.2.1. The run modules and conditions and the data analysis were performed according to the NGM™ PCR kit protocol instructions.

Results and Discussion

Quantification

The quantification of DNA samples extracted by both methods (Table 1) reveals that only degraded DNA was obtained using the DNAzol® method. By contrast, using the PrepFiler BTA™ extraction method, it was possible to recover non-degraded DNA as reflected in the data obtained for samples 2, 5, 13, and 16. These results show that the extraction method using the PrepFiler BTA™ lysis buffer delivered better quality DNA than the DNAzol® extraction method.

Sample	BTA Extraction Method		DNAzol® Extraction Method	
	Non-Degraded DNA Yield (ng)	Degraded DNA Yield (ng)	Non-Degraded DNA Yield (ng)	Degraded DNA Yield (ng)
1	0.04	0	0.4	0
2	0.10	0	0.5	0
5	0.10	0.4	1.4	0
13	0.04	0.5	1.1	0
16	0.04	0	0.9	0

Table 1. Total DNA yield obtained from the BTA and DNAzol® extraction methods. This data is estimated based on the real-time PCR results.

STR Profiles

The comparison of the profiles obtained for different samples using the NGM™ PCR Amplification kit (Table 2) shows non-complete profiles with DNA obtained by DNAzol® extraction method, while four complete and a partial profile are obtained with the DNA extracted using the PrepFiler BTA™ method.

Sample	Number of STR Loci Amplified using the NGM™ Kit	
	BTA Extraction Method	DNAzol® Extraction Method
1	11/16	12/16
2	16/16	8/16
5	16/16	14/16
13	16/16	8/16
16	16/16	6/16

Table 2. Number of STR loci amplified using the AmpF/STR® NGM™ PCR Amplification Kit (Applied Biosystems) from DNA extracts obtained from the PrepFiler BTA™ and DNAzol® extraction methods.

The PrepFiler BTA™ method resulted in a far greater number of loci being recovered for the majority of the samples compared to the DNAzol® method. Even in situations where the DNAzol® method delivered a higher amount of DNA (e.g., Sample 2), the quality of the profile was higher as reflected in the profiles obtained (Figures 2, 3). These results confirmed what we had

observed in the quantification results—that the DNA extracted using the PrepFiler BTA™ method was of higher quality than the DNA extracted using the DNAzol® method.

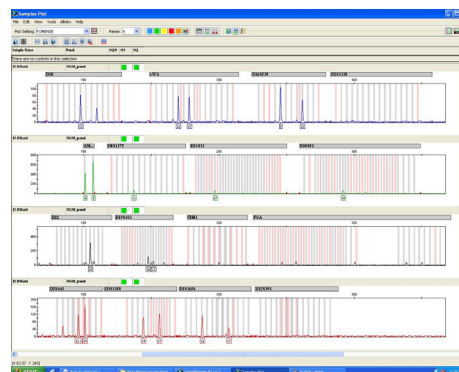


Figure 2. Electropherogram of sample 2 DNAzol® extracts amplified using 0.5ng DNA.

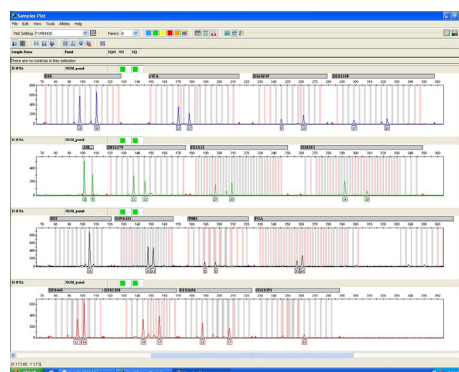


Figure 3. Electropherogram of sample 2 BTA extracts amplified using 0.3ng DNA.

Conclusions

The PrepFiler BTA™ method is a very efficient extraction method for obtaining high-quality DNA from ancient bones. The small amount of starting material required (50 mg) is another of its advantages as well as a reduction in processing time.

Moreover, most laboratories perform DNA extraction of ancient samples using homebrew methods. Using a commercial kit such as the PrepFiler BTA™ method will allow standardization of tests and therefore, standardization of results.

References

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