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DNA typing from skeletal remains using GlobalFiler™ PCR amplification and Investigator® 24plex QS kits

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ABSTRACT

Since the beginning of our work in 2003 our laboratory has focused exclusively on STR DNA from bone, a powerful tool in missing person cases. In cases such as mass disasters or missing persons, human remains are challenging to identify as they may be fragmented, burnt, recovered from water, degraded, and/or contain inhibitory substances. To address these challenges, this study has evaluated the performance of relatively new STR kits Investigator® 24plex QS kit (Qiagen) and GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific) by comparing it with current uses of the AmpFLSTR® Identifiler® Plus kit (Applied Biosystems) to obtain genetic information from skeletal remains. We analyzed 20 bone samples of skeletal remains from routine casework submitted for body identifications by law enforcement corresponding using Investigator® 24plex QS kit and GlobalFiler™ PCR Amplification kit, previously analysed AmpFLSTR® Identifiler® Plus kit (Thermo Fisher Scientific). The data indicates that the STR profiles obtained using the GlobalFiler™ and Investigator® 24plex QS kit for analysis of skeletal remains has shown results in an increased number of reportable genetic loci, and provide greater power of discrimination in comparison to the Identifiler® Plus Kit. Advanced extraction and purification techniques, together with more sensitive and robust new amplification kits allowed us to overcome the challenges associated with processing compromised skeletal remains and ultimately obtain full STR DNA profiles in 99% of the bones.

1. Introduction

In cases involving charred remains, missing persons, and mass disasters, highly degraded bone fragments are often the only obtainable physical evidence for human identification [1–3]. Obtaining DNA from bone samples is often challenging due to low levels of endogenous DNA, environmental conditions, bacterial materials, and post-mortem DNA damage, as well as the coextraction of the PCR inhibitors naturally present in bone [4,5]. All of these factors can negatively impact the PCR amplification and the quality of the DNA profiles generated. Thus, reliable genotyping of challenging bone sample requires an efficient DNA extraction method and a very sensitive genotyping method.

This study evaluated the sensitivity and performance of the GlobalFiler™ PCR Amplification and Investigator® 24plex QS kits with 20 bone samples of skeletal remains from routine casework in our laboratory, each with different age and environmental exposure [6,7]. The two kits being tested are both 6-dye multiplex kits each with 24 markers, including the mini-STR loci with amplicon size falling below 220 bps, which was designed to maximize performance on degraded samples and robust PCR buffer can tolerate certain levels of PCR inhibitors that may be present in DNA extracts. However, GlobalFiler™ and Investigator® 24plex QS could potentially be the optimum amplification choice for the limited amounts of DNA obtained from challenged bone samples.

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2. Material and methods

2.1. Samples and DNA extraction

This study analysed 20 bones, 18 femurs and 2 skulls, submitted for routine casework body identifications by law enforcement. Samples were processed individually and extracted in parallel with a reagent blank control that accompanied the sample throughout testing. First, all bones were cleaned from the remnant soft tissue and all soil traces. The cut bone fragments were washed and air dried [3]. The resulting sample was pulverized into fine powder in mill MM 301 (Retsch).

Total DNA was isolated from bone samples followed two different protocols: phenol chloroform isoamyl alcohol (PCIA) organic extraction method [6] and PrepFiler® BTA Forensic DNA Extraction Kit (Applied Biosystems) [7]. Modifications to both extraction methods have been introduced in order to improve our laboratory success rate with identification of these skeletal remains [6,7].

2.2. PCR amplification and typing

DNA was quantified with an ABI Prism® 7000 Sequence Detection System (Applied Biosystems) using Quantifiler™ Human DNA Quantification kit. Amplifications were performed on the GeneAmp PCR System 9700 Gold Plate (Applied Biosystems) using the AmpFLSTR® Identifiler® Plus kit (Applied Biosystems), GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific) and Investigator® 24plex QS kit (Qiagen) following the manufacturers' protocols. Amplified products are separated and detected on ABI 3500 Genetic Analyzer (Applied Biosystems).

3. Results

Twenty DNA extractions were completed on skeletal remains from routine casework and the results are presented in Table 1. Three different groups of bones were selected based on exposure of skeletal remains to different environmental influences which may have led to degradation of DNA: burned bodies (5 samples), remains recovered from water (7 samples) and remains recovered from fields (8 samples) (Table 1). Seventeen samples, from each amplification kits produced full STR profiles beside on extraction method are used (Table 1). The results showed that in 3 samples which were partial with Identifiler® Plus, with GlobalFiler™ and Investigator® 24plex QS gave a greater number of loci producing better STR profiles (Table 1). In 2 of the 3 samples, both amplification kits gave a complete, 24 locus profile (Case #7 and Case #9) regardless detection PCR inhibition by Case #7 and confirm DNA degradation by Case #9. Sample Case #18 was still considered a partial profile, but the amount of reportable loci increased usinig GlobalFiler™ and Investigator® 24plex QS on 22 out of 24 loci available in those multiplex, while applying AmpFℓSTR® Identifiler® Plus kit obtained only 9 out of 16 loci. For sample Case #18 amplified with GlobalFiler®, the loci that were not amplified were primarily the longest loci CSF1PO and SE33, while with Investigator® 24plex QS loci that were not amplified were D2S1338 and SE33. Regardless of the DNA input, dropped out the same loci also observed by other authors [8].

Nuclear DNA quantity; the efficiency of autosomal STR typing (AmpFℓSTR® Identifiler® Plus Amplifiction Kit) expressed as the number of successfully typed STRs; efficiency of GlobalFiler™ expressed as the number of successfully typed number of successfully typed STRs Y-indel, and a DYS391; Investigator® 24plex QS, expressed as the number of successfully typed number of successfully typed STRs, DYS391and a Quality Sensor in bones from 20 human identification cases.

4. Discussion

The results of this research suggest that the GlobalFiler® kit is slightly more sensitive than the Investigator® 24plex QS kit, producing more complete and balanced STR profiles with peak heights at least 2-fold greater, supporting the results of previous work [9]. The two kits produced concordant STR profiles for all samples.

Kits that can analyse 24 loci provide a greater power of discrimination and provide DNA analysts with greater confidence in associations made between skeletal remains and family reference samples. With this ability to obtain increased genetic information from the skeletal remains with Investigator® 24plex kit, we could to detect PCR

Table 1
Nuclear DNA quantity; the efficiency of autosomal STR typing (AmpFℓSTR® Identifiler® Plus Amplifiction Kit) expressed as the number of successfully typed STRs; efficiency of GlobalFiler™ expressed as the number of successfully typed number of successfully typed STRs Y-indel, and a DYS391; Investigator® 24plex QS, expressed as the number of successfully typed number of successfully typed STRs, DYS391and a Quality Sensor in bones from 20 human identification cases.

Bone sample (Q)/casework	Environmental exposure	Extraction method	Quantity (ng/μl)	Efficiency of STR typing		
				Identifiler® Plus	GlobalFiler™	Investigator® 24plex QS
Femur/Case #1	Buried	PrepFiler® BTA[a]	13.337	16/16	24/24	24/24
Femur/Case #2	Burned	PrepFiler® BTA[a]	1.089	16/16	24/24	24/24
Femur/Case #3	Water	PrepFiler® BTA[a]	2.981	16/16	24/24	24/24
Femur/Case #4	Buried	PrepFiler® BTA[a]	0.163	16/16	24/24	24/24
Femur/Case #5	Burned	Organic[b]	2.989	16/16	24/24	24/24
Skull /Case# 6	Buried	PrepFiler® BTA[a]	11.262	16/16	24/24	24/24
Femur/Case #7	Buried	Organic[b]	0.127	13/16	24/24	24/24
Femur/Case #8	Water	PrepFiler® BTA[a]	0.382	16/16	24/24	24/24
Femur /Case #9	Buried	PrepFiler® BTA[a]	0.121	12/16	24/24	23/24
Skull /Case #10	Buried	PrepFiler® BTA[a]	0.302	16/16	24/24	24/24
Femur/Case #11	Buried	PrepFiler® BTA[a]	0.188	16/16	24/24	24/24
Femur /Case#12	Water	PrepFiler® BTA[a]	0.281	16/16	24/24	24/24
Femur/Case #13	Buried	PrepFiler® BTA[a]	2.132	16/16	24/24	24/24
Femur/Case #14	Water	Organic[b]	25.647	16/16	24/24	24/24
Femur/Case #15	Water	PrepFiler® BTA[a]	0.258	16/16	24/24	24/24
Femur/Case #16	Buried	PrepFiler® BTA[a]	0.180	16/16	24/24	24/24
Femur/Case #17	Water	Organic[b]	0.321	16/16	24/24	24/24
Femur/Case#18	Burned	Organic[b]	0.009	9/16	22/24	22/24
Femur/Case #19	Buried	Organic[b]	0.282	16/16	24/24	24/24
Femur/Case #20	Water	Organic[b]	0.116	16/16	24/24	24/24

[a] PrepFiler® BTA Forensic DNA Extraction Kit [7].
[b] PCIA organic extraction method [6].

inhibition or confirm DNA degradation and amplification success in general.

5. Conclusion

This study was aimed at improving identification techniques based on the analysis of genomic DNA. The data indicated that the GlobalFiler™ and Investigator® 24plex QS are extremely sensitive multiplex STR amplification systems and can produce greater quality DNA profiles from skeletal remains compared to the Identifiler® Plus kit. They have been successfully used to obtain multilocus STR profiles from bone samples with minimal amounts (pg) of human DNA, highly inhibited, and degraded challenging samples.

Our results have shown that simple modifications to extraction techniques, together with more sensitive and robust new amplification kits with the mini-STR loci that are more tolerant to common inhibitors, allowed us to overcome the challenges associated with processing compromised skeletal remains and lead to the identification of more missing individuals.

Declaration of Competing Interest

None.

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