A Study of Skeletal Remains using GlobalFiler™

Almohammed. E [1*, 2*]; Dragana Zgonjanin [3*]; Hadi. S[1*]

[1]School of Forensic and Applied Sciences, University of Central Lancashire, Preston, UK; [2]Ministry of Interior of Qatar, Doha, Qatar;
[3]Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

*Corresponding author: eida-k-al@hotmail.com

Abstract

DNA analysis from human remains is of immense relevance in missing persons identification and disaster victim identification (DVI). DNA degrades gradually in hard tissues, such as bones and teeth under a high temperature, humidity, pH, geochemical properties of the soil, the presence of microorganisms and all other factors that affect the preservation of DNA in skeletal remains. The GlobalFiler™ kit simultaneously amplifies and detects 21 autosomal loci including CODIS extended set of STR loci. The kits was designed to combine all 21 autosomal STR loci along with a novel male specific Y insertion/deletion marker the sex-determining marker, amelogenin. Thus, GlobalFiler™ combines the CODIS extended set of loci and includes seven autosomal STR loci from the expanded European Standard Set of Loci (ESSL). The kit also includes the highly discriminating SE33 locus. The aim of this study was to profile the old skeleton remains using GlobalFilerTM for use in disaster victim identification that previously profiled using Identifiler Plus kit using 3500 capillary electrophoresis (CE). This study investigated the success rate of short tandem repeat (STR) typing from different types of bone samples observed a higher STR success rate using GlobalFiler™ PCR kit, which contains more than 10 loci with a maximum size of 200 bp. Thus, GlobalFiler™ could potentially be the optimum amplification choice for the limited amounts of DNA obtained from challenged bone samples. The performance of the GlobalFiler™ kit and the profile quality was examined for the degraded bone samples to determine if it could generate more robust genetic information.

Keywords: GlobalFiler ™; Autosomal STR; Validation; Forensic; Qatar.

1. Introduction

1.1 Nuclear DNA Degradation &GlobalFiler™ kit

DNA can be damaged by adverse environmental conditions that are ubiquitous in nature. Some of these include ultraviolet irradiation, humidity and water, low pH and enzymes called nucleases, which can result in fragmented DNA. This degradation can impact STR profiling in various ways. Moreover, DNA examination has become an important tool for identifying victims from DVI mass disasters. Skeletal samples thus become a choice in personal identification through DNA profiling. Of course, mitochondrial DNA (mtDNA) analysis has been the first choice in old archaeological remains, due to its high copy number per cell [1]. The victims from several wars and natural disasters including those from Chile, Argentina and Guatemala who died in the 1970s and 1980s and those from recent war conflicts including Iraq or Libya have been identified using DNA profiling methods [2]. In addition, DNA analyses was successful in identifying the victims of natural disasters such as Hurricane Katrina and the Asian Tsunami disaster in 2004 and 2005, respectively. Similarly, DNA profiling helped to identify missing victims from the World Trade Centre in September 2001 and Madrid bombing in March 2004 [3]. The DNA quantity, presence of inhibitors and degradation level can now be detected using the real time PCR kits such as Quantifiler Trio and Hyres [8, 13]. The aim of this study was to investigate the success rate of short tandem repeat (STR) typing in bone samples exposed to various conditions for different periods of time using GlobalFiler™ PCR kit. DNA analysis from human remains is of immense relevance in missing persons identification and disaster victim identification (DVI) [9, 10].

2. Materials and Methods

2.1 Human Identification Casework

This study employed a total of 79 bone samples from, Serbia were used example of cases were shown in (Fig1 A-B). The samples burial periods ranged from approx. 2-70 years. All studies on bone samples were carried out in the Serbian laboratories in Belgrade.

2.2 Skeletal Fragment Cleaning, Drilling & Bone Powdering & Decalcification

Sizes of 3 cm x 2 cm of the bone samples surface were prepared by removing a section of bone. Then, 1-2 mm of bone was removed from the superficial surface of each bone by sanding band attachment. The bone section was then cleaned using 5% (w/v) sodium hypochlorite solution followed by water. The bone specimen was placed in a clean weighing boat. The bone was drilled to generate 0.5 to 5 grams of shavings. The fragments

were placed in a labelled weigh boat until dry. Once dried, the bone fragments were added to a blender cup and blended for approximately 20 seconds to a powder form. Approximately 0.2 g of powder was transferred to a cleaned, irradiated 50 ml conical tube, which was previously cleaned with 70% (v/v) ethanol (Fig 1 A-B). Bones contain a hard matrix network and hydrated collagen fibrils [12]. The de-mineralization procedure was used to break the fibril network via the formation of hydroxyapatite crystals that encapsulated the fibrils using the EDTA which helped to get rid of all external DNA materials. The bone samples were incubated with EDTA overnight for the mineralization process.

2.3 Extraction, Quantification, PCR Amplification & Capillary Electrophoresis

The bone samples were extracted using different methods of extraction using the QIAamp® extraction DNA (QIAMaxi) protocol or PrepFiler kit on Automate Express according to the instructions from the Manufacturer. The quantification of the bone samples was carried out with the Quantifiler™ Trio DNA Quantification kit according to the instructions from the Manufacturer (Thermo Fisher Scientific). PCR amplification reactions were used in this study following the PCR setup proportions recommended by the GlobalFiler™ PCR kit user guide. A full volume PCR of 25 µl was used for DNA samples, extraction negative, positive control 2800M (Promega), and 007® male DNA control (Thermo Fisher Scientific). Amplified samples were prepared for fragment analysis in capillary electrophoresis in 8 capillary (50 cm length) arrays on the ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) filled with POP-4™ polymer.

3. ***Results***

3.1Typing Success on Skeletal Remains Cases

A total of 79 human bone samples were extracted and assessed for degradation using the GlobalFiler™ kit. These samples included bone and teeth. All samples were quantified using Quantifiler™ Trio quantification. IPC CT values were used to assess PCR inhibition. The IPC CT values of each sample were within ±1 of the IPC CT of the NTC (Appendix-E). The IPC values for some samples were above 32 CT for showing the presence of inhibitors. Degradation indices were calculated for each of the bone extracts and results suggested that in most samples had degraded DNA (Table A-B Supplementary Material). GeneMapper® ID-X Software v1.4 was used to analyse the data. The level of degradation in each bone sample was estimated using Degradation Index (DI) and GlobalFiler profiles results, which were analysed for peak heights and allele calls. The results obtained for the small amplicon of the Quantifiler™ Trio kit were used to determine the DNA quant value. The ratio between the small and large amplicons generated the Degradation Index (DI). The results reveal that higher degradation indices, indicated greater the levels of degradation. In this study, DI values ranging 1.5 to >1000 were detected showing non-degraded to highly degraded samples. The results of the DI were compared to the number of alleles called for GlobalFiler™ kit (Table A-B Supplementary Material). With increasing DI values, the differences between the two fragments become significant $p<0.05$ suggesting degradation in the samples. Thirteen samples had DI above thirty and nine samples had DI above 30. Ten samples had moderate degradation and showed a DI between 1 to 5.

4.1 Evaluation of Quantifiler™ Trio Kit Degradation Index (D) and GlobalFiler™ Kit Allele Recovery

The Quantifiler™ Trio kit and GlobalFiler™ kit was used in order to evaluate the effectiveness of the kit when dealing with degraded bone samples. Four ranges of DI values corresponding to four distinct classifications of DNA degradation were used. The data are presented in. These degradation categories were introduced in order to identify a common strategy for processing samples that share a similar level of DNA degradation. Figures 3 to 6 show examples of profiles belonging to each proposed degradation category. Figure (1E-F) shows four EPGs with a moderate and severe degradation with different degradation index. The allele recovery for these samples ranged from 40-90% on the average. Fifty-six bone samples had an allele recovery above 70%. Only six samples had 40% allele recovery. The average cycle threshold (CT) for the internal positive control (IPC) was 29 for the controls. Twenty-one samples had an IPC CT value of 28-29 cycles, with 31 samples having a CT of 27, 15 having a CT of 31-35 and six had more than 35-38. The lowest CT was from 19 for 7 samples.

The most inhibited samples showed a CT. above 29 cycles. In nearly every case, where the DNA extract showed inhibition, also had the lower yield.

5. Discussion

5.1 GlobalFiler™ Profiles and Degradation Index (DI)

In this study, the DI value obtained for the skeletal remains during quantification results reflects the ratio of the amplification of target regions of the long and short amplicons. The data show that DI was reflected in the profile, affecting the peak heights of various alleles. In order to correlate the DI to the quality of the STR profile obtained with the GlobalFiler™ kit, the peak heights of alleles were analysed against the DI. For each sample, the average peak heights of alleles observed in the range of 80-224 bp were calculated. The averages of peak heights for non-degraded samples and degraded samples (DI > 5) were comparable quite different and the peak heights decreased to about 30% in degraded samples (5000- 1085 RFU and 1860- 720 RFU), respectively for comparison). The data showed a lower allele recovery, but an average peak height of <1000 RFU at SE33, TPOX, CSFPO, D2S338. SE33 is complex hypervariable STR that exists with numerous non-consensus alleles that differ in both size and sequence and are therefore challenging to genotype reproducibly especially in degraded bone samples [13, 14]. PCR inhibitors may reduce PCR amplification efficiency for some alleles and/or simple STRs loci [15].

These results were expected since of input DNA was based on the small amplicon (80 bp) of the Quantifiler™ Trio kit. The results revealed a strong link between DI and average peak heights and allele recovery of GlobalFiler™ kit. These demonstrated the effectiveness of DI in reflecting the quality of the STR profile. The average peak heights of the alleles of less than 214 bp resulted in a higher RFU in samples having a DI of 1.5 or less. The analysis showed that, when the optimal input using 0.5 ng of DNA was available, a total of 80% of called loci was obtained with samples having a DI of up to 1.5. This percentage gradually decreased with increasing degradation index down to an average value of <40% for severely degraded samples with a DI of >5. The number of alleles recovered for the GlobalFiler™ kit markers decreased (from a total of 21autosomal STR markers) for samples with DI >1.5 for the skeletal samples. However, in few samples despite high DI a reasonable partial profile was obtained. Perhaps, these results were due to non-inhibition and a good quant value. Remarkably, only eight of the markers in GlobalFiler™ kit (D2S441, AMEL, D22S1045, D10S1248, D3S1358, D8S1179, D19S433 and FGA) were constantly profiled in most of the samples, regardless of the degradation level/index/category. For the profiles obtained with a lower input DNA (<0.5 ng and >45 pg), the percentage of loci with at least one allelic peak above the analytical threshold declined more rapidly with increasing degradation. In the non-degraded bone samples (DI < 1.49), 84 % of the markers were observed including samples with quant values as low as 10 pg/µl. Only four bone samples achieved a success rate of <100% as only one or two alleles dropped out. Forty-eight samples had 80% or more loci amplified and rest had less percentage age of loci amplified. They were eight bone samples which had <40% amplified loci and these were reflected by the DI and also the inhibitor status. The bone samples demonstrated the increased tolerance of GlobalFiler™ PCR buffers and enzyme to the effect of PCR inhibitors and DNA degradation. Nuclear STR profiling has been quite successful in mass disaster victim identification [16, 17]. None of the samples in this study showed contamination however, there are a few samples that gave high IPC, which might indication the presence of inhibitors. There are many factors that can play a role in the success of STR typing from skeletal remains. Obtaining DNA from skeletal remains can be challenging due to limited amounts of DNA available, which may be fragmented or degraded, and the presence of inhibitors that could co-purify with extracted DNA and prevent amplification by the polymerase chain reaction (PCR). In the field of ancient DNA testing, the temporal age can affect the DNA when much there is a much a longer period of time of a sample [18]. The successful PCR amplification depends on polymerase enzyme to cooperate to the DNA template creating new strand. It is now well established that damage to the DNA template can partially inhibit this process and in turn it can affect to generate reliable data [12].

6. Conclusion

The results for this study showed the effectiveness of both Quantifiler™ Trio and GlobalFiler™ kits for the analysis of DNA samples in old skeletal remains. The results demonstrated that the DI is effective in characterizing a degraded sample giving good indication of amplification success though CT and DNA quant

values can also affect amplification efficiency. The study also showed that despite the samples being inhibited and the IPC values being below thresholds, all samples could be amplified with varying levels of success. The GlobalFiler™ kit was proved tolerant to inhibitors in a much more efficient manner than older kits. Thus, IPC and DI when assessed together could be used to estimate the quality of the expected profile and to decide how to process the samples in order to maximize allele recovery. Thus, Quanifiler™ trio represents a valid tool in dealing with casework samples in a forensic laboratory. This is important in order to save time/resources and enhancing the efficiency of DNA profiling results.

7. *Acknowledgements*

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8. *References*

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