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Comparative study of STR loci for typing old skeletal remains with modified protocols of AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR Kits

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This study highlights a comparative study of short tandem repeat (STR) loci with modified protocols of AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR Kits for typing 27 old skeletal remains collected from 100–1000-year-old mass graves in Pakistan. DNA profiles were obtained from minute quantities of DNA (even from ≤ 10 pg/μL) with modified protocols of these kits, which is a significant achievement in this study. Consensus profiles were produced for each bone sample. A comparison was carried out between Identifiler and Minifiler successfully genotyped STR loci. Full concordance was perceived in 97.33% (146/150) of the compared STR loci, while discordant STR loci were 2.67% (4/150) of the total successfully genotyped STR loci due to either or both allele drop-out or drop-in. Finally, it was observed that the AmpFlSTR MiniFiler kit promoted the recovery of locus/alleles that failed to type with the AmpFlSTR Identifiler kit and more informative DNA profiles were obtained from old skeletal remains with the AmpFlSTR MiniFiler STR kit compared with the AmpFlSTR Identifiler STR kit.

Keywords: DNA typing; old skeletal remains; AmpFlSTR Identifiler STR kit; MiniFiler STR Kit; concordant STR loci; discordant STR loci

Introduction

STR markers are consistently used in case studies for human identification and paternity testing[1,2]. Genotyping with STR loci produces results quickly and with high discriminatory power, yet there is a need to extend this technique to obtain the most informative DNA profiles from highly degraded DNA samples[3]. The amplicon size of the STR markers that are used for DNA profiling usually ranges between 100–450 base pairs[4]. Due to DNA degradation, the longer fragments often cannot be amplified, resulting in partial DNA profiles with lower discrimination power. The autosomal STR multiplex, Identifiler kit, amplifies 15 STR loci and the amelogenin locus in one reaction and has been extensively used within forensic case studies[5]. However, during the analysis of highly degraded DNA, the larger loci in the AmpFlSTR Identifiler kit show allelic/locus drop-out; Therefore, to increase the success rate in analysing highly degraded DNA, the MiniFiler kit is used to overcome the problem of allelic/locus drop-out[6,7,8].

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The MiniFiler STR kit (ABI) has the ability to interpret genotypes from the eight largest loci (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, and FGA) as well as Amelogenin present within the AmpFlSTR Identifiler PCR amplification kit. The AmpFlSTR MiniFiler kit endorses the recovery of alleles that failed to type with the AmpFlSTR Identifiler kit and shows a reduction in stochastic effects due to an overall decrease in amplicon size as MiniFiler alleles range from 70 bp to 283 bp in length[9]. The aim of this study was to compare AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler STR loci for typing highly Degraded DNA, in order to get more informative DNA profiles from old bone samples. The concordant and discordant STR loci between AmpFlSTR MiniFiler and AmpFlSTR Identifiler STR kits were examined and proved that AmpFlSTR MiniFiler kits promoted the recovery of locus/alleles that failed to type with the AmpFlSTR IdentiFiler STR kit.

Materials and methods

Collection of bone samples

In this study, 27 different kinds of human bones (degraded DNA samples) ranging in age from 100 to 1000 years old, collected from old mass graves in Khyber Pakhtunkhwa province of Pakistan, were analysed for comparative study of IdentiFiler and MiniFiler STR loci. Approval for sample collection was obtained from the ethical review committee of the Centre of Excellence in Molecular Biology, University of the Punjab Lahore Pakistan. The samples were photo-documented, labelled and kept at – 20°C until use.

Sample cleaning, pre-treatment and maceration

Sample cleaning, pre-treatment and maceration were carried out in the Forensic Research Laboratory of the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan. The outer surface of these samples was scraped with a motor drill and a dental bur to remove the latent contamination. Each bone sample was fragmented into small pieces using a dental diamond disc and exposed to ultraviolet (UV) light for 30 min. The bone fragments were treated with a scalpel, surgical blades, a Dremel tool, 10% bleach, ddH_2O and 95% ethanol to remove contamination and were placed in a sterilised fume hood overnight. The samples were macerated into fine powder using liquid nitrogen and a SPEX 6750 Freezer / Mill (SPEX CertiPrep, Metuchen, NJ). The bone powder were transferred to 15 mL falcon tube and kept at – 20°C until DNA extraction.

DNA extraction

The extraction of DNA was conducted with a modified silica-column-based complete demineralization extraction method according to the reported protocol of Zar *et al.*[10]. 0.5 g bone powder of each sample was added to a 50 mL falcon tube and 15 mL of extraction buffer (0.5 M EDTA and 0.5% SDS) plus 150 µL of 20 mg/mL Proteinase K was poured into each tube to dissolve the bone powder. Tubes were mixed well and incubated at 56°C for almost 48 h. After first incubation, an additional 150 µL of 20 mg/mL Proteinase K was poured into each tube and incubated at 56°C for 1 h. 7.5 mL of the supernatant was taken from each tube and added to another 50 mL falcon tube.

38 mL of PB buffer (QIAquick PCR purification kit, Qiagen) was added and mixed well. Each tube was centrifuged at 3200×g for 5 min. The mixture of each sample was passed through a QIAamp Blood Maxi column (Qiagen) using QIAvac 24 Plus connecting system (Qiagen). Maxi columns were cleaned by pouring 15 mL PE buffer (QIAquick PCR purification kit, Qiagen) into each column. Each column was placed in a 50 mL collection tube. The tubes were centrifuged at 3200×g for 5–6 min to eradicate the remaining PE buffer. Collection tubes were discarded and each QIAamp Maxi column was kept in a new 50 mL falcon tube. 1 mL of nuclease-free double distilled water (ddH_2O) was added to each QIAamp Blood Maxi column (Qiagen), which after the cap was closed was kept for 5 min at room temperature. Columns in tubes were centrifuged at 3200×g for 5 min. This step was repeated to attain 2 mL of eluted DNA of each sample. 10 mL of the PB buffer was added to each tube containing eluted DNA and mixed well. The mixture of each sample was passed through the QIAamp Mini spin columns (Qiagen) using a QIAvac 24 Plus connecting system (Qiagen). Mini columns were cleaned by pouring 750 µL of PE buffer (QIAquick PCR purification kit, Qiagen) into each column. Each column was placed in a 2 mL collection tube. The tubes were centrifuged at 14,000 rpm for 3 min. Collection tubes were discarded and each QIAamp Mini column was placed in a 1.5 mL Eppendorf tube. 100 µL of nuclease-free double distilled water (ddH_2O) was added to each QIAamp Mini column and incubated for 5 min at room temperature. Each column was centrifuged at 8000 rpm for 1 min. The QIAamp Mini columns were discarded and eluted DNA was stored at –20°C until use. All extractions were accompanied by negative controls.

DNA quantitation

Concentration of DNA was determined by Real Time PCR with Quantifiler Human DUO DNA Quantification kit[11] and the ABI Prism 7500 Sequence Detection System (SDS) as per the recommendations in the Quantifiler User's Manual.

PCR amplification

Amplification of DNA (<100–200 pg/µL) was performed twice with both Identifiler and MiniFiler PCR amplification STR kits. Identifiler PCR amplification conditions were as follows: initial incubation at 95°C for 11 min, denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1 min and a final extension at 60°C for 60 min with a final hold at 4°C. The number of PCR cycles was kept at 33 instead of the standard 28 during all experiments. Amplification conditions were conducted with reduced volume reaction mixtures containing 2.0 µL of Primer Mix, 1.7 µL dH_2O, 0.5 µL AmpliTaq Gold DNA Polymerase (5.0 U/µL), 3.8 µL of PCR Reaction Mix and 2 µL of template DNA in a final reaction volume of 10 µL.

AmpFlSTR MiniFiler PCR amplification was conducted under the following conditions: 95°C for 11 min, 94°C for 20 s, 59°C for 2 min, 72°C for 1 min and a final extension at 60°C for 45 min. The number of PCR cycles was kept at 33 instead of the standard 30 during all experiments. Amplification conditions were carried out with reduced volume reaction mixtures consisting of 1.7 µL H_2O, 2.0 µL of primer mix, 4.0 µL of PCR mix, 0.3 µL (5 U/µL) of AmpliTaq Gold DNA Polymerase and 2.0 µL of low template DNA (<100–200 pg/µL) in a final reaction volume of 10 µL.

Capillary electrophoresis and data analysis

Experiments were run on an ABI Prism 3130 Genetic Analyzer. The injection time and electrokinetic voltage were the same for both Identifiler and MiniFiler PCR products. 1.0 μL of amplified product was combined with 10.0 μL of Hi-Di formamide and 0.2 μL of LIZ GeneScan 500 size standard. The samples were loaded into a 96-well plate, denatured for 5 min at 95 °C and snap cooled for 3 min before running on a genetic analyser. The data were analysed with GeneMapper ID software (Version 3.2). The minimum analysis threshold for scoring allelic peak height was 100 RFU (Relative Fluorescent Unit). Consensus DNA profiles (collecting common alleles from two replicate reactions of each sample) were produced for each bone sample. DNA profiles of all members of the laboratory staff were also produced with AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler STR kits, in order to exclude the chances of any possibility of internal contamination, by comparing the staff DNA profiles against the results obtained from the bones.

Results and discussion

Construction of consensus DNA profiles

In the current study, a modified silica-column-based complete demineralization extraction method was used for DNA extraction. It might be due to the fact that complete demineralization followed by silica binding is highly successful for the extraction and recovery of DNA profiles from degraded old skeletal remains[12]. The quantity of DNA was ≤ 100–200 pg /μL from 0.5 g bone powder of each old skeletal remains; therefore, an increased number of PCR cycles was used for the amplification of low-template DNA using both AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler STR kits. The quantity of PCR product was increased with an extended number of PCR cycles, but stochastic effects also appeared; therefore, rules of low-template DNA interpretations (consensus approach) were applied for the analysis of low template DNA[13,14]. In this study two replicates were produced independently for each of the old bone samples using both AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler STR kits. Consensus profiles were made from two replicates of each bone sample as shown in Tables 1 and 2. All PCR amplification reactions were accompanied by negative controls. The negative controls showed no allele or locus drop-in (Figures 1 and 2) confirming the authenticity of the results, and there was no indication of staff contamination when comparing the staff's DNA profiles against the results obtained from the bones.

Concordance and non-concordance between AmpFlSTR Identifiler and AmpFlSTR MiniFiler successfully genotyped STR Loci

The most significant challenge to interpretation in DNA profiling of highly degraded DNA samples arises when either or both allele drop-in and drop-out create discordances[15,16]. The AmpFlSTR IdentiFiler STR kit simultaneously amplifies 15 autosomal STR loci (D8S1179, D21S11, D7820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA) and a sex determining marker (amelogenin), while the AmpFlSTR MiniFiler PCR amplification kit (ABI) simultaneously amplifies eight mini-STR loci D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA and the sex determining amelogenin loci, shared with the AmpFlSTR IdentiFiler STR kit, but with shorter amplicons. In this study,

Table 1. Consensus profiles produced with AmpFlSTR Identifiler STR kit.

Sample ID	Loci Replicates	D8S1179	D21S11	D7820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
DFL 1	Replication # 1	13, 14	29, 32.2	8	10,12	15	6, 9.3	8, 9	12, 13	18	14, 16.2	16, 18	9, 11	13, 21	X	10, 12	20, 24
	Replication # 2	13, 14	29, 32.2	8	10,12	15	6, 9.3	8, 9	12, 13	18	14, 16.2	16, 18	9, 11	13, 21	X	10, 12	20, 24
	Consensus Profile	13, 14	29, 32.2	8	10, 12	15	6, 9.3	8, 9	12, 13	18	14, 16.2	16, 18	9, 11	13, 21	X	10, 12	20, 24
DFL 2	Replication # 1	13	28	11, 12, 15	12	15, 16, 17	6	11, 12	9, 11	19, 20	13	14.2	-	14, 16	X, Y	-	21
	Replication # 2	13	28	11	10, 12	15, 16	-	11, 12	9, 11	20	13, 16, 15	14.2	-	16	X, Y	11, 12	21
	Consensus Profile	13	28	11	12	15, 16	-	11, 12	9, 11	20	13	14.2	-	16	X, Y	-	21
DFL 3	Replication # 1	-	33.2	11	10, 12	15, 16	9.3	11, 13	11, 12	19, 20	13	17	8	16	X	12, 13	21, 24
	Replication # 2	13	33.2	9, 11	10, 12	15, 16	8, 9.3	11, 12	11,12	19, 20	13	17	8	16	X	12, 13	21
	Consensus Profile	-	33.2	11	10, 12	15, 16	9.3	11	11, 12	19, 20	13	17	8	16	X	12, 13	21
DFL 4	Replication # 1	13	30	-	-	14, 15	-	-	11, 12	-	13.2, 15.2	11	-	-	X	-	23
	Replication # 2	-	29, 30	-	-	13, 14, 15	-	-	11	-	13.2, 15.2	-	7, 8	-	X	11	23
	Consensus Profile	-	30	-	-	14, 15	-	-	11	-	13.2, 15.2	-	-	-	X	-	23

DFL 5	Replication # 1	14, 15	26, 30	12	10, 11	15, 17	9.3	8, 11, 12	11, 13	20, 24	14, 15	18, 19	8, 9	15, 17	X, Y	11	22, 24
	Replication # 2	14, 15	26, 30	12	10, 11	15, 17	9.3	8, 12, 13	11, 13	20, 24	14, 15	18, 19	8, 9	14, 15, 17	X, Y	11	22, 24
	Consensus Profile	14, 15	26, 30	12	10, 11	15, 17	9.3	8, 12	11, 13	20, 24	14, 15	18, 19	8, 9	15, 17	X, Y	11	22, 24
DFL 6	Replication # 1	10, 15	30.2, 31.2	11	10, 13	16, 17	6, 9	8, 12	11, 12	20, 23	13, 15.2	16, 18	8, 9	13, 17	X	9, 11	19, 21
	Replication # 2	10, 15	30.2, 31.2	11	10, 13	16, 17	6, 9	8, 12	11, 12	20, 23	13, 15.2	16, 18	8, 9	13, 17	X	9, 11	19, 21
	Consensus Profile	10, 15	30.2, 31.2	11	10, 13	16, 17	6, 9	8, 12	11, 12	20, 23	13, 15.2	16, 18	8, 9	13, 17	X	9, 11	19, 21
DFL 7	Replication # 1	14	30, 32.2	8, 11	12	15, 17	8, 9	8	11	23, 25	13, 14	16, 18	8, 11	13, 17	X, Y	12, 13	21, 22
	Replication # 2	14	30, 30.2, 32.2	8, 11	12	15, 17	8, 9	8	11	23, 25	13, 14	16, 18	8, 11	17	X, Y	9, 12, 13	20, 21, 22
	Consensus Profile	14	30, 32.2	8, 11	12	15, 17	8, 9	8	11	23, 25	13, 14	16, 18	8, 11	17	X, Y	12, 13	21, 22
DFL 8	Replication # 1	10, 14	30, 31.2	10	11, 12	14, 18	6, 8	8, 11	8, 11	18, 22	14, 15.2	16, 18	8, 10	17, 19	X, Y	10, 11	20, 24
	Replication # 2	10, 14	30, 31.2	10	11, 12	14, 18	6, 8	8, 11	8, 9, 11	18, 22	14, 15.2	16, 18	8, 10	17, 19	X, Y	10, 11	20, 24
	Consensus Profile	10, 14	30, 31.2	10	11, 12	14, 18	6, 8	8, 11	8, 11	18, 22	14, 15.2	16, 18	8, 10	17, 19	X, Y	10, 11	20, 24
DFL 9	Replication # 1	15	-	-	12	16	-	-	-	-	16	17	-	15	X	12	-

(*Continued*)

Table 1. (*Continued*).

Sample ID	Loci Replicates	D8S1179	D21S11	D7820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
	Replication # 2	15	-	-	-	16, 17	6	11	-	-	14.2, 15.2	17	-	15	X	11	-
	Consensus Profile	15	-	-	-	16	-	-	-	-	-	17	-	15	X	-	-
DFL 10	Replication # 1	13,14	28, 30	8, 13	12	17, 19	7, 8	12, 13	13	23, 24	13, 16.2	14, 16	11	13, 14	X, Y	11, 13	20, 25
	Replication # 2	14	28, 30	8, 13	10, 12	17, 19	8	12	13	23, 24	13, 16.2	14, 16, 18	11, 12	13, 14	X, Y	11, 12	20, 25
	Consensus Profile	14	28, 30	8,13	12	17, 19	8	12	13	23, 24	13, 16.2	14, 16	11	13, 14	X, Y	11, 12	20, 25
DFL 11	Replication # 1	10	28, 30	8, 11	10, 11	16, 18	7, 9	11, 13	12	20, 22	13, 15	17	8, 12	14, 17	X, Y	12, 13	23, 26
	Replication # 2	10, 15	28, 30	8, 11	10, 11	16, 18	7, 9	11, 13	9, 12	20, 22	13, 15	16, 17	8, 12	14, 15, 17	X, Y	11, 12, 13	23, 26
	Consensus Profile	10	28, 30	8, 11	10, 11	16, 18	7, 9	11, 13	12	20, 22	13, 15	17	8, 12	14, 17	X, Y	12, 13	23, 26
DFL 12	Replication # 1	13,16	28, 33.2	11, 12	10, 11	14, 16	6, 9	8	10, 12	19, 24	13, 14	15, 16	8	13, 15	X, Y	10, 13	19, 23
	Replication # 2	13, 16	28, 33.2	12	10, 11	14, 16	6, 9	8	10, 12	19, 24	13, 14	15, 16	8	13, 15	X, Y	10, 13	19, 23
	Consensus Profile	13, 16	28, 33.2	12	10, 11	14, 16	6, 9	8	10, 12	19, 24	13, 14	15, 16	8	13, 15	X, Y	10, 13	19, 23
DFL 13	Replication # 1	12, 13	30, 30.2	10, 12	12, 13	16, 17	9	8, 10	10, 11	18, 20	15	14, 19	11	14, 15	X	12, 13	19, 24
	Replication # 2	12, 13	30, 30.2	10, 12	12, 13	16, 17	9	8, 10	10, 11	18, 20	15	14, 18, 19	11	14, 15	X	12, 13	19, 24
	Consensus Profile	12, 13	30, 30.2	10, 12	12, 13	16, 17	9	8, 10	10, 11	18, 20	15	14, 19	11	14, 15	X	12, 13	19, 24

DFL 14	Replication # 1	-	-	11	-	18	-	11	12	-	13	17, 18	8, 9, 11	15	X, Y	12	-
	Replication # 2	10, 16	-	8,9,11	10	-	-	11	8, 11	-	13,16	17	8,9	16	X, Y	-	-
	Consensus Profile	-	-	11	-	-	-	11	-	-	13	17	8,9	-	X,Y	-	-
DFL 15	Replication # 1	17	28	11	-	-	6	-	9	-	14	-	8	-	X, Y	-	21
	Replication # 2	13	-	11.2	-	16	6	13	-	-	12, 13	-	-	-	X,Y	-	21
	Consensus profile	-	-	-	-	-	6	-	-	-	-	-	-	-	X,Y	-	21
DFL 16	Replication # 1	-	-	-	-	16	-	11	-	-	-	-	-	-	-	-	20
	Replication # 2	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	21
	Consensus Profile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFL 17	Replication # 1	13	28, 33.2	11	11	15, 16	9.3	11	9, 11	17, 20	13, 16	19	-	15, 16	X, Y	11, 12	21
	Replication # 2	10, 11, 13	28, 29	8	9.2, 11	17	9.3	11	-	-	13, 16	17, 19	-	16	X,Y	12	21
	Consensus Profile	13	28	-	11	-	9.3	11	-	-	13, 16	19	-	16	X,Y	12	21
DFL 18	Replication # 1	13	-	-	-	16	-	-	-	20	11	-	-	-	Y	-	-
	Replication # 2	-	28	11	-	-	7	-	-	20	11	17	-	-	Y	12	-
	Consensus Profile	-	-	-	-	-	-	-	-	20	11	-	-	-	Y	-	-

(*Continued*)

Table 1. (*Continued*).

Sample ID	Loci Replicates	D8S1179	D21S11	D7820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
DFL 19	Replication # 1	-	-	-	-	16	-	-	-	-	-	-	-	20	-	-	-
	Replication # 2	-	-	10	-	-	-	11	-	-	-	-	-	-	-	12	-
	Consensus Profile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFL 20	Replication # 1	-	-	10	12	-	-	-	-	-	-	17	11	14, 18	X	-	23
	Replication # 2	12	-	11	-	-	-	12	-	-	-	-	-	-	-	-	-
	Consensus Profile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFL 21	Replication # 1	11, 14	28, 32.2	12	11	15	7, 8	12	11	22	13, 14.2	14, 15	8	14, 16	X, Y	11, 12	21
	Replication # 2	11, 14	28, 32.2	12	11	15	7, 8	11, 12	-	22	13, 14.2	14, 15	8, 9	14, 15	X, Y	11, 12	21, 23
	Consensus Profile	11, 14	28, 32.2	12	11	15	7, 8	12	-	22	13, 14.2	14, 15	8	14	X, Y	11, 12	21
DFL 22	Replication # 1	13, 14	29, 30	8, 11	11, 12	15, 17	9	12	9	19	13, 14	14, 15	10	-	X	-	24
	Replication # 2	14	-	-	11	-	8	8, 12	9	25	-	15	10	-	X	10	24
	Consensus Profile	14	-	-	11	-	-	12	9	-	-	15	10	-	X	-	24

DFL 23	Replication # 1	15	30	8	-	-	6	12	-	-	-	-	-	-	-	20
	Replication # 2	14	-	-	-	-	-	-	-	-	-	14, 18	-	-	Y	-	-
	Consensus Profile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFL 24	Replication # 1	10	29, 31	8	13	15, 16, 17	-	8	-	-	13, 15.2	16, 19, 20	-	-	X	11, 13	22
	Replication # 2	10	29,31	8	13	16, 17	-	8	-	-	13, 15.2	16, 20	-	-	X	11, 13	22
	Consensus Profile	10	29,31	8	13	16, 17	-	8	-	-	13, 15.2	16, 20	-	-	X	11, 13	22
DFL 25	Replication # 1	-	-	-	-	-	-	12	-	-	-	-	-	-	X		21
	Replication # 2	-	-	-	-	-	-	12	-	-	-	-	-	-	X		21, 22
	Consensus Profile	-	-	-	-	-	-	12	-	-	-	-	-	-	X		21
DFL 26	Replication # 1	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-
	Replication # 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Consensus Profile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DFL 27	Replication # 1	11	29	12	10	16	6	8	10	19	15	14, 17	9	12	X	13	21
	Replication # 2	11	29	12	10, 13	16	6	8	10	19	15	17	9	12	X	13	21
	Consensus Profile	11	29	12	10	16	6	8	10	19	15	17	9	12	X	13	21

Table 2. Consensus profiles produced with AmpFlSTR MiniFiler STR kit.

Sample	Replications	D13S317	D7S820	AML	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
DFL 1	Replication # 1	8, 9	8	X	18	29, 32.2	12, 13	13, 21	10, 12	20, 24
	Replication # 2	8, 9	8	X	18	29, 32.2	12, 13	13, 21	10, 12	20, 24
	Consensus Profile	8,9	8	X	18	29, 32.2	12, 13	13, 21	10, 12	20, 24
DFL 2	Replication # 1	11,12	11	X, Y	19, 20	26.2, 30, 33.2	9, 11	16	12	21, 24
	Replication # 2	11, 12	9, 11	X, Y	20	26.2	9, 11, 12	15, 16	10, 12	21
	Consensus Profile	11, 12	11	X, Y	20	26.2	9, 11	16	12	21
DFL 3	Replication # 1	11	11	X, Y	19, 20	33.2	11, 12	15, 16	10, 12	21, 24
	Replication # 2	11, 12	11, 12	X, Y	19, 20	33.2	8, 11, 12	15, 16	10, 11, 12	21
	Consensus Profile	11	11	X, Y	19, 20	33.2	11, 12	15, 16	10, 12	21
DFL 4	Replication # 1	11	10, 11	X	16	30	11	14, 15	10, 11	18.2, 23
	Replication # 2	11	10, 11	X	24	30	11, 12	14, 15	10	18.2, 23
	Consensus Profile	11	10, 11	X	-	30	11	14, 15	10	18.2, 23
DFL 5	Replication # 1	8, 12	11, 12	X, Y	20, 24	26, 30	11, 13	13, 15, 17	10, 11, 12	21, 22, 24
	Replication # 2	8, 12	12	X, Y	20, 24	26, 30	9, 11, 13	14, 15, 17	10, 11	22, 24
	Consensus Profile	8, 12	12	X, Y	20, 24	26, 30	11, 13	15, 17	10, 11	22, 24
DFL 6	Replication # 1	8, 12	11	X	20, 23	30.2, 31.2	11, 12	13, 17	10, 13	19, 21
	Replication # 2	8, 12	11	X	20, 23	30.2, 31.2	11, 12	13, 17	10, 13	19, 21
	Consensus Profile	8, 12	11	X	20, 23	30.2, 31.2	11, 12	13, 17	10, 13	19, 21
DFL 7	Replication # 1	8	8, 11	X, Y	23, 25	30, 32.2	11	17, 19	12	21, 22
	Replication # 2	8	8, 11	X, Y	23, 25	30, 32.2	11	17, 19	12	21, 22
	Consensus Profile	8	8, 11	X, Y	23, 25	30, 32.2	11	17, 19	12	21, 22
DFL 8	Replication # 1	8, 11	10	X, Y	18, 22	30, 31.2	8, 11	17, 19	11, 12	20, 24
	Replication # 2	8, 11	10	X, Y	18, 22	30, 31.2	8, 11	17, 19	11, 12	20, 24
	Consensus Profile	8, 11	10	X, Y	18, 22	30, 31.2	8, 11	17, 19	11, 12	20, 24

DFL 9	Replication # 1	11, 12,13	-	X	20	30	11, 12, 13	15	10, 11, 12	21
	Replication # 2	13	-	X	25	31.2	11	15	10, 11	-
	Consensus Profile	13	-	X	-	-	11	15	10, 11	-
DFL 10	Replication # 1	8, 12, 13	8, 13	X, Y	23, 24	28, 30	13	13, 14	12	20, 25
	Replication # 2	12, 13	8, 13	X, Y	23, 24	28, 30	12, 13	13, 14	12	20, 25
	Consensus Profile	12, 13	8, 13	X, Y	23, 24	28, 30	13	13, 14	12	20, 25
DFL 11	Replication # 1	11, 13	8, 11	X, Y	20, 22	28, 30	12	14, 17	10, 11	26
	Replication # 2	11, 13	8, 11	X, Y	20, 22	28, 30	9, 12	14, 17	10, 11	26
	Consensus Profile	11, 13	8, 11	X, Y	20, 22	28, 30	12	14, 17	10, 11	26
DFL 12	Replication # 1	8	11, 12	X, Y	19, 24	28, 33.2	10, 12	13, 15	10, 11	19, 23
	Replication # 2	8	11, 12	X, Y	19, 24	28, 33.2	10, 12	13, 15	10, 11	19, 23
	Consensus Profile	8	11, 12	X, Y	19, 24	28, 33.2	10, 12	13, 15	10, 11	19, 23
DFL 13	Replication # 1	8, 10	10, 12	X	18, 20	30, 30.2	10, 11	14, 15	12, 13	19, 24
	Replication # 2	8, 10	10, 12	X	18, 20	30, 30.2	10, 11	14, 15	12, 13	19, 24
	Consensus Profile	8, 10	10, 12	X	18, 20	30, 30.2	10, 11	14, 15	12, 13	19, 24
DFL 14	Replication # 1	11, 12	11, 12	X, Y	26	-	-	-	11, 12	21, 22
	Replication # 2	11	11	X, Y	20	33.2	9	15	10, 12	-
	Consensus Profile	11	11	X, Y	-	-	-	-	12	-
DFL 15	Replication # 1	12	-	X, Y	18, 25	33.2	8, 11	16, 17	10	24
	Replication # 2	-	-	X, Y	20, 23	28	9, 10	16, 17	10	24
	Consensus Profile	-	-	X, Y	-	-	-	16, 17	10	24
DFL 16	Replication # 1	-	-	-	-	28	-	19	-	24
	Replication # 2	-	-	-	-	-	-	-	12	-
	Consensus Profile	-	-	-	-	-	-	-	-	-

(*Continued*)

Table 2. (*Continued*).

Sample	Replications	D13S317	D7S820	AML	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
DFL 17	Replication # 1	11, 12	11	X, Y	29	28	6	16, 19	11	21
	Replication # 2	11	8, 10	X, Y	18	28, 30	11	16	11	21
	Consensus Profile	11	-	X, Y	-	28	-	16	11	21
DFL 18	Replication # 1	-	9, 10	Y	20	32.2, 34	-	15	10	-
	Replication # 2	-	-	Y	20	-	-	16	11, 12	24
	Consensus Profile	-	-	Y	20	-	-	-	-	-
DFL 19	Replication # 1	11	-	X	-	-	-	-	12	20
	Replication # 2		11	-	-	31	10, 11	19	-	-
	Consensus Profile	-	-	-	-	-	-	-	-	-
DFL 20	Replication # 1	-	11	X, Y	20	29	6	18	-	-
	Replication # 2	-	10	X, Y	20	-	8, 10, 12	-	-	-
	Consensus Profile	-	-	X, Y	20	-	-	-	-	-
DFL 21	Replication # 1	12	11, 12	X, Y	22	28, 32.2	9, 12	12, 14	10, 11	21, 22
	Replication # 2	11, 12	11, 12	X, Y	22	28, 32.2	9, 12	12, 14	10, 11	21, 22
	Consensus Profile	12	11, 12	X, Y	22	28, 32.2	9, 12	12, 14	10, 11	21, 22
DFL 22	Replication # 1	12	8	X	17, 20	-	11	14, 16	11, 12	23
	Replication # 2	8, 12	10	X	-	33.2	11	16	11	22, 23
	Consensus Profile	12	-	X	-	-	11	16	11	23
DFL 23	Replication # 1	12	-	-	-	29	-	-	-	25
	Replication # 2	-	11	-	-	-	9	-	12	20, 21
	Consensus Profile	-	-	-	-	-	-	-	-	-
DFL 24	Replication # 1	8, 11	8, 9	X	20, 25	29, 31	10, 12	12, 14	12, 13	22, 26
	Replication # 2	8, 11	8	X	24, 25	29, 31	10, 12	12, 14, 19	12, 13	22
	Consensus Profile	8, 11	8	X	25	29, 31	10, 12	12, 14	12, 13	22

DFL 25	Replication # 1	9, 12		X		28				21, 24
	Replication # 2	9, 12		X		-				21, 24
	Consensus Profile	9, 12		X		-				21, 24
DFL 26	Replication # 1	11		XY						21
	Replication # 2	11		XY						21, 23
	Consensus Profile	11		XY						21
DFL 27	Replication # 1	8, 9	12	X	19, 25	29	10	12, 15	10	21
	Replication # 2	8, 9	12	X	19	29	10	12, 15	10	21, 22
	Consensus Profile	8, 9	12	X	19	29	10	12, 15	10	21

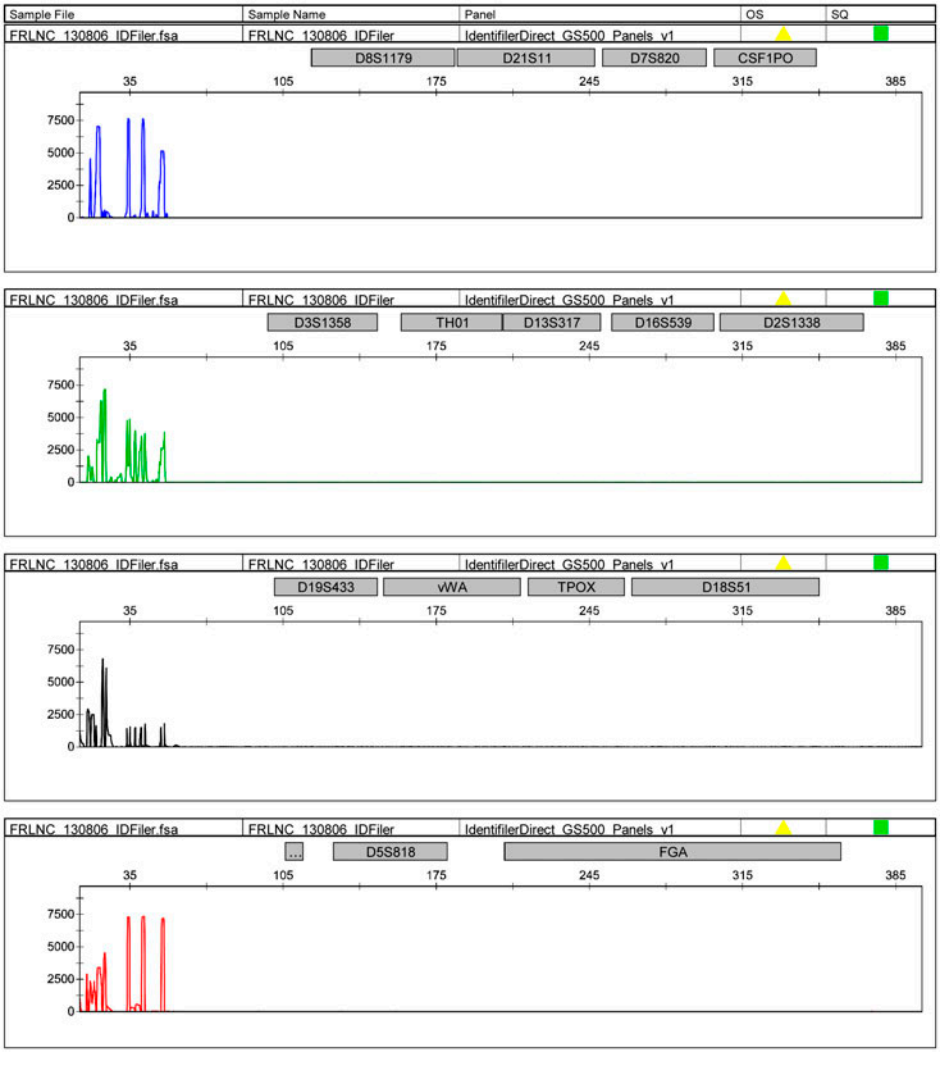


Figure 1. Negative control with AmpFlSTR Identifiler STR kit.

27 highly degraded old bone samples were evaluated with modified protocols of Identifiler and MiniFiler STR kits. Nine STR loci are common in both AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler STR kits; therefore, concordance and non-concordance was determined on the basis of these common STR loci. Full concordance between AmpFlSTR MiniFiler and AmpFlSTR IdentiFiler successfully genotyped STR loci was perceived in 97.33% (146/150) of the compared STR loci, while discordant STR loci were 2.67% (4/150) of the total STR loci, due to either or both of allele drop-out or drop-in (Table 3). Similar kinds of findings (99.7% and 99.88% full concordance), have been reported by Hill *et al.*[17] and Alenizi *et al.*[18], respectively, for typing fresh blood samples using AmpFlSTR MiniFiler and AmpFlSTR IdentiFiler STR kits, while in the current study old skeletal remains have been used. Oh *et al.*[8] have investigated eight human femurs (200–400 years old) for comparative analysis of STRs and mini-STRs

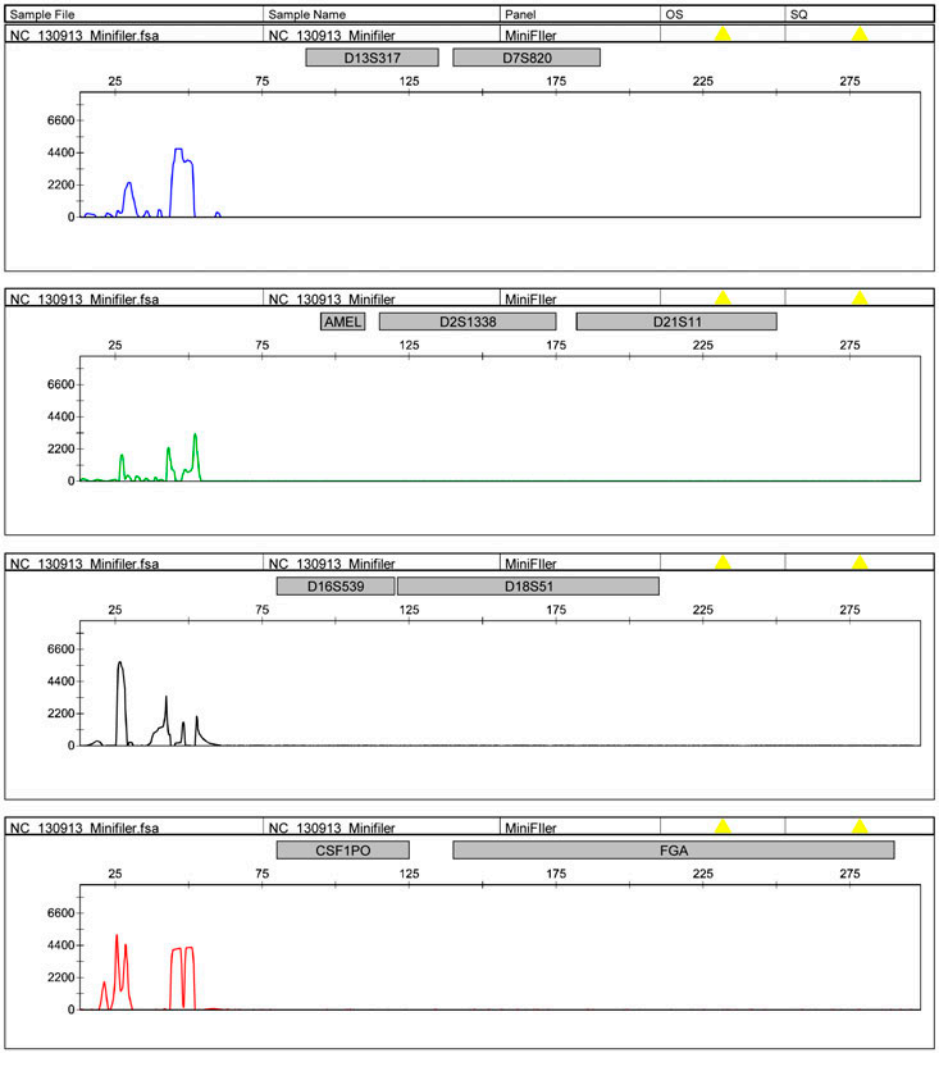


Figure 2. Negative control with AmpFlSTR MiniFiler STR kit.

loci, while in the current study 27 different kinds of old skeletal remains have been used. Considering these points, it is recommended that forensic DNA experts strongly consider modified protocols of IdentiFiler and MiniFiler STR kits for typing degraded and old skeletal remains.

Comparison of DNA profiles obtained with AmpFlSTR IdentiFiler and AmpFlSTR MiniFiler kits from highly degraded old bones

A strategy of comparing DNA profiles obtained with AmpFlSTR MiniFiler and AmpFlSTR Identifiler PCR amplification kits for typing highly-degraded DNA revealed more genetic information with the MiniFiler kit compared with the Identifiler kit. In total, 27 DNA samples, 14 full DNA profiles, 10 partial and three no profiles were

Table 3. Concordance and non-concordance of STR loci using AmpFlSTR Identifiler and AmpFlSTR Minifiler STR kits.

Sample ID	Name of Kits	Loci D13S317	D7S820	AMEL	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
DFL 1	IdentiFiler	8, 9	8	X	18	29, 32.2	12, 13	13, 21	10, 12	20, 24
	MiniFiler	8,9	8	X	18	29, 32.2	12, 13	13, 21	10, 12	20, 24
DFL 2	IdentiFiler	11, 12	11	X, Y	20	28	9, 11	16	12	21
	MiniFiler	11, 12	11	X, Y	20	26.2	9, 11	16	12	21
DFL 3	IdentiFiler	11	11	X	19, 20	33.2	11, 12	16	10, 12,	21
	MiniFiler	11	11	X, Y	19, 20	33.2	11, 12	15, 16	10, 12	21
DFL 4	IdentiFiler	-	-	X	-	30	11	-	-	23
	MiniFiler	11	10, 11	X	-	30	11	14, 15	10	18.2, 23
DFL 5	IdentiFiler	8, 12	12	X, Y	20, 24	26, 30	11, 13	15, 17	10, 11	22, 24
	MiniFiler	8, 12	12	X, Y	20, 24	26, 30	11, 13	15, 17	10, 11	22, 24
DFL 6	IdentiFiler	8, 12	11	X	20, 23	30.2, 31.2	11, 12	13, 17	10, 13	19, 21
	MiniFiler	8, 12	11	X	20, 23	30.2, 31.2	11, 12	13, 17	10, 13	19, 21
DFL 7	IdentiFiler	8	8, 11	X, Y	23, 25	30, 32.2	11	17	12	21, 22
	MiniFiler	8	8, 11	X, Y	23, 25	30, 32.2	11	17, 19	12	21, 22
DFL 8	IdentiFiler	8, 11	10	X, Y	18, 22	30, 31.2	8, 11	17, 19	11, 12	20, 24
	MiniFiler	8, 11	10	X, Y	18, 22	30, 31.2	8, 11	17, 19	11, 12	20, 24
DFL 9	IdentiFiler		-	X	-	-	-	15	-	-
	MiniFiler	13	-	X	-	-	11	15	10, 11	-

DFL 10	IdentiFiler	12, 13	8, 13	X, Y	23, 24	28, 30	13	13, 14	12	20, 25
	MiniFiler	12, 13	8, 13	X, Y	23, 24	28, 30	13	13, 14	12	20, 25
DFL 11	IdentiFiler	11, 13	8, 11	X, Y	20, 22	28, 30	12	14, 17	10, 11	23, 26
	MiniFiler	11, 13	8, 11	X, Y	20, 22	28, 30	12	14, 17	10, 11	26
DFL 12	IdentiFiler	8	12	X, Y	19, 24	28, 33.2	10, 12	13, 15	10, 11	19, 23
	MiniFiler	8	11, 12	X, Y	19, 24	28, 33.2	10, 12	13, 15	10, 11	19, 23
DFL 13	IdentiFiler	8, 10	10, 12	X	18, 20	30, 30.2	10, 11	14, 15	12, 13	19, 24
	MiniFiler	8, 10	10, 12	X	18, 20	30, 30.2	10, 11	14, 15	12, 13	19, 24
DFL 14	IdentiFiler	11	11	X, Y	-	-	-	-	-	-
	MiniFiler	11	11	X, Y	-	-	-	-	12	-
DFL 15	IdentiFiler	-	-	X,Y	-	-	-	-	-	21
	MiniFiler	-	-	X, Y	-	-	-	16, 17	10	24
DFL 16	IdentiFiler	-	-	-	-	-	-	-	-	-
	MiniFiler	-	-	-	-	-	-	-	-	-
DFL 17	IdentiFiler	11	-	X, Y	-	28	-	16	11	21
	MiniFiler	11	-	X, Y	-	28	-	16	11	21
DFL 18	IdentiFiler	-	-	Y	20	-	-	-	-	-
	MiniFiler	-	-	Y	20	-	-	-	-	-
DFL 19	IdentiFiler	-	-	-	-	-	-	-	-	-
	MiniFiler	-	-	-	-	-	-	-	-	-

(*Continued*)

Table 3. (*Continued*).

Sample ID	Name of Kits	Loci D13S317	D7S820	AMEL	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
DFL 20	IdentiFiler	-	-	-		-	-	-	-	-
	MiniFiler	-	-	X, Y	20	-	-	-	-	-
DFL 21	IdentiFiler	12	12	X, Y	22	28, 32.2	-	14	11	21
	MiniFiler	12	11, 12	X, Y	22	28, 32.2	9, 12	12, 14	10, 11	21, 22
DFL 22	IdentiFiler	12	-	X	-	-	9	-	11	24
	MiniFiler	12	-	X	-	-	11	16	11	23
DFL 23	IdentiFiler	-	-	-	-	-	-	-	-	-
	MiniFiler	-	-	-	-	-	-	-	-	-
DFL 24	IdentiFiler	8	8	X	-	29, 31	-	-	13	22
	MiniFiler	8, 11	8	X	25	29, 31	10, 12	12, 14	12, 13	22
DFL 25	IdentiFiler	12		X						21
	MiniFile	9, 12		X						21, 24
DFL 26	IdentiFiler	-	-	-	-	-	-	-	-	-
	MiniFile	11		XY						21
DFL 27	IdentiFiler	8	12	X	19	29	10	12	10	21
	MiniFile	8, 9	12	X	19	29	10	12, 15	10	21

Table 4. DNA quantity and number of STR loci successfully genotyped with the AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR Kits from old skeletal remains.

Sample ID	Type of sample	DNA quantity (pg/μL)	Identifiler STR loci	Minifiler STR loci	Sample ID	Type of sample	DNA quantity (pg/μL)	Identifiler STR loci	Minifiler STR loci
DFL (1)	Humerus	113	16/16	9/9	DFL (15)	Radius	Not detected	3/16	4/9
DFL (2)	Tibia	6.0	13/16	9/9	DFL (16)	Femur	Not detected	0/16	0/9
DFL (3)	Ulna	5.0	15/16	9/9	DFL (17)	Tibia	5.0	11/16	6/9
DFL (4)	Metacarpal	Not detected	6/16	8/9	DFL (18)	Radius	2.0	3/16	2/9
DFL (5)	Tibia	70.0	16/16	9/9	DFL (19)	Femur	Not detected	0/16	0/9
DFL (6)	Ulna	106.0	16/16	9/9	DFL (20)	Humerus	Not detected	0/16	2/9
DFL (7)	Ulna	24.0	16/16	9/9	DFL (21)	Metacarpal	23.0	13/16	9/9
DFL (8)	Radius	40.0	16/16	9/9	DFL (22)	Fibula	Not detected	8/16	6/9
DFL (9)	Radius	3.0	5/16	5/9	DFL (23)	Radius	Not detected	0/16	0/9
DFL (10)	Skull	122.0	16/16	9/9	DFL (24)	Metacarpal	7.0	11/16	9/9
DFL (11)	Tibia	145.0	16/16	9/9	DFL (25)	Humerus	Not detected	3/16	3/9
DFL (12)	Femur	165.0	16/16	9/9	DFL (26)	Skull	Not detected	0/16	3/9
DFL (13)	Ulna	110.0	16/16	9/9	DFL (27)	Fibula	75.0	16/16	9/9
DFL (14)	Ulna	4.0	6/16	4/9					

Identifiler Kit: Full DNA profile (16/16 STR loci); partial DNA profile (<16/16 STR loci); no profile (0/16 STR Loci).
Minifiler Kit: Full DNA profile (9/9 STR loci); partial DNA profile (<9/9 STR Loci), no profile (0/9 STR loci).

produced with the MiniFiler kit, while in the case of the Identifiler kit, 10 full DNA profiles, 12 partial profiles and five no profiles were produced, as shown in Figure 5. Full, partial and no profiles were made on the basis of the number of STR loci successfully genotyped with the AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR kits from old skeletal remains as shown in Table 4. Comparison of STR loci highlights the ability of the MiniFiler STR kit to recover more informative DNA profiles than the

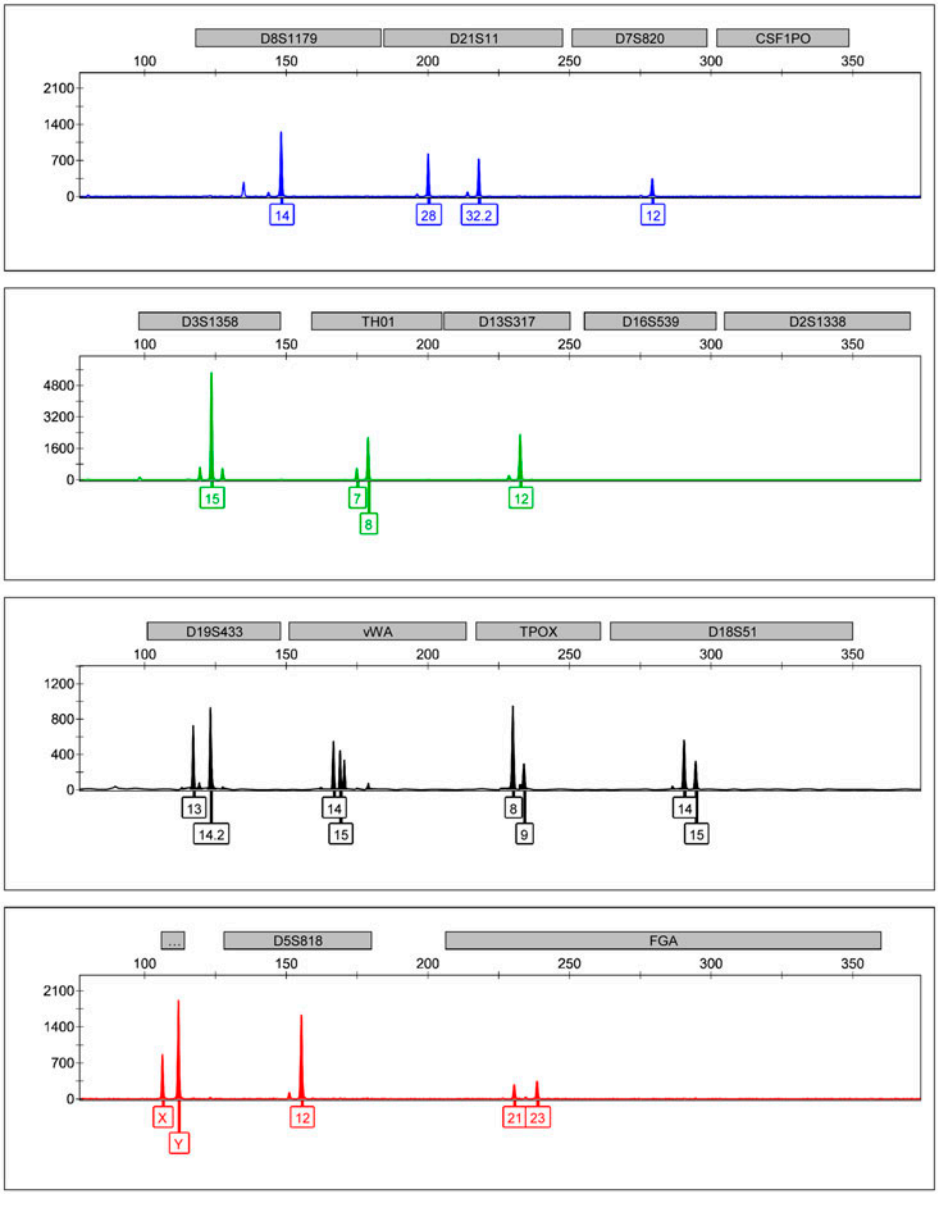


Figure 3. Partial DNA profile obtained with AmpFlSTR Identifiler STR kit from bone sample (DFL 21).

Identifiler kit from the same bone samples, as shown by one example in Figures 3 and 4. It might be because the primers of the MiniFiler STR loci yield smaller amplicons compared with the conventional Identifiler STR loci, which recover STR loci/alleles that failed to type with the Identifiler STR kit[8]. Similar kinds of finding have been reported by Coble and Butler[19]).

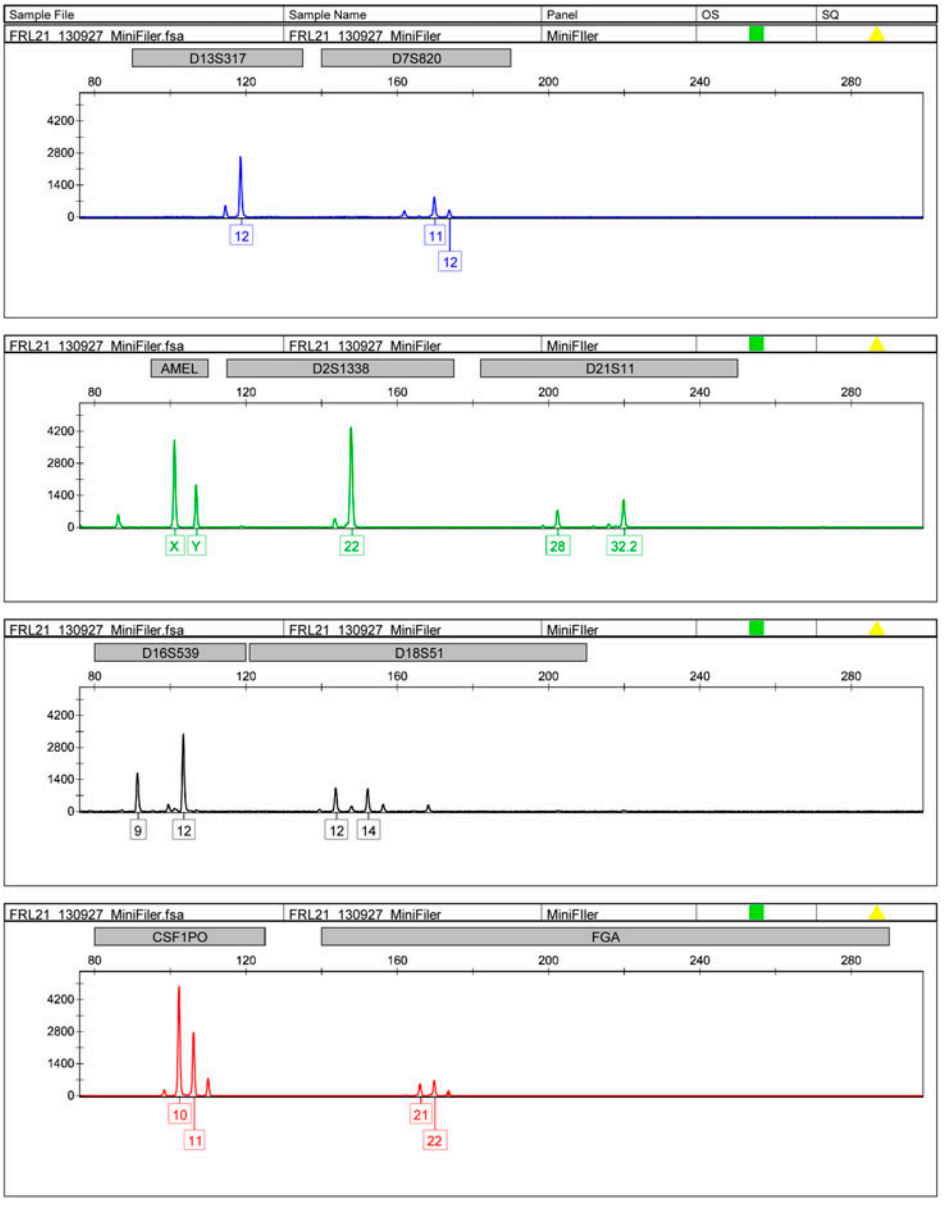
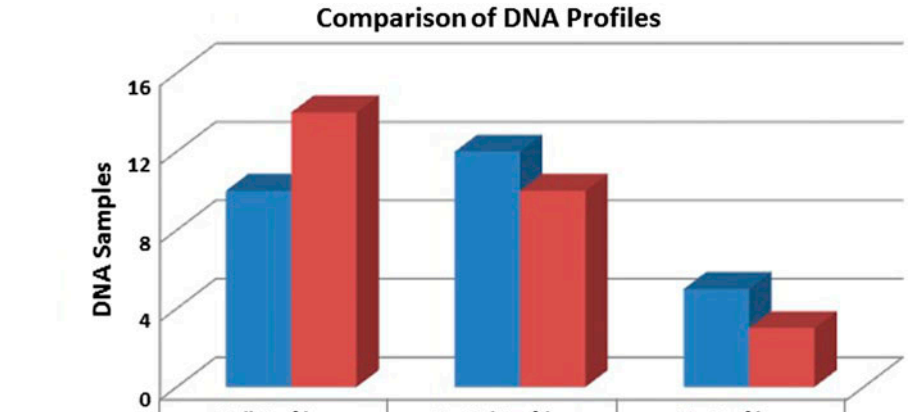


Figure 4. Full DNA profile obtained with AmpFlSTR MiniFiler STR kit from bone sample (DFL 21).



	Full Profiles	Partial Profiles	No Profiles
IdentiFiler Kit	10	12	5
MiniFiler Kit	14	10	3

Figure 5. Comparison of DNA profiles obtained with AmpFlSTR Identifiler and AmpFlSTR Minifiler STR kits.

Conclusions

The present study showed the importance of both AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR Kits for typing of old skeletal remains obtained from 100–1000-year-old mass graves in Pakistan. Promising DNA profiles were obtained from old skeletal remains using both AmpFlSTR Identifiler and AmpFlSTR MiniFiler STR kits with optimised PCR amplification conditions and extended PCR cycles. DNA profiles were obtained from a minute quantity of DNA (even from ≤10 pg/μL) with modified protocols of these kits, which is a significant achievement. Discordant STR loci were perceived in DNA profiles of a few samples due to either or both allele drop-in or drop-out, while most of the STR loci were concordant. Finally it was proved that the AmpFlSTR MiniFiler kit promoted the recovery of locus/alleles that failed to type with the AmpFlSTR Identifiler kit and more informative DNA profiles were obtained from old skeletal remains with the AmpFlSTR MiniFiler STR kit compared with the AmpFlSTR Identifiler STR kit.

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