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Typing short amplicon binary polymorphisms: Supplementary SNP and Indel genetic information in the analysis of highly degraded skeletal remains

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ABSTRACT

Two sets of short amplicon binary markers (SABs): 50 single nucleotide polymorphisms (SNPs) and 38 insertion/deletion polymorphisms (Indels) were used to genotype bones of 35 years "post-mortem". Typing results of these binary markers were compared with those obtained for standard commercial STR and mini-STR DNA typing kits. We observed SAB marker performance to be better compared with conventional STR and mini-STR genotyping in degraded bone sample analysis. Furthermore, additional genetic information provided by these 88 binary markers, 50 SNPs and 38 Indels, combined with classical markers gave very high discrimination power even in severely degraded specimens, with all tested bone samples showing Random Match Probabilities (RMPs) higher than 1019. Missing person and disaster victim identification by kinship analysis is considerably strengthened by the addition of SAB markers since they can be successfully typed on degraded bone samples while adding considerable extra genetic data when poor or incomplete information is available from conventional forensic markers for the analysis of family pedigrees.

1. Introduction

Short tandem repeat (STR) typing is the most informative tool for individualization of biological specimens in forensic genetic laboratories [1–3]. Commercially available multiplex STR typing kits, producing amplicons ranging in size from 100 to 450 base pairs (bp) with an elevated discriminatory power, are frequently used to type the samples [4–6]. However, the success of STR profiling depends on the quantity and quality of the DNA that can be extracted from the forensic sample to be typed. Challenging samples are common in forensic analysis with three main problems frequently present when trying to determine a profile: low template DNA levels; presence of PCR inhibitors, and; highly fragmented DNA. DNA fragmentation can be produced by biochemical, bacterial or oxidative processes [7,8]. As a consequence, allele or locus dropout becomes a common phenomenon, particularly in the larger sized loci with more than 250–300 bp amplicons, producing partial or no STR profile when using commercial STR typing kits [9,10]. Since important genetic information is lost when obtaining partial STR profiles, the power of discrimination is considerably diminished in many low template and/or degraded samples. Aged skeletal remains frequently present such challenging samples in which the success of DNA profiling is profoundly influenced by the types of bone specimen available, the characteristics of the soil and levels of humidity in which they are found, the postmortem taphonomic process and the number of years they have been buried. Mitochondrial DNA analysis is generally applicable in this type of severely degraded bone samples because of its characteristic high copy number but it presents the disadvantage of having a relatively low discrimination power in comparison with that of STR analysis. Disaster victim identification (DVI) and missing person identification (MPI) generally require the application of adequate DNA typing technologies for challenging samples, such as decomposed tissues or degraded bone remains, both likely to produce partial STR

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profiles. Therefore the need to extend DNA typing to include markers from much smaller amplicon PCR products to successfully type a sample is clearly evident.

The re-design of the primers closer to the repeat region, reducing the flanking regions and producing short length PCR products, has in part overcome the problem of obtaining partial STR profiles in degraded samples [11–15]. This "mini-STR" typing generates amplicons ranging from 70 to 200 bp – diminishing allele and locus dropout and improving the chances of successful analysis of degraded DNA from compromised forensic material [12–18]. Mini-STR based commercial kits are now available for use with degraded forensic material and inhibited DNA extracts allowing more complete genetic profiles to be obtained in comparison with standard typing kits [19–22] with the added advantage of using the laboratory's same allele frequency databases. Furthermore, mini-STR loci additional to CODIS and ESS sets have been characterized and their typing provides useful extra genetic information [14–23]. The increase in the number of genetic markers analyzed as well as their analysis from miniaturized amplicons are particularly applicable to DVI or MPI cases when poor reference data is available to allow reliable reconstruction of a pedigree. However, even when using mini-STR technology, many highly degraded forensic samples, particularly aged bones, can still present partial profiles so extra genetic information is required to achieve satisfactory levels of discrimination.

Single nucleotide polymorphism (SNP) typing has been successfully used in forensic human identification [24–27]. One advantage of SNP typing is its successful application to typing highly degraded material, since nearly all SNPs are typed from amplicons smaller than 150 bp [26–28]. Additionally, the low mutation rate of SNPs makes them particularly appropriate for kinship/parentage analysis in DVI and MPI cases. SNPs have been very useful in resolving relationship investigations in cases with ambiguous STR results [29].

The use of another type of short amplicon binary marker in human identification has recently been published: the typing of insertion/deletions polymorphisms (Indels) [30]. These Indels are analyzed in amplicons shorter than 160 bp so they are equally applicable to analyzing degraded material [31], although the initial reported results were obtained in a single aged bone sample and in paraffin-embedded tissues. For this reason it is desirable to comprehensively analyze this Indels set in more extensive challenging sample cohorts to confirm these findings.

During the decade of the 1970s, a military dictatorship ruled Argentina, abducting and killing thousands of people for political reasons. Since 1984, the Argentine Forensic Anthropology Team (EAAF) has exhumed over 1000 buried skeletons recorded as 'John Doe' (unidentified). In 2007, EAAF began the Latin American Initiative for the Identification of the "Disappeared" Project (LIID) to improve the identification of human right victim's remains [32]. The Forensic DNA EAAF laboratory of Cordoba, Argentina has been carrying out part of the genetic studies needed to identify the victims by kinship analysis, using reference genetic information from their relatives. As bone remains studied in the LIID project are 30–35 years post-mortem, many of them show degradation characteristics, making it important to compare the performance of the two available short amplicon binary marker typing multiplexes: the 52-plex SNP*for*ID set and the 38-plex Indels set in comparison with the two commercial Applied Biosystems (AB) STR kits of Identifiler™ and Minifiler™.

Table 1
Reportable markers on the 30 bone samples analyzed with different genotyping kits: Identifiler™, Minifiler™, 38-Indelplex and 50-plex SNPs.

Sample*	Specimen	DNA qPCR (ng/μl)	ID	ID + MNF*	38-Indelplex	50-plex SNPs	Total binary
1	Tooth	0.04	15	15	36	50	86
2	Femur	0.16	15	15	38	50	88
3	Femur	0.15	14	15	38	50	88
4	Tooth	1.17	12	15	34	50	84
5	Femur	Undet**	15	15	36	48	84
6	Femur	0.01	15	15	37	48	85
7	Femur	0.06	15	15	36	46	82
8	Femur	0.11	15	15	38	46	84
9	Femur	0.015	11	15	35	45	80
10	Tooth	0.015	14	15	23	44	67
11	Femur	0.47	12	14	38	50	88
12	Femur	0.07	8	13	36	47	83
13	Femur	0.018	11	13	33	43	76
14	Femur	Undet**	11	13	30	42	72
15	Femur	0.005	8	12	21	45	66
16	Humerus	0.033	8	12	31	44	75
17	Ulna	0.005	10	12	26	41	67
18	Fibula	0.013	8	12	22	40	62
19	Femur	0.18	5	11	33	45	78
20	Vertebrae	0.03	7	11	32	40	72
21	Ileon	<0.001***	10	11	24	28	52
22	Tooth	0.004	7	9	28	45	73
23	Ulna	0.013	7	9	19	37	56
24	1er metatarsal	0.001	6	8	33	49	82
25	Humerus	0.001	5	8	26	35	61
26	Femur	<0.001***	5	7	16	36	52
27	Femur	0.001	4	4	23	37	60
28	Femur	0.006	3	3	25	30	55
29	Tooth	0.01	3	3	11	27	38
30	Scapula	Undet**	2	3	13	25	38

MNF = Minifiler™; ID = Identifiler™, total binary = Indels + SNPs reportable loci.

* Bone samples are listed according to the number of reportable markers obtained with Identifiler™ and MiniFiler™ kits and classified as full profile (15 STR reportable markers in column ID + MNF), partial profile (11–14 reportable STR markers) and poor profile results (less than 11 reportable STRs). Numbers represent the amount of reportable loci with the different kits assayed. DNA qPCR: DNA quantification using Quantifiler™ kit. Indet: Indeterminate result for Quantifiler™ kit.

** Undet: highly inhibited samples unable to be quantified.

*** < 0.001: Non-inhibited samples with DNA concentration below 0.001 ng/μl.