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SNP*for*ID 52-plex in casework samples: "Cracking" bones and other difficult samples

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ABSTRACT

Casework samples can present difficulties to forensic scientists in criminal and identification investigations. Some challenging samples like bones, teeth and crime scene samples, often contain low DNA quantity which can even be degraded. In these cases, obtained STR profiles are many times incomplete or even null. This is partly due to relative bigger size of commonly used STRs in forensic analysis. In order to bypass this problem, other strategies of analysis have been developed in the past based on mini-STRs and biallelic markers, such as Indels and SNPs. Although each marker type has its advantages and disadvantages, SNPs benefit from the fact of having smaller amplification products and its analysis can be realized analyzing simultaneously a great number of loci using large multiplexes. One of such multiplex is SNP*for*ID 52-plex which analyzes 52 loci, providing good results, as reported by some authors. Taking this in consideration, we compared the amplification success of 53 real casework samples from our casuistic consisting of bones, teeth and other samples using the 52-plex and the Identifier® Plus kit. Mean amplification success rate by loci was of 73% and 43% respectively and 16 out of 36 samples in which STR profiles were not obtained or in which these were poor, generated complete or almost complete SNP profiles. We conclude that the 52-plex can be a valuable tool in the analysis of different types of challenging forensic samples when STRs fail to provide necessary genetic information for identification.

1. Introduction

In routine casework, forensic scientists have to deal with different kinds of samples where type, availability, and state of its preservation can make difficult to obtain a genetic profile [1]. Samples such as bones, teeth and crime scene samples often present challenging situations due to their DNA quantities and their degradation state, usually when it is no longer possible to obtain more sample for further studies. In these cases, obtained STR profiles can be incomplete or even null due to amplicon sizes of commonly used STRs in forensic routine. In order to overcome this problem, other genetic markers have successfully started to being used: mini-STRs and biallelic markers, such as Indels and SNPs [2]. Advantages of SNP typing in forensic genetics are well known and includes the use of smaller PCR products and the possibility of multiplexing many loci in a single PCR reaction. Sanchez et al. developed the SNP*for*ID 52-plex assay [3], that allows simultaneously amplification of 52 autosomal SNPs and which was validated for forensic use [4]. This multiplex was reported as having an higher performance when compared to STRs and even to Indels [2], resulting in full profiles from DNA extracts that yielded no or few STR loci during challenging criminal samples analysis [5].

The aim of this work was to study suitability of the SNP*for*ID 52-plex to complement standard STRs methodologies in bone and teeth analysis but also in other challenging samples related to criminal and individual identification. Taking this in consideration we compared amplification success of 53 real casework samples from our casuistic consisting of bones, teeth and other challenging samples using the 52-plex and the Identifier® Plus® kit.

2. Material and methods

A total of 53 samples were investigated comprising 24 bones, 16 teeth and 13 diverse crime scene and identification samples. Different extraction methods were used: Phenol-Chloroform,

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1875-1768/

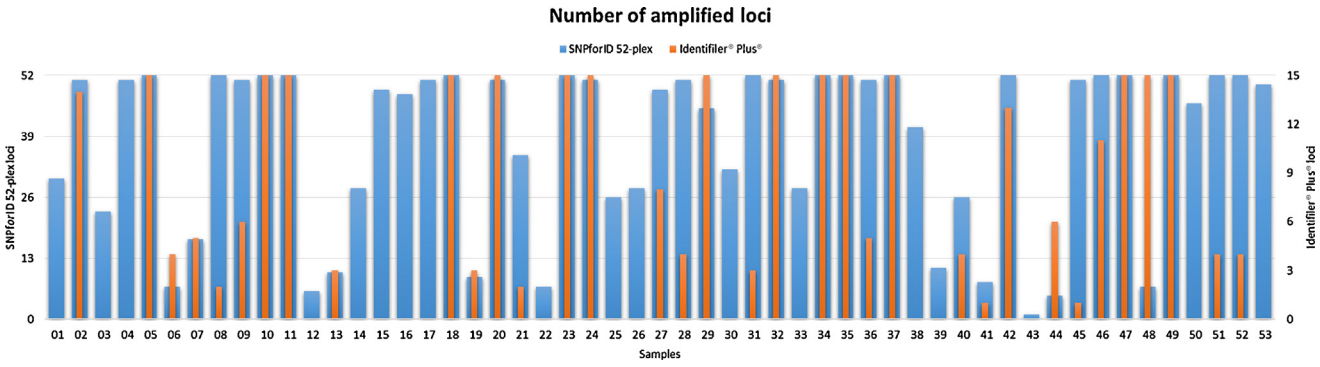

Fig. 1. Number of amplified loci for each one of tested samples, with 52-plex and Identifiler® Plus®.

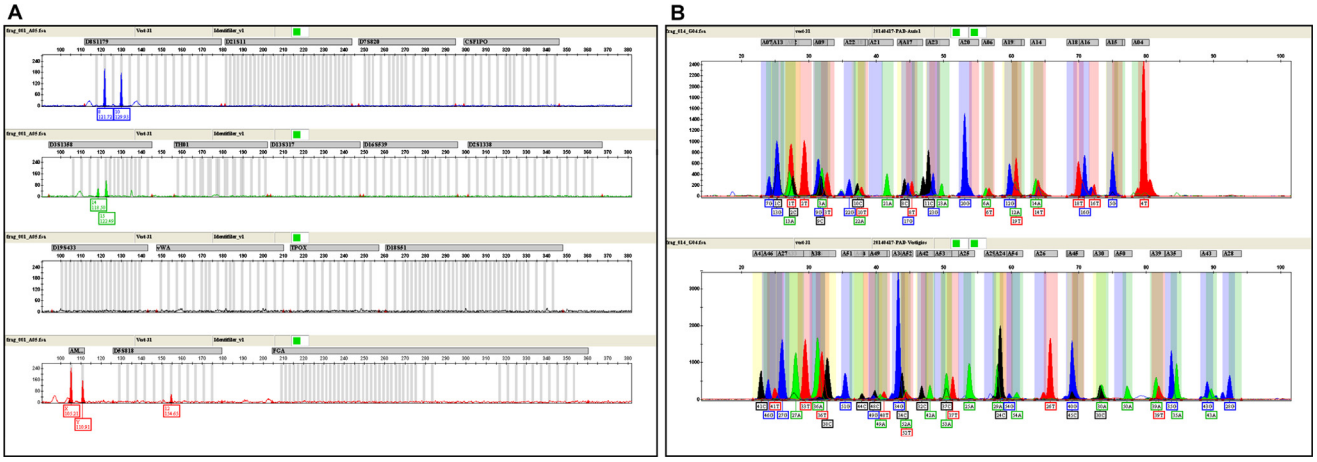

Fig. 2. Bone sample analyzed with Identifiler® Plus® (A) and with 52-plex (B) [sample 31, concentration of 0.02 ng/μl].

Machery Nagel Nucleospin, Qiagen Mini, Micro and Investigator kits depending on type, availability and quality of samples presented for analysis. Same extract was used both for STR and SNP amplification. DNA quantification was performed using Quantifiler Duo DNA Quantification kit in an Applied Biosystems® (AB) 7500 Real Time PCR System. STR typing was done using Identifier® Plus® (AB) and SNPs were typed with SNaPshot® Multiplex kit (AB) using SNP*for*ID 52-plex [3]. All amplifications were performed in AB GeneAmp® PCR System 9700 (gold block) and capillary electrophoresis was accomplished in AB 3130/3130xl Genetic Analyzers. Obtained data was analyzed with GeneMapper® ID v3.2.1 (AB).

3. Results and discussion

Mean amplification success rate of the 52-plex and the Identifiler® Plus® kit by loci was of 73% and 43% respectively, and 16 out of 36 samples in which STR profiles were not obtained or in which these were poor, generated complete or almost complete SNP profiles, as can be visualized in Fig. 1 (e.g. samples 04, 08 and 09).

An example of these samples is presented in Fig. 2, where a poor profile was obtained with Identifiler® Plus® but a full profile was obtained with 52-plex.

This superior amplification success of the 52-plex relatively to Identifiler® Plus® kit was observed in most bone samples (e.g. 08 and 09), teeth samples (e.g. 04 and 15) and also in crime scene samples (52 and 53). There were two termination of pregnancy samples tested which presented opposite results – 48 and 50 – what makes believe these must be the result from preservation methods used. Nevertheless, better results were obtained with 52-plex where full profiles were obtained from DNA extracts that generated no or few STR loci.

4. Conclusion

This study demonstrates utility of SNP analysis as a complement or as an alternative to STR typing in challenging samples analysis such as bones, teeth and other problematic samples where STR amplification fails.

Conflict of interest

None.

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