

Research article

Mini-SGM multiplex in degraded samples

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Abstract

A five Mini-SGM multiplex which encompasses TH01, FGA, D18S51, D16S539 and D2S1338, common STR markers in human identity testing, have been performed. Two cases with different biological tissues were selected to illustrate the usefulness of this technique in forensic casework. The use of routine methodology can sometimes give only a partial genetic profile or no profile at all. However, using the Mini-STR technique, a full profile was obtained for the majority of the degraded samples. We conclude that the Mini-SGM methodology is more sensible than routine methodology for degraded samples, although a full genetic profile is not obtained in all cases as results are still very much sample-dependent. This Mini-SGM multiplex can be considered a useful tool to complement conventional STR analysis in degraded samples.

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1. Introduction

In forensic casework, extraction of high quality DNA is not always possible. The first observation that small PCR products had a much higher success rate with degraded DNA was reported in 1995. However, Mini-STR designation was introduced, for the first time, in 2001 [1]. More recently, other authors have demonstrated that this technique can be useful to recover genetic information from degraded samples [2,3].

To implement Mini-STR methodology in forensic cases, a Mini-SGM multiplex has been studied [1], with chosen *loci* from the STR panel used in routine forensic investigations. When studying much degraded samples, the amplification products from D2S1338, D16S539, D18S51 and FGA *loci*, due to their high molecular weight, can give no results with the routine multiplex normally used in forensic laboratories. The application of Mini-STR methodology to degraded samples will permit the identification of these samples with a higher number of genetic loci studied.

2. Materials and methods

More than 30 samples with degraded DNA from several biological materials were studied. Two cases performed with different biological tissues were selected to exemplify this methodology. All samples have been also previously routinely studied with PowerPlex[®]16 System (Promega) and/or AmpF/STR Identifiler Multiplex Kit (Applied Biosystems).

DNA has been extracted by QIAmp method and/or phenol/chloroform method followed by Microcon[®] Amicon purification. PCR amplification was performed with TH01, Amelogenin, FGA, D18S51, D16S539 and D2S1338 primers, according to Butler et al. [1], in a GeneAmp 9700 (Applied Biosystems). A 12.5 µl total volume was used with products and ladder from the AmpFISTR SGM Plus kit according to Coble and Butler protocols [4]. PCR products were detected in an ABI Prism[®] 3130 Genetic Analyser with POP7[™] and analysed with GeneMapper[®] 3.2 (Applied Biosystems).

3. Results and discussion

Two special cases have been selected to illustrate the usefulness of mini-STR methodology in forensic casework: case 1—a tissue sample preserved in a paraffin block and case

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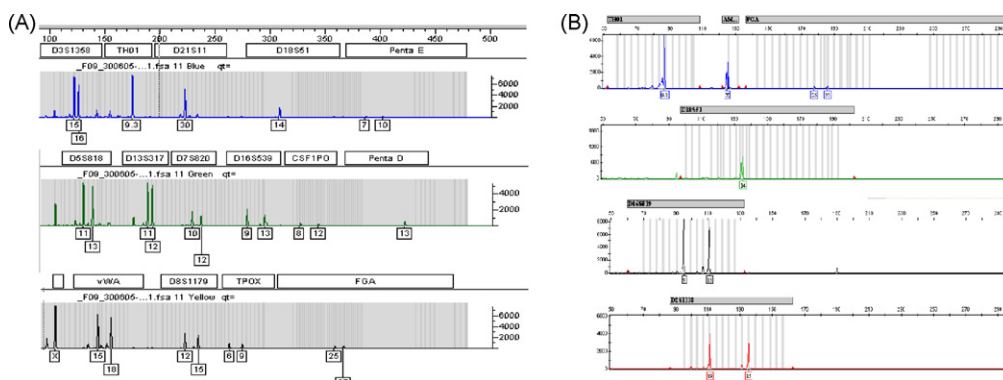


Fig. 1. PCR amplification from an embryonic material in a paraffin block: (A) PowerPlex[®] 16 (1 µl DNA) and (B) Mini-STRs (1 µl DNA diluted 1:20).

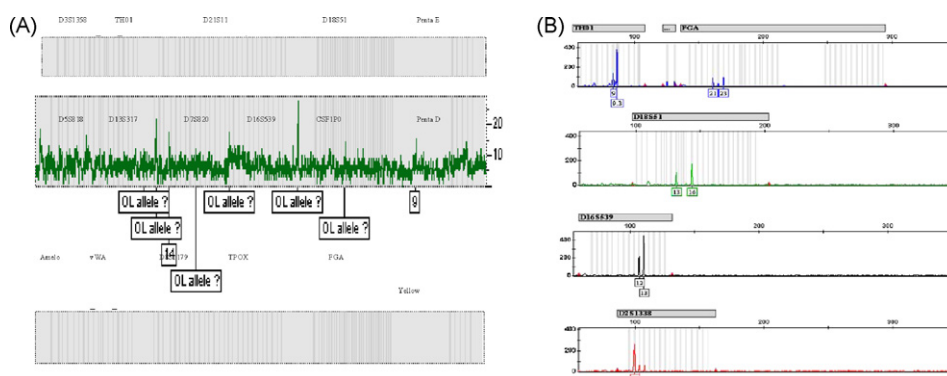


Fig. 2. PCR amplification from a much degraded bone which have been immersed in lake water for more than a year: (A) PowerPlex[®] 16 (5 µl DNA); (B) Mini-STRs (2.5 µl DNA diluted 1:5).

2—a much degraded sample bone which have been immersed in lake water.

In the first case, two paraffin blocks with embryonic material were sent to the forensic laboratory to perform a paternity investigation. PCR amplification was performed with 1 µl DNA with PowerPlex[®] 16 System and the same extraction was used with Mini-STR methodology—1 µl diluted 1:20 (Fig. 1). In both results, the amelogenin locus points to a female. In Fig. 1A, there is a substantial decrease of allele sizes greater than 300 bp, normally encountered when studying degraded samples. In Fig. 1B, the loci profile is well defined till 170 bp, and the dilution of the sample shows the great sensibility of this methodology.

The second case exemplifies the genetic profile obtained for the identification of a corpse immersed in lake water for 15 months. PCR amplification was performed with 5 µl DNA with PowerPlex[®] 16 System and the same extraction was studied with Mini-STR methodology—2.5 µl diluted 1:5 (Fig. 2). This degraded bone gave no results with PowerPlex[®] 16. With Mini-STR methodology, a STR profile identical to a tooth sample from the same person was obtained.

Mini-STR methodology can be considered a useful tool to complement STR analysis of degraded samples giving partial STR profiles or no profiles at all when performing routine forensic methodology.

Conflict of interest

None.

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