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Research article

AmpFlSTR® MiniFiler™ PCR amplification kit: The new miniSTR multiplex kit

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Abstract

The AmpFlSTR® MiniFiler™ PCR amplification kit (Applied Biosystems), a new available 8-miniSTR and the sex determining marker Amelogenin multiplex, includes the most common problematic loci (above 200 bp) of the AmpFlSTR® Identifiler™ PCR amplification kit: FGA, D21S11, D18S51, D13S317, D7S820, D16S539, CSF1PO and D2S1338.

Several casework samples with different DNA contents were tested.

Results allowed to complete partial Identifiler™ profiles and additional information was achieved in low copy number (LCN) samples, revealing that this miniSTR kit can improve identification of compromised samples.

Keywords: MiniSTRs; LCN

1. Introduction

The ability to recover DNA sequence and STR data from bones and teeth exposed over time to a variety of environmental conditions has become a valuable tool for the identification of missing individuals and unknown remains [1]. Hair samples (without intact bulbs) are one of the most problematic casework samples; being the only biological material left in some crimes, a genetic profile should be determined.

One way to improve the success rate for degraded DNA is to utilize redesigned STR primers that generate shorter amplicons. Several STR systems with size-reduced fragments were used in collaborative European exercises on artificially degraded DNA and gave complete and correct results [2]. To increase success rates in STR recovery, short amplicon STR multiplexes have been devised and implemented [3].

MiniFiler™ is a 9-plex miniature STR amplification kit, expected to revolutionize the way forensic scientists process casework samples by significantly increasing the ability to obtain information from DNA evidence, specially inhibited and/or degraded samples, that previously would have yielded limited or no genetic data. In compromised samples, the largest molecular weight loci of Identifiler™, including FGA, D21S11, D18S51, D13S317, D7S820, D16S539, CSF1PO and D2S1338 most often fail to amplify. By combining innovative primer design, improved PCR amplification conditions and a properly mastermix, MiniFiler™ provides increased sensitivity, robust results in the presence of inhibitors and improved discrimination for casework samples. Working with other kits, MiniFiler™ can recover more complete DNA data from challenging samples, enabling more crime and missing person cases to be solved [4].

2. Materials and methods

Sixteen compromised samples (blood, saliva, bone, tooth and hair) were amplified with Identifiler™ and MiniFiler™ in order to test the new miniSTR kit. DNA was extracted from blood stains or saliva swabs by phenol/chloroform extraction, from bones/teeth by Puregene DNA Isolation™ tissue kit (Gentra Systems) and from hairs by Tissue and Hair™

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Table 1
Compromised samples analysed with Identifiler™ and MiniFiler™

Samples/(quantity (ng/μl))	Identifiler™	MiniFiler™	No coincident results (false homozygotes detected with Minifiler)
Hair (0.0017)	–	1 locus	–
Hair (0.0036)	–	1 locus	–
Hair (0.0054)	–	3 loci	–
Hair (0.0139)	–	4 loci	–
Hair (0.0140)	–	9 loci	–
Hair (0.0216)	–	5 loci	–
Hair (0.0332)	–	1 locus	–
Hair (0.0491)	–	8 loci	–
Bone (0.0015)	12 loci	8 loci	–
Bone (0.0247)	8 loci	9 loci	D13S317, D18S51, HUMFIBRA/FGA
Bone (0.0286)	12 loci	8 loci	D2S1338, D21S11
Bone (0.0480)	13 loci	9 loci	–
Bone (0.1600)	11 loci	9 loci	D18S51, HUMFIBRA/FGA
Tooth (0.0380)	13 loci	9 loci	CSF1PO, D18S51, HUMFIBRA/FGA
Degraded saliva (0.0380)	8 loci	8 loci	D16S539
Degraded blood (0.0660)	11 loci	9 loci	–

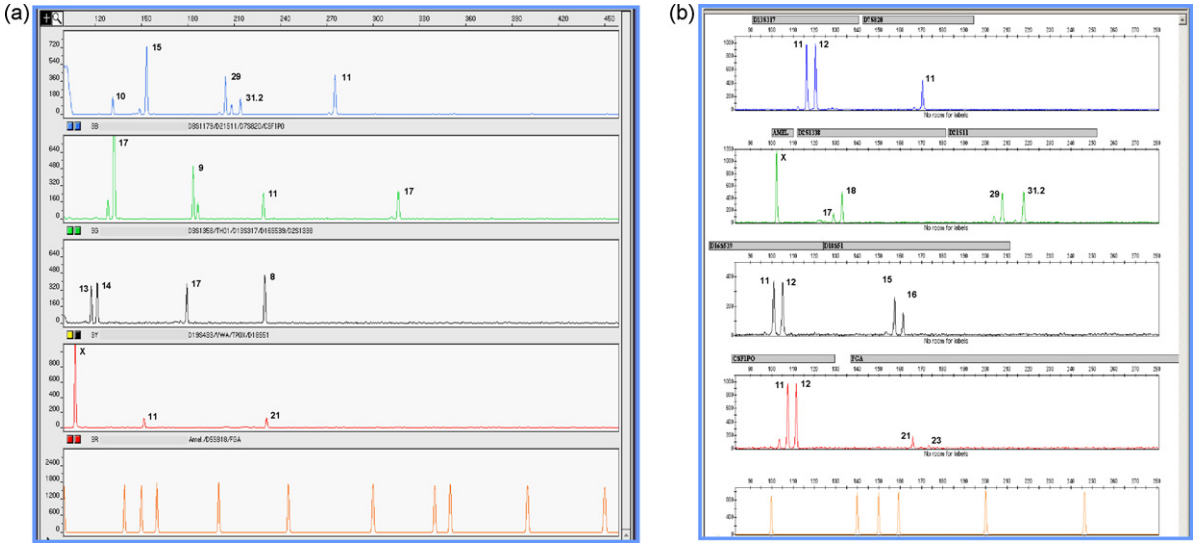

Fig. 1. Compromised sample (bone, 0.0247 ng/μl; Table 1) amplified with Identifiler™ (a) and MiniFiler™ (b).

extraction kit (Promega), in accordance with the manufacturer's recommendations.

DNA quantity was determined with the Quantifiler™ Human DNA quantification kit, using the ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

Identifiler™ PCR amplifications were performed in a total volume of 12.5 μl, in a GeneAmp® PCR System 2700 (Applied Biosystems).

MiniFiler™ PCR amplifications were performed in a total volume of 25 μl, containing, if possible, 0.5–0.75 ng/μl of DNA, following the recommendations described in the manual, in a GeneAmp® PCR System 2700 (Applied Biosystems). Samples with lower DNA amounts were also amplified.

Fragments were detected by electrophoresis in an ABI 310 Genetic Analyzer (POP-4 polymer). Data were analysed using Genotyper® V2.5 and GeneMapper® ID V3.2 software.

Negative controls were tested, to despite contamination. All amplifications were done twice.

3. Results

Table 1 shows the compromised samples analysed with Identifiler™ and MiniFiler™ and Fig. 1 shows the compromised sample (bone, 0.0247 ng/μl; Table 1) amplified with Identifiler™ (a) and MiniFiler™ (b).

4. Discussion and conclusions

MiniFiler™ PCR amplification kit has been demonstrated to yield the greatest amount of information from samples that have previously produced partial profiles or no profile at all using other existing commercially available autosomic amplification

kits. In the presence of PCR inhibitors, MiniFiler™ outperforms other kits with regard to genetic information recovery. The use of a dual-amplification strategy (MiniFiler™ and Identifiler™) is an adequate strategy to deal with compromised samples, allowing to achieve information from the most common database autosomic markers, without the need of further population studies (Table 1 and Fig. 1).

Comparing biological samples of hair and, for example, bones, with similar amounts of DNA, the genetic profiles that are obtained have different qualities; hair samples did not allow the determination of complete DNA profiles, not even with MiniFiler™ (Table 1).

Important advantages of this new kit are also the possibility to verify the presence of false homozygotes and artefact peaks defined through Identifiler™; MiniFiler™ reduces stochastic effects produced by improved amplifications in LCN samples.

Conflict of interest

None.

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