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Introduction of the DNase in forensic analysis

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Abstract. Laboratory DNA contamination is a frequent problem in forensic genetics, especially when extra PCR cycles are used to obtain acceptable amplified products from low copy number DNA samples. We have evaluated the use of the DNase I for forensic genetics studies. Initially we introduced known genomic DNA in the PCR reaction mix in order to simulate DNA contamination. Different conditions of action an inactivation of the DNase I were tested. Finally, we performed amplifications of low copy number samples employing homemade and commercially available STR amplification systems; in both cases decontamination was successful. We demonstrate that DNase I may be useful to reduce laboratory contamination in forensic genetics labs. Our protocol may be easily introduced in standard PCR protocols of commercial kits in order to be used routinely in low copy number DNA amplification.

Keywords: Low copy number DNA; DNase I; Forensic analysis

1. Introduction

Increasing the number of amplification cycles can expand the utility of DNA profiling in forensic casework. However, this procedure may favour laboratory-based contamination most of the times unavoidable, also under stringent conditions of cleanliness [1]. Low concentrated templates are prone to be contaminated by DNA from numerous other sources (reagents, plastic, tubes, etc.) used in forensic laboratories. Many authors have suggested that the use of DNase I may reduce the possibility of DNA contamination [2,3]. Unfortunately, these approaches are normally limited for anthropological DNA studies. Here, we have evaluated the use of DNase I to eliminate contamination in laboratory materials and reagents prior to perform the PCR reaction. This protocol could be easily adapted to standard PCR protocols of commercially available kits for forensic genetics analyses.

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2. Materials and methods

2.1. DNA samples

Serial dilutions of K562 cell line control DNA (Promega, MA, USA) and Quantifiler Human DNA standard (Applied Biosystems, CA, USA) were initially used to simulate contamination. DNA recovered from gloves and touched objects and quantified by Real-time PCR with Quantifiler™ Human DNA Quantification Kit (Applied Biosystems), was employed in simulated experiments of forensic casework. Negative controls were always used in all the experiments.

2.2. Decontamination with the enzyme

Determination of the best conditions of decontamination by DNAase was performed using a home-made STR amplification system [4]. Initially, to simulate DNA contamination, 0.9 μl of genomic DNA corresponding to 1 ng of genomic DNA, was added into PCR sterile tubes containing 5.8 μl of the QIAGEN® Multiplex PCR Kit (Qiagen, Germany). One microliter of DNase I (10 mg/ml) (Roche, cat. 104 159) was then added in each contaminated mix, followed by incubation at room temperature. Different incubation times were used covering a range from 1 to 10 min, in order to verify the most efficient time of action of the enzyme. After initial incubation, inactivation was performed in a thermocycler (PTC-100, MJA Research) at 70 °C and 80 °C for 20 min. 5.8 μl of primers were added to the mix just after DNase I inactivation. Finally, PCR reaction was performed using 40 cycles of amplification and amplified products were analysed by electrophoresis in a polyacrylamide gel as described [4]. Activity was considered effective when no amplification products were present; inactivation was considered effective when primer fluorescence was observed in the polyacrylamide gel.

2.3. PCR amplification

Using the most efficient conditions of activation and inactivation of the enzyme (10 min at room temperature and 80 °C for 20 min, respectively), we performed the amplification of genomic DNA and simulated low copy number DNA samples, extracted from gloves and touched objects from known subjects. The PCR reactions were performed with experimentally contaminated reaction mixes (with K562 DNA) from a homemade STR amplification system and the AmpF*l*STR® Profiler Plus™ [5]. Primer mixes and target DNA were added after the DNase I inactivation in order to avoid their degradation. The PCR was performed with 34 cycles of amplification.

3. Results

The efficiency of decontamination by the DNase I treatment in simulated forensic samples was evident just after 2 min at room temperature. In fact, samples incubated under these conditions for 2 or more minutes showed no bands of amplification in the polyacrylamide gel. Times shorter than 2 min showed amplification bands with intensities inversely proportionate to the time of incubation. Degradation of DNase I was correctly performed after incubation at 80 °C for 20 min. When a lower incubation temperature was

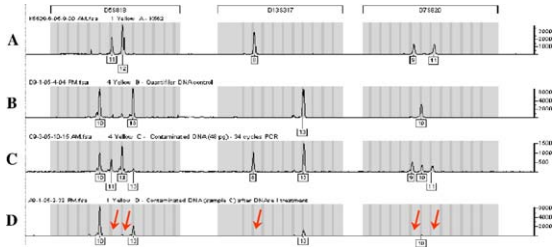

Fig. 1. Example of DNA genotyping of genomic DNA with and without DNase I treatment (34 cycles—NED dye): (A) K562 DNA profile; (B) Quantifiler Human DNA standard profile; (C) profile of artificially contaminated sample (K562+Quantifiler Human DNA standard); (D) profile of sample *C* (K562+Quantifiler Human DNA standard), after decontamination by DNase I. The arrows show the positions of missing alleles of K562 control DNA after decontamination. Unbalanced profile was observed in contaminated and decontaminated samples for D5S818 locus.

used for the same time (70 °C for 20 min), degradation of primers occurred, as shown by the absence of the primer fluorescence in the autoradiogram-like image. Incubation at room temperature with DNAase for 2–5 min followed by inactivation at 80 °C for 20 min does not seem to interfere with the efficiency of the amplification in any of both amplification systems employed in this work. Fig. 1 shows the results obtained analysing a genomic DNA sample with and without DNAase decontamination. Contaminated sample treated with DNAase I shows the expected profile of the target DNA, while the profile of the contaminator was completely absent.

4. Conclusion

Using this protocol in simulated forensic samples, we compared performance results obtained with and without use DNase I decontamination procedure. We argue that the use of DNase I may be useful to reduce the possibility of laboratory DNA contamination when low copy number DNA samples are amplified. The two steps needed for the decontamination of the PCR mix are easily introduced into the standard PCR for homemade and commercially available kits. The DNase I-based protocol presented here may eliminate DNA contamination in all cases when external DNA is introduced prior to the addition of primers or template. Obviously this protocol fails whenever contamination is introduced with the primers or with the template that can be contaminated during extraction procedures.

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