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Rapid Extraction of Human DNA Containing Humic Acid

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The identification process of dead bodies or human remains is nowadays conducted in numerous fields of forensic science, archeology and other judicial cases. A particular problem is the isolation and DNA typing of human remains found in mass graves, due to the degradation process, as well as post mortal DNA contamination with bacteria, fungi, humic acids, metals, *etc*. In this study, the influence of humic acid (HA) on the DNA extraction and typing is investigated. If present in the amplification reaction mix, humic acid inhibited DNA amplification, but the addition of 50 mg PVPP (polyvinil-polypyrrolidone) to the reaction mixture before extraction appeared to be optimal in overcoming this inhibition.

Keywords
human DNA
extraction
humic acid
DNA inhibition

INTRODUCTION

The polymerase chain reaction (PCR) is a very powerful and sensitive analytical technique with applications in many diverse fields, including molecular biology, clinical diagnosis, population genetics and forensic analysis. Analysis of deoxyribonucleic acid (DNA) by short tandem repeats (STR) polymorphisms has had a major impact on identity testing. Exhumation and war victims identification have a special connotation. Different identification methods are used depending on the case circumstances and the grade of the post mortal body changes.[1,2] One of the methods is the identification by DNA typing of different biological samples (genotyping). Most common problems that forensic scientists encounter while working with DNA extracted from bones and teeth samples recovered from mass graves or mass disasters are DNA degradation and/or contamination.[3,4] Another particular problem is the isolation and DNA typing of human remains found in mass graves, usually heavily degraded as well as post mortally contaminated with bacteria, fungi, metal ions and humic acids (HA). Extraction of DNA from bones may result in co-extraction of HA, which then interferes with DNA detection and measurement.[5] This contamination can inhibit PCR amplification. Many scientists have reported that PCR inhibitors were frequently co-purified with the DNA extracted from a mass grave bone or an ancient bone.[6,7] Also, scientists have reported on extraction and purification methods of DNA from different samples, the sources of samples not being human. Tebbe and Vahjen, Cullen and Hirsch developed a protocol for the extraction and purification of total DNA from soil samples.[8] Tsai and Olson found that the best results were obtained when crude DNA extracts containing HA were purified by using Sephadex G-200 spin columns.[9] Howeler *et al.* described extraction and purification of DNA from compost.[10] Some authors reported that HA could be removed by treatment with polyvinil-polypyrrolidone (PVPP) resin.[11]

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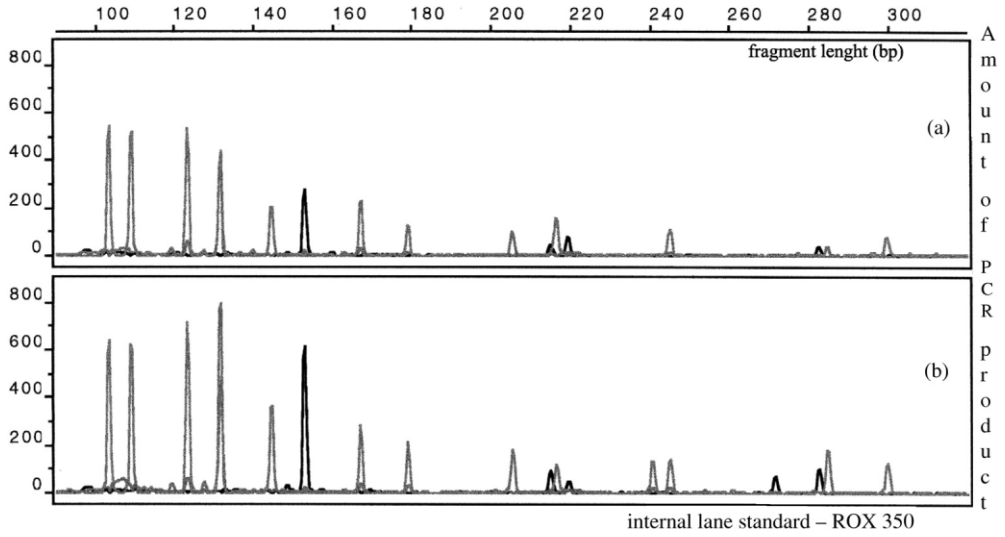

Figure 1. Electropherogram of the influence of HA on PCR amplification (DNA from fresh bone extracted by Chelex resin) with the AmpfISTR ProfilerPlus™ Amplification kit: (a) with addition of 0.5 g HA/mg dry bone sample, (b) with addition of 0.5 g HA/mg dry bone sample and addition of 25 mg PVPP.

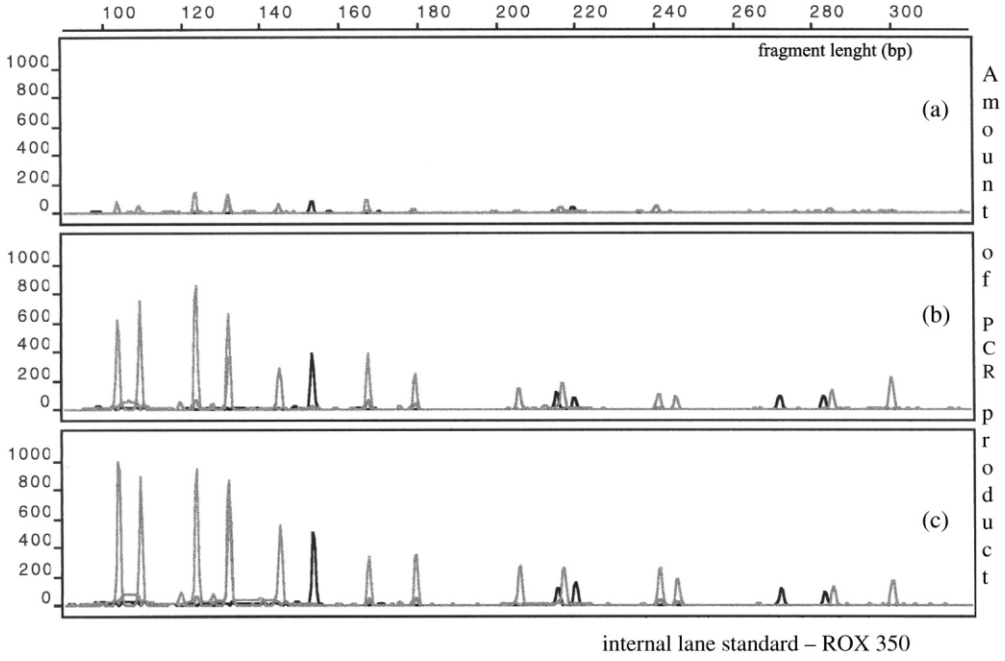

Figure 2. Electropherogram of the influence of HA on PCR amplification (DNA from fresh bone extracted by Chelex resin) with the AmpfISTR ProfilerPlus™ Amplification kit: (a) with addition of 5 g HA/mg dry bone sample, (b) with addition of 5 g HA/mg dry bone sample and addition of 25 mg PVPP, (c) with addition of 5 g HA/mg dry bone sample and addition of 50 mg PVPP.

EXPERIMENTAL

We developed a rapid method for the extraction of human DNA containing HA. It could be useful for forensic DNA analysis of biological evidence materials containing very small amounts of DNA, as used in criminal investigations. For this reason, we investigated the differences between organic extraction using phenol/chloroform/isoamyl alcohol (25/24/1) and inorganic extraction using Chelex resin with addition of PVPP resin. A model of inhibition samples was used to investigate the effects of HA on human DNA extraction. The model system employed a known HA impurity that may be present in bone samples. All our DNA samples for *in vitro* tests were DNA extracted from 50 mg of ten fresh bones. Bone preparation and DNA extraction were done as described by Burgi[12] and Walsh[13]. HA was a product of Fluka Inc. (Taufkirchen, Germany). PVPP was purchased from MERCK (Darmstadt, Germany).

In this study, we describe the effect of PVPP on DNA inorganic extraction by Chelex resin and PCR inhibition. The influence of HA on the PCR efficiency of DNA extracts

was monitored with 0.5 and 5 μg of HA/mg dry bones and different amounts of PVPP (25 and 50 mg per assay). PCR amplification was performed on a Perkin-Elmer Thermal Cycler 9600 using AmpFlSTR ProfilerPlus™ PCR Amplification from Applied Biosystems, according to the recommended protocols.[14] The amount of DNA was from 1–5 ng. Thermal cycling conditions were: 95 ºC for 11 min, 28 cycles of [94 ºC for 1 min, 59 ºC for 1 min, 72 ºC for 1 min] and 60 ºC for 45 min. Typing of PCR products was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with the Data Collection Software. Electropherogram data were analyzed with the GeneScan® Software and Genotyper® Software v.2.5.2. for use with the Macintosh operating system. Internal standard was Rox-350.[15]

RESULTS AND DISCUSSION

Humic acid had no effect on DNA extraction and PCR amplification by organic extraction. All ten loci of STR ProfilerPlus kit were successfully amplified (data not shown). In samples containing 0.5 μg of HA/mg dry bones, and extracted by Chelex (inorganic extraction), several STR loci longer than 230 base pairs (bp) could not be amplified and typed (Figure 1a). PVPP was shown to remove co-extracted humic compounds from soil extracted DNA.[11] PVPP removes HA with phenolic groups from crude DNA extracts *via* hydrogen bounding and formation of PVPP-phenolic complexes. By adding, before extraction, 25 mg PVPP to the reaction mixture, all of the ten loci of STR ProfilerPlus kit were successfully amplified (Figure 1b). Signals of loci longer than 230 bp were not as high as signals shorter than 230 bp, but signals could be determined in every locus.

In the experiment with a higher amount of HA, 5 μg of HA/mg dry bones, amplification after extraction was completely inhibited (Figure 2a). A small reduction in PCR inhibition was noted after 25 mg of PVPP was added into the reaction mixture before extraction (Figure 2b). Addition of 50 mg PVPP to the reaction mixture appeared to be optimal in overcoming HA inhibition (Figure 2c).

In summary, simple addition of >10 mg PVPP into the reaction mixture before inorganic extraction effectively overcomes inhibition of amplification by HA. It is necessary in samples containing very small amounts of DNA and is becoming widely used in criminal investigations. However, co-extracted HA strongly impairs DNA amplification. Using this method, the DNA was successfully amplified in all loci. Routine forensic DNA analysis, based on STR markers, can be performed successfully on most evidence materials found at a crime scene.

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SAŽETAK

Brza ekstrakcija humane DNA iz uzoraka onečišćenih humusnom kiselinom

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Jedno od važnijih dostignuća primjene molekularne biologije u sudskoj medicini je određivanje identiteta osoba DNA tipizacijom iz bioloških uzoraka. Posebno se to odnosi na identifikaciju pomoću uzoraka kosti ili

zuba osoba stradalih u masovnim katastrofama ili ekshumiranih tijela koja se ne mogu obaviti drugim klasičnim metodama. Kod takvih identifikacija poseban problem predstavlja izolacija i dobivanje genotipa iz skeletnih ostataka pronađenih u masovnim grobnicama u kojima dolazi do truležne razgradnje kao i posmrtnog onečišćenja DNA bakterijama, gljivicama, metalima i humusnim kiselinama. U ovom je radu ispitan utjecaj humusne kiseline na ekstrakciju i umnažanje humane DNA. Rezultati su pokazali da humusna kiselina, ako je prisutna u reakcijskoj smjesi, inhibira umnažanje DNA dok je u prisutnosti 50 mg PVPP, dodanog u uzorak prije ekstrakcije, dobiven rezultat kao u odsutnosti inhibitora.