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Enzyrim: A new additive to increase the DNA yield from different materials such as teeth, blood or saliva

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Abstract. Enzyrim (Arte Copia, Zürich, Switzerland) is an enzyme mixture normally used for bone maceration. It is cheap, easy to handle, non-toxic and disposal is simple. When extracting DNA from Enzyrim treated teeth we discovered that the amount of extracted DNA was unexpectedly high. We then systematically investigated different biological materials using three extraction kits, the Invisorb Forensic kit, the PSP Spin Swab kit (both Invitek, Berlin, Germany) and the NucleoSpin Blood Quick pure kit (Macherey Nagel, Düren, Germany). DNA was extracted from buccal swabs, dried blood spots on filter paper, whole blood and toothpowder. All DNA extractions were performed according to the manufacturer's recommendations as well as after addition of Enzyrim to the lysis step of each kit. DNA quality and quantity were tested on ethidium bromide stained agarose gels. Absolute quantification was done using real time PCR. The DNA samples were also employed to genetic fingerprinting using the Powerplex ES and the AmpF/STRIdentifiler kits. The application of Enzyrim greatly improves the DNA yield from forensically important materials and does not hamper DNA amplification. Thus Enzyrim apparently is a very useful additive for the optimisation of DNA extraction in the forensic routine. © 2005 Published by Elsevier B.V.

Keywords: Enzyrim; Forensic analysis; DNA extraction

1. Introduction

When human remains are found that are in advanced stages of decomposition or skeletonized, forensic anthropologists or forensic odontologists may try to establish a

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potential identification by using dental records or skeletal features. If these methods do not lead to a positive identification, DNA typing would be another possibility.

In such cases, DNA has to be extracted from bone material, that has already been cleaned and analyzed by the forensic anthropologist or odontologist. Usual maceration techniques such as boiling or bleaching may lead to a decrease in DNA yield [1,2].

Bone maceration can also be performed using enzyme mixtures such as e.g. Enzyrim (Arte Copia, Zürich) [3]. When extracting DNA from Enzyrim treated teeth we discovered that the amount of extracted DNA was significantly higher in comparison with the amount of DNA extracted from teeth macerated with usual techniques. The aim of this study therefore was to find out whether the application of Enzyrim generally leads to an improvement of DNA yield from forensically important materials such as teeth, blood or saliva.

2. Material and methods

2.1. Preparation of Enzyrim

20 g of Enzyrim was applied to 1 l of distilled water, pH-value was adjusted to 8.5 using sodium carbonate. The temperature optimum for the enzymes in Enzyrim lies between 55 °C and 60 °C, therefore Enzyrim was added to a step in the extraction protocol, in which such temperatures are needed, e.g. during the lysis step.

2.2. Samples and DNA extraction

Altogether, 54 samples were extracted, including 6 buccal swabs, 8 dried blood spots on filter paper, 20 whole blood samples and 20 toothpowder samples. DNA extraction from one half of the samples was extracted using the abovementioned kits according to the manufacturer's recommendations. These samples served as untreated controls. The other half of the samples was extracted using the kits after addition of 50 μl to 1 ml of Enzyrim to each lysis step. Enzyrim was also used for DNA purification purposes of 6 samples of Standard DNA (Human Genomic DNA, Promega GmbH, Mannheim, Germany) before ethanol precipitation.

DNA extraction was performed using the following kits: Nucleo Spin Blood quick pure (Macherey Nagel, Düren, Germany) for whole blood samples, Invisorb Forensic kit (Invitek, Berlin, Germany) for dried blood spots on filter paper and toothpowder and the PSP Spin Swab kit (Invitek, Berlin, Germany) for saliva applied on cotton swabs.

2.3. Testing for DNA quality and quantity and genetic typing

10 μl extracted DNA from each sample was separated on ethidium bromide stained 1% agarose gels. Relative DNA quantity was analyzed by densitometry (GelDoc, Bio-Rad Laboratories Inc.). Absolute quantification was performed using real time PCR based on SYBRGreen [4].

STR typing of DNA samples was performed using the Powerplex ES (Promega GmbH, Mannheim, Germany) and the AmpF*l*STRIdentifiler kits (Applied Biosystems, Weiterstadt, Germany) according to the manufacturers' instructions. PCR products were analyzed using an ABI Prism310 DNA sequencer (Applied Biosystems).

3. Results and discussion

3.1. DNA extraction from toothpowder and dried blood spots (Invisorb Forensic kit)

Toothpowder: incubation with 1 ml Enzyrim at 56°C prior to lysis resulted in 20% increase of extractable DNA. The simple addition of 300 μl Enzyrim to the lysis step even led to 40% increase of DNA yield.

Dried blood spots: addition of 200 μl Enzyrim to the lysis step resulted in 50% increase of DNA yield.

3.2. DNA extraction from whole blood (Nucleo Spin Blood quick pure kit) and saliva (PSP spin swab kit)

Enzyrim showed no significant effect on DNA yield when applied to these samples.

3.3. Purification of standard DNA

Incubation of Standard DNA with Enzyrim for 24 h at 60 °C followed by ethanol precipitation led to an increase of DNA retrieval of 70%.

The application of Enzyrim to the abovementioned DNA extraction methods did not hamper STR genotyping but resulted in substantially better DNA typing results.

DNA from forensically important materials is often dramatically degraded, typically resulting in poor sample quality and quantity for STR examination. Major challenges when extracting DNA from such degraded materials therefore are to maximize the DNA yield and to obtain DNA of high quality. Using Enzyrim as a simple and easy to use additive e.g. to the Invisorb Forensic kit produces a significantly higher DNA yield and substantially better STR genotyping results, making it a useful tool for the optimisation of DNA extraction in the forensic routine.

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