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Research article

Rapid genomic DNA extraction (RGDE)

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Abstract

Hyperpolymorphic short tandem repetitive DNA sequences, STRs or microsatellites, have become widely used in human identification, particularly in criminal cases and in mass disasters. In such cases the substrates for the analyses may be decomposed as a biological material, a fact that has to be taken into account when choosing the appropriate casework methods. Nowadays expanded windows have been opened to the world especially in the area of genetic and biology science by performance of big projects such as human genome project. In this regard, one of the primary and important steps for all is DNA extraction with high quality and quantity in minimum time from biological material. By using RGDE method, genomic DNA with high quality and quantity can be acquired in the shortest time which has been presented in the world up to now. In this paper we report the evaluation of DNA extraction in this method.

Keywords: Rapid extraction; Genomic DNA; Forensic genetics

1. Introduction

Nowadays expanded windows have been opened to the world especially in the area of genetic and biology science by performance of big projects such as the human genome project. Production of great bio banks and DNA banks in national and international levels is one of the development pivots, which is very important in medical, agricultural, economical and forensic genetic fields. In this regard, one of the primary and important steps for all is DNA extraction with high quality and quantity in minimum time from cells. By using RGDE method, genomic DNA with high quality and quantity can be acquired in the shortest time which has been presented in the world up to now. For DNA extraction from blood, tissue, sperm, tooth and bone several methods have been examined and demonstrated so far, also this subject has been developed by the improvement of science. While continuing to address questions about prehistory, ancient DNA (aDNA) research has begun to focus more on the methodological problems associated with the extraction and analysis of DNA from sub-optimal sources. This includes recent research on contamination, postmortem DNA damage, and DNA preservation. A less explored topic in aDNA research is the existence of polymerase chain reaction (PCR) inhibitors that are often co-extracted with aDNA. When present, these substances make the amplification of aDNA difficult, if not impossible, and are often cited, but not demonstrated, as the cause of PCR failure in aDNA studies. In these cases DNA extraction is very important for molecular diagnostic methods.

2. Methods

We developed a rapid method for the DNA extraction from biological material. It could be useful for forensic DNA analysis of biological evidence materials containing very small amounts of DNA, as used in criminal investigations.

New protocol using system has recently been developed for the extraction and isolation of DNA from blood, tissue and bone samples. The RGDE method, which does not use novel paramagnetic particles to purify DNA, has a strong denaturing agent that disrupts many sample types in the preparation of DNA purification. This approach has been applied to liquid

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blood, blood on various materials, and other samples, such as buccal and saliva swabs, and differentially extracted sperm and epithelial cells.

In the RGDE method, lysis buffer does not lyse cells in the interior of tissue masses, including hair and bone, so pretreatment with proteinase K is required to ensure complete lysis. These preprocessing steps do significantly add to the user's ''hands-on'' time and still provide the extraction and isolation capabilities of the RGDE method to remove PCR inhibitors and deliver DNA for use in downstream assays.

All our DNA samples for in vitro tests were DNA extracted from fresh blood and even old bone samples.

For rapid genomic DNA extraction method pour 500 μl or 0.5 g of biological material (e.g. blood) into a 1.5 ml microfuge tube and add 1000 μl of cell lysis buffer whose components have been presented in Table 1. Shake microfuge tube gently, then centrifuge it for 2 min at 6000 rpm. Remove and discard supernatant and repeat this step two or three more times according to the sample.

In DNA extraction from bone, 50 μl proteinase K (20 mg/ml) was added followed by gentle mixing at 56 °C for 3 h. Add 300 μl of nuclei lysis buffer, whose components have been mentioned in Table 2, to the microfuge tube and keep the tube in room temperature for 2 min to prevent clot formation. Add 100 μl of saturated NaCl and 600 μl of Chloroform to the microfuge tube; shake it gently then quickly; then centrifuge it for 2 min at 6000 rpm. Transfer 300–500 μl of supernatant to a new 1.5 ml microfuge tube.

Add 600 μl of cold isopropanol or absolute ethanol to it; shake it gently then quickly. Centrifuge the microfuge tube for 1 min at a maximum speed in a cold environment and subsequently dried up before it was made soluble in 50 ml of a TE buffer of pH 7.6 (10 mM Tris, 1 mM EDTA). Keep the microfuge tube of DNA at −20 °C for later uses.

It should be noted that just for preparation of bone samples following method is necessary: in each case an approximately 5 cm rectangular piece of the femur shaft was cut with 3 a saw. Soft tissue was removed with a scalpel before freezing the sample at −20 °C the clean bone was exposed by use of a coarse sandpaper under running water while the sample was thawing. The bone was dried with a paper towel and subsequently clamped in a small vice. A 0.3 ± 0.4 cm thick section (weight approximately 1 g) was cut off using a clean hacksaw blade. The bone fragment was powdered with liquid nitrogen and then stored at −20 °C.

All bone samples were subjected to DNA extraction by the RGDE method. About 0.5 ± 1.0 g of bone powder was added to 1000 μl of cell lysis buffer.

Table 1
Components of cell lysis buffer for RGDE method

Materials	Quantity	Density
Tris–HCl	1.57 g	10 mm/l
Sucrose	110 g	11% (w/v)
$MgCl_2$	1.01 g	5 mm/l
Triton X-100	10 ml	1% (v/v)

Note: add Triton in the final section, pH 8.

Table 2
Components of nuclei lysis buffer for RGDE method

Materials	Quantity (g)	Density
Tris–HCl	1.57	10 mm/l
SDS	10	1% (w/v)
EDTA	3.75	10 mm/l
Sodium citrate	2.94	10 mm/l

Note: pH 8.

The quality of DNA extraction using the RGDE method was evaluated by STR analysis with the AmpF£STR®Identifiler™ PCR Amplification Kit (Applied Biosystems). Amplification products were electrophoresed on an ABI 3130 Genetic analyzer (Applied Biosystems) and evaluated using the GeneScans version 3.2 and Genotypers version 3.2 software (Applied Biosystems).

3. Results

The quality and quantity of extracted genomic DNA was controlled. All the samples (including fresh blood and even old bone samples) were able to profile for STR zones.

4. Quality evaluation

Agarose gel 0.8% was used for quality control of genomic DNA (Fig. 1).

5. Quantity evaluation

Spectrophotometry method was used for quantity control of genomic DNA (Table 3).

6. Discussion

DNA extraction from different biological material is used widely medical genetic laboratories for diagnosis of genetic disease and in forensic and research centers and laboratories. In RGDE method, genomic DNA can be extracted in the least time and with high quality and quantity by using simple materials and without any need to use the special equipment and apparatus.

The present work on different DNA extraction procedures was conducted to improve the routine forensic laboratory service in cases involving the analyses of degraded human tissue. This topic

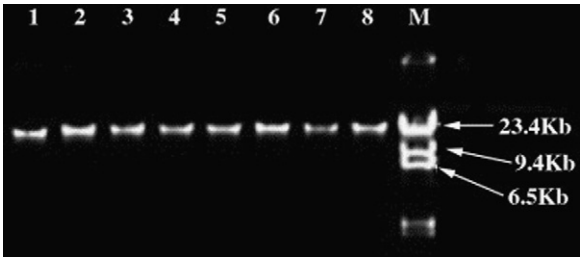

Fig. 1. Shows extracted genomic DNA by RGDE method on agarose gel 0.8%. Genomic DNA from fresh blood samples (lines 2, 4, 6, and 8) genomic DNA from old bone samples (lines 1, 3, 5, and 7). Marker of molecular weight γ phage DNA/HindIII (line M).

Table 3
Quantity control of genomic DNA from fresh blood and old bone samples by RGDE method

Sample	Volume of biological sample used	DNA yield (μg)	A260/A280 ratio	Time of isolation
Blood	500 μl ($n = 20$)	4.36 ± 2.53	1.91 ± 0.13	∼10 min
Bone	0.5 g ($n = 20$)	4.13 ± 1.37	1.69 ± 0.16	∼24 h

has in particular the reference to human identification, either of single deceased individuals or of mass disaster victims.

7. Conclusion

This method (RGDE) has different advantages such as: economical spending, no need of the special and expensive equipment, spending little time, no need of the experimented and experienced staff, etc. In this method genomic DNA with high quality and quantity can be acquired from different biological sources such as blood, tissue, sperm, tooth and bone. Time of extraction of genomic DNA in RGDE method is less than 10 min.

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Conflict of interest

None.