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DNA Extraction from Human Remains through Different Methods

Poonam Moon[1], Gunjan Jindal[2], Dr. Ranjeet Kumar Singh[2]

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Abstract:

In recent days, there is constantly increasing or developing the techniques for human identification. From degraded human remains, DNA typing is a big task or challenge for forensic DNA experts not only in prospective of purification of DNA but also in data manipulation and interpretation of establishing the profiles of DNA, specifically in mass fatalities. Forensic anthropologists processed the human remains which are used for the purpose of genetic analysis. In future analysis, DNA's condition (deoxyribonucleic acid) which is found in human remains, may become an issue. Scanning electron microscope (SEM) identified the bone's damage which is indicates differences that may exists in quantity and quality of DNA extracted. DNA profiling has been developed the core of human identification with new era of revolution of DNA, molecular biology and PCR techniques. In this paper, discussed about the various methods for DNA extraction from human remains samples.

Keywords: *DNA Extraction, PCR Amplification, Human Remains, Scanning Electron Microscope (SEM)*

Authors:

1. *Government Institute of Forensic Science, Nagpur, Maharashtra, INDIA*

Introduction

In ancient time, DNA has suffered from so many problems, it is preserved only in numerous states of degradation and small amounts **(Rohland and Hofreiter, 2007)**. Forensic anthropologist, forensic pathologist and forensic geneticists faced with the human remains which are observed in buried, decomposed and skeletonized condition. He used various investigative techniques due to which established the potential identification in which includes the details of skeletal by their relative and special features includes scar or tattoo mark, fingerprint impression and dental profile. These techniques are required for the purpose of comparison between informative and huge ante-mortem (AM) and post-mortem (PM) data of human remains but in cases of missing person and mass casualty, the information of ante-mortem is not valuable or less informative. In some cases, such conventional methods have to be used but they cannot provide the positive result then used the DNA typing method which provide positive identification of human. It is very rapid test and analyzed and performed by automatic machines **(Arismendi, Baker and Matteson, 2004; Siriboonpiputtana, 2018)**. In some cases, human remain samples includes many types of sample such as bone, blood, nail, hair and tissue etc., considered as a biological evidence which are used for the purpose of analysis and identification of cadaver victims.

In some cases, human remains (such samples) may degraded due to which faces some serious problem, it has been occur due to rigorous environment. If body was buried then humus acid taken from soil which interfere with the process of extraction and prevent the amplification of extracted DNA **(Abuidress, Alhamad and Alsaadany, 2016)**.

Skeletal remains' DNA involves a set of difficulties which is derived from the bad molecular preservation and low template DNA. For the purpose of analysis of DNA, first step is DNA extractions in which obtain the good quality of DNA extraction **(Palomo-Diez et.al. 2017)**. Burnt bones are submitted to the laboratories for the identification of human remains in which bones contains physical and chemical properties both. Physical changes includes fragmentation and deformation due to heat-induced contraction, changes the morphological indicators such as stature, sex, age and species estimation. Physical alteration in which occur the heat process which is also induces chemical alteration of bones due to pyrolysis and combustion of chemical substances **(Imaizumi, 2015)**. In both unidentified remains and mass disaster cases, DNA profile is generated from bones and teeth which is an important part for identification **(Latham and Miller, 2018)**.

Prevention and Pretreatment of Sample

After the collection of bone, extraction process should be carried out under the condition of sterile with proper precaution including protective clothing (mask, double latext gloves and sterile disposable coat), equipment and different working areas or surfaces which's treated with irradiated UV light and bleach. The material of bone surface was recovered using surgical blade and three times washed with bi-distilled or sterile water, detergent and finally cleaned with 95% ethanol. At 56º C, cleaned bones were desiccated in an incubator for overnight. Sample should be handled or performed into the cabinets of laminar flow which is equipped with UV lights and HEPA filters using disposable sterile filters tips, dedicated pipettes and sterile tubes. Blank controls were used for the purpose of preventing of samples from contamination in which including all stages. Sample of bones were separated on the basis of physically and temporally from analyses of sample **(Pajnic, 2017; Siriboonpiputtana, 2018)**.

DNA Extraction

The term of success or failure represents the ability to extract the DNA from bone in which failure term is used for 'degraded sample'. Human decomposition promoted by environments which is also contributed to DNA degradation. DNA degradation influenced by the various factors of environmental and taphonomic includes soil microbes, mold, humidity, temperature, postmortem interval, ultraviolet light, fire, water and storage condition (Mundroff, Davoren and Weitz).

For the extraction of bone, first bone samples should be cleaned mechanically using brush or rotary sanding tool, washed chemically with ethanol or 5% alconox detergent, dried and grinded with nitrogen which is in liquid form (Cryomill) (Abuidress, Alhamad and Alsaadany, 2016). DNA extraction was conducted by different-different methods from different bones which are as follows:

Manual Organic Method

- This method is conducted with 500mg of bone powder which is taken from clavicle and femur bone. It is described by the Hochmeister and Jakubowska scientists. In this method, bones were decalcified for 5 days and then digested the bone powder for overnight. This digested DNA sample is extracted using PCI and concentrated with the centrifugal filters of Amicon.
- Added 500µl digestion buffer such as 50mM EDTA, 0.1% SDS and 20mM Tris; and 5µl proteinase K in 1.5ml tubes which is filled with bone powder.
- At 56º C, this solution was vortexed and incubated overnight.
- Phenol was added in this solution following by vortexing for 15 seconds and centrifuging for 5 minutes.
- The upper layer was transferred into the 1.5ml tubes and then added the 500µl chloroform.
- Again vortexing and centrifugation were occur for 15 seconds and 5 minutes.
- Again upper layer was transferred into the 30kDa Amicon filters and centrifugation occur at 14,000x g for 10 minutes.
- Discarded the flow-through and washed the columns with 300µl Tris EDTA and centrifuge the solution at 14,000x g for 10 minutes.
- Again discarded the flow-through and washed the column with same TE. This step is repeated again.
- Then, solution transferred into the columns and centrifugation occurs at 14,000x g for 10 minutes.
- Finally, inverted the columns into new tubes of Amicon and conducted the centrifuged at 1,000x g for 3 minutes and collected the retentates.

Automatic Magnetic Beads coated with Silica

This method is conducted with 500mg of bone powder which is taken from clavicle and femur bone. In this method, extraction was conducted using Qiagen (EZI advanced XL) and also using DNA investigation kit includes silica spin filters. Investigator should be careful from contamination, and precautions in keep in mind (Abuidress, Alhamad and Alsaadany, 2016; Hebda, 2013; Pajnic, 2017).

- Added the buffer ATE at the center of membrane and incubated for 5 minutes at room temperature before centrifugation.
- Two times repeated the elutions, total three elutions are there.

DNA IQ System

- Added the 0.5-2.0g decalcified bone powder in 4ml of bone incubation buffer such as 100mM NaCl, DW to 200 ml, 10mM Tris pH 8.0, 50mM EDTA.
- At 56º C, it is incubate for overnight.
- Through centrifugation, remaining bone powder was removed at 4,000rpm for 10 minutes.
- Then, extracted the DNA from supernatant (Siriboonpiputtana, 2018).

Power Soil Extraction Method

This method is based on the patent inhibitor removal technology which is used to remove the humic substances and isolate DNA using silica spin filter. All columns and tubes were not UV radiated on each side while solutions were not.

- Added the approximately 100mg of bone powder in tubes of power beads for digestion.
- Then, incubates for 1 hour at 70 degree C while vortexing for 10 minutes.
- Remaining supernatant were transferred into 2ml tubes following incubation.
- Extracted the DNA by adding 75µl of TE (Tris EDTA) at 55º C and centrifuge at 10,000x g for 30 seconds.

- Extraction of bone concentrated using 30kDa Amicon with ultra-0.5ml centrifugal filters.
- Also using the 495µl Tris EDTA and 5µl salmonid DNA before using it which is added to Amicon filters and centrifuge for 10 minutes at 14,000x g.
- Discarded the supernatant and centrifuge at 14,000x g for 5 minutes by added it to Amicon filters.
- Finally, extracted the DNA from this filters.

Soil Master Extraction Method

This method involves a resin-filled column chromatography, salting-out protein precipitation, DNA precipitation using spermidine and hot detergent lysis. All columns and tubes were not UV radiated for 5 minutes on each side while reagent were not.

- Approximately 100mg bone powder should be used for extraction of DNA.
- Powder should be contain in columns and tubes, and this solution were incubated at 70º C for cell lysis.
- Remaining supernatants were transferred into the 1.5ml microcentrifuge tubes for the purpose of incubation.
- Vacuum dried the pellets for 15 minutes using vacuum pump of Maxima C Plus and resuspended in 25µl of Tris EDTA **(Hebda, 2013)**.

FDEB Method

- Decalcify the 300mg of pulverized bone in 10ml of EDTA (Ethylene Diamine Tetra-acetic Acid) at 4º C in a shaker for overnight.
- Then, solution was centrifuge and discarded the supernatant, and decalcified the remaining pellets.
- Added the 5µl DTT (dithiothreitol) and 30µl proteinase K and 300µl cell lysis buffer to lower sediment for digestion purpose and incubated at 56º C for 3 hours and continuous shaking the solution.
- Now, added the 100µl nucleue lysis buffer and again incubated at 70º C for 30 minutes.
- Solution was spun at 4,000 rpm for 5 minutes using centrifuge after completion of digestion and transferred the supernatant to 2ml microtube.
- At this position, added the 20µl RNase enzyme at 37º C for fresh bones to remove RNA molecules.
- After adding the RNase, one stage came called salting-out also known as salt-chloroform which is carried out such as: equal volume of chloroform and 100µl NaCl, and then vortexed and centrifuge the solution for 5 minutes at 14,000rpm.
- Now, removed the aqueous solution and remaining solution transferred into 2ml microtube.
- Then, added the 0.1 volume sodium acetate and 1.5 volume ethanol and kept for 3 hours at -20º C.
- Solution was centrifuged for 10 minutes at 14,000rpm and completely removed the supernatant.
- Added 0.1 volume tris solution and 170µl chelex solution, and incubated for 1 hour at 56ºC.
- Now, transferred the supernatant to 1.5ml microtube and added the 100 µl ethanol and centrifuged for 10 minutes at 14,000rpm.
- Then, completely removed the ethanol and added the 30 µl deionized water to residue.
- Finally, samples or solutions were prepared for PCR technique (Mohammadi, et.al, 2017).

Conclusion

In most of the forensic cases, human remains' identification in which specially determining the sex from decomposed human remains. Forensic

investigator faced so many challenges for extraction of DNA from human remains which's affects with the environmental condition such as bacterial effects, environmental chemical and physical degradation, and environmental inhibitors. All extraction methods are producing the better results in a shorter time, it is applied in the laboratories.

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