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Original Research Article

Forensic genetic analysis of bone remain samples

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ABSTRACT

DNA typing from degraded human remains is still challenging forensic DNA scientists not only in the prospective of DNA purification but also in the interpretation of established DNA profiles and data manipulation, especially in mass fatalities. In this report, we presented DNA typing protocol to investigate many skeletal remains in different degrees of decomposing. In addition, we established the grading system aiming for prior determination of the association between levels of decomposing and overall STR amplification efficacy. A total of 80 bone samples were subjected to DNA isolation using the modified DNA IQ™ System (Promega, USA) for bone extraction following with STR analysis using the AmpFLSTR Identifiler® (Thermo Fisher Scientific, USA). In low destruction group, complete STR profiles were observed as 84.4% whereas partial profiles and non-amplified were found as 9.4% and 6.2%, respectively. Moreover, in medium destruction group, both complete and partial STR profiles were observed as 31.2% while 37.5% of this group was unable to amplify. Nevertheless, we could not purify DNA and were unable to generate STR profile in any sample from the high destroyed bone samples. Compact bones such as femur and humerus have high successful amplification rate superior than loose/spongy bones. Furthermore, costal cartilage could be a designate specimen for DNA isolation in a case of the body that was discovered approximately to 3 days after death which enabled to isolate high quality and quantity of DNA, reduce time and cost, and do not require special tools such as freezer mill.

1. Introduction

Human identification strategies of decomposing samples are still challenging forensic pathologists, forensic odontologists and forensic geneticists in our world today. In some extreme conditions such as natural disasters (e.g. earth quakes, tsunami, volcanoes, and avalanches) and human made catastrophes (e.g. wars, terrorists, political crisis, plane clashes, and bombings), the degree of destroyed/decomposed samples largely depends on the specific type of natural casualty and the difference in tactics of the criminal to hide/destroy the crime scene evidences [1,2]. Many methods have been used to identify human remains depending on the circumstances and the state of remains. The common human identification methods are including; the victim data giving by living witnesses or deceased relatives such as direct facial and special feature recognitions, tattoo, scar or mark and their belongings; the matching of fingerprints (provided pre-mortem inked prints are available) or matching of dental profiles (provided pre-mortem dental records are available) [3]. Consequently, these techniques as described above are required for the comparison between huge and informative ante-mortem (AM) data and the post-mortem (PM) data of the remains. However, in most mass casualty cases and missing person identification, AM information is not available or less informative for several victims to generate the match. Moreover, in extremely disasters such as 9/11 and MH37, the physical appearances of several victims were extremely destroyed and the organs of victims were not intact to their bodies. Thus, using those data to identify human remains in case of mass catastrophes and decomposed samples are very difficult and still stimulating forensic scientists.

DNA typing methods have been used in forensic laboratories worldwide for human identification as well in mass fatalities (e.g. 9/11 World Trade Center Attack, USA; 2001, India ocean Tsunami; 2004). The stunning roles of DNA typing technologies for human identification in extreme mass catastrophes and high degree of decomposing samples are including the test is not restricted to any

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particular one to one body landmark comparison (e.g. friction ridge details in fingerprints) and DNA profile matching can be conducted to associate separated remains or body parts. In addition, DNA typing techniques are able to identify human remained samples in a variety degree of destroyed status. Moreover, low amount of samples is required and many types of sample (blood, hair, nail, tissue, bone etc.) could be selected as a source for DNA isolation depending on forensic scenarios. DNA typing is a very rapid test and can be performed and analyzed by automatic machines. Furthermore, the applications of population DNA database and biostatistics calculation for missing person scenario help forensic geneticists have more confidence to generate and report the DNA matching results [1].

DNA as genetic material encodes many crucial proteins to drive human being through a life. Human remains such as body fluid, bone, teeth, and hair are the source of DNA. Recently, many reports have been focused on the development of a powerful method to isolate a tiny amount of DNA molecules from remain samples. In several forensic cases, human remains including bone samples have been found on the ground in different degree of decomposition, embedded in soil for some years or immersed in water or sea. Many factors that commonly inhibit PCR amplification of DNA isolated from remained samples are following, the originate environment of the remains, hydroxyapatite and contamination from another organism (e.g. bacteria or fungi). Three common sources of contamination in DNA extraction from bone samples are including co-extracted surface contamination, contamination during laboratory practice, and PCR carryover. Therefore, the preliminary cleaning processes of decomposing samples, decontaminating and removing PCR inhibitor are necessary to perform the successful PCR amplification and generate the complete DNA profile. Various methods to remove PCR inhibitors and decontamination have been reported such as surface washing, surface removing by physical methods, interior part extraction, surface washing with acid, irradiating with UV light, exposing to high concentrated ethanol, exposing to a bleach (NaOCl), and the combinations of those above techniques. The criteria to choose a decontamination method are largely depending on the laboratory personal experiences, the nature of contaminations, time, cost and previous reports [2]. Moreover, laboratory experiences, specialized equipment such as cutting, sanding and powdering tools as well as freezer mill are still necessary to generate the appropriate amount of started bone material for efficient DNA isolation. In addition, to avoid contamination occurred, the scientists should have a separate area for dealing with bone, teeth and decomposed samples from other laboratory function [2]. Several effective methods to extract DNA from decomposed human samples have been reported in various publications. Those are including phenol-chloroform extraction [4–8], silica-based extraction [7,9–13], chelex extraction [14–16], and commercial kits as well as QIAamp® DNA mini Kit (QIAGEN) and DNA IQ™ (Promega, USA). In this report, we proposed routinely effective DNA extraction and amplification protocol for human identification from decomposed bone samples registered from 2007 to 2014.

2. Materials and methods

2.1. Samples

A total of 80 human skeletal samples in variety degrees of decomposing were included in this study. Samples were grouped into three categories based on the degrees of decomposing; high (cannot identify bone origin), medium (bone fragments of known origin) and low (complete bones) degree. This criteria was also including the physical examination such as colors, burned marks, water or soil immerged bone.

2.2. Pretreatment and decontamination

Bone samples were carried out under the sterile condition using groves, mask and separated working areas (biosafety cabinets). Bone surface material was removed by using surgical blade and washed with sterile water for three times and finally washed with 95% ethanol. The cleaned bones were dried in an incubator at 56 °C for overnight.

2.3. Bone powdering

Bone samples were divided to approximately 0.5–1.0 cm long by using electrical bone surgery instrument. Bone powder was generated by using Freezer/Mill®, model 6750 (Spex/Mill, Spex, Metuchen, NJ) and weighted to 0.5–2.0 g in 15 ml centrifuge tube. To prevent contamination, before cutting the next bone sample, the electrical bone surgery instrument was cleaned with 70% ethanol and subsequently decontaminated with UV treatment. Additionally, the surgical blade was detached and washed with water, 70% ethanol, treated with UV and autoclaved prior used.

2.4. DNA extraction

DNA extraction was applied from the bone extraction protocol with DNA IQ™ System (Promega, USA) as following, add decalcified 0.5–2.0 g bone powder in 4 ml of bone incubation buffer (10 mM Tris pH 8.0, 100 mM NaCl, 50 mM EDTA, 0.5% SDS, DW to 200 ml), incubate at 56 °C for overnight, and remove the remaining bone powder by centrifugation at 4000 rpm for 10 min. Then, follow modified DNA IQ procedure, November 2001 (split lab) to isolate DNA from the supernatant.

2.5. DNA quantification

The extracted DNA quantity was measured by the Quantifiler™ Human DNA Quantification Kit (Life Technologies), and worked with ABI7500 Fast Real-Time PCR (Life Technologies).

2.6. DNA purification and concentration

Some extracted DNA solution with quantity lower than 0.1 ng/μl were passed through YM-100 MICROCON® (Millipore, USA) to concentrate the DNA amount and to remove non-requisite materials in DNA solution that can impact on PCR amplification process.

2.7. PCR amplification and genotyping

The 28-cycle standard multiplex STR analysis recommended from the AmpFlSTR Identifiler PCR Amplification kit (Life Technologies) was performed on samples with DNA concentration higher than or equal to 0.1 ng/μl. The low copy number (LCN) protocol with 32-cycle combined with double amount of AmpliTaq Gold® (Life Technologies) was performed on samples with DNA amount lower than 0.1 ng/μl. A total 25 μl of PCR reaction was amplified on GeneAmp® PCR System 9700 thermal cycler (Life Technologies). Genotyping was performed with 3130 Genetic Analyzer and analyzed by GeneMapper® software (Life Technologies).

2.8. Analysis of data

The acceptance criteria of DNA profiles were concordant to the standard operating procedure (SOP) obtained from various DNA laboratories in Thailand (Thailand Tsunami Victim Identification; TTVI) and used at the Information Management Center (IMC) at