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Mitochondrial DNA and Methods for Forensic Identification

Gazi Nurun Nahar Sultana*and Mohammad Zakir Sultan

Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, Bangladesh

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***Corresponding author:** Gazi Nurun Nahar Sultana, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, Bangladesh, Tel: 880966192959/4628; Email: nngazi@du.ac.bd

Abstract

A growing area of forensic mitochondrial DNA (mtDNA) has been appreciated by forensic scientists and law enforcement agencies in many countries. Crime scene investigators, law enforcement agencies, and prosecuting attorneys have recommended that this form of testing may successfully advance the investigation and prosecution of cases with limited biological evidences, such as bones, hairs and degraded skeletal remains. Defense attorneys are also increasingly requesting testing of samples with advancement of methods that may aid in exonerating their clients. MtDNA an extra nuclear genome, has certain features that make it desirable for forensics; namely, high copy number, lack of recombination, matrilineal inheritance, heteroplasmy, expression variability, and mitotic segregation.

MtDNA typing has become routine in forensic biology since mid of 1980 and is a last resort for testing highly degraded biological debris. Further, the high mutation rate of the human mitochondrial genome (mtDNA) has potentiality become a promising biomarker even to differentiate between monozygotic twins with rare SNPs for law enforcement agency because monozygotic twins cannot be separated by short tandem repeat (STR) profiling. With the advancement of Sanger Sequencing, Next-Generation Sequencing, Mass Spectroscopy, MALDI-TOF technology, it is now possible to characterize minor difference of the mtDNA genomes in routine identification of skeletal of missing persons, mass disaster casualty, and identification of twins. This short article reviews the role of mtDNA and its advanced methodologies in Forensic investigations.

Introduction

Human DNA is the genetic material found in every cell except in erythrocytes [1]. DNA traces can be found is body fluids; including saliva, blood, semen, vaginal secretion, bones, teeth, hair and perspiration [2]. DNA is unique to every individual and DNA typing methodologies are continuously subjected to scientific and legal scrutiny [3]. Most of these typing methods are dedicated to nuclear DNA. DNA has been used as unique investigation material for forensic purpose since after Alec Jeffrey's who first introduced restriction fragment length polymorphism (RFLP) in 1985 to identify the unique markers in the genetic material [4]. The method has been improved with the discovery of polymerase chain reaction (PCR) in the mid-1980's a critical molecular biology technique [5]. With the advancement of technology, time required for DNA testing has been reduced from days to hours which made it possible to reduce the process of forensic investigation and judgment [6].

Mitochondria and Mitochondrial DNA

Mitochondria are specialized sub cellular organelles unique to the cells of animals, plants and fungi. They serve as power hub for various powering functions of the cells and organisms as a whole. Energy requiring cells have higher number of mitochondria and differ from cell to cell. They are situated close to the part of the cell that shows highest energy requirement [7]. They are self replicating and they increase in number by division throughout inter phase, and their division is not synchronized with the cell cycle. Cambridge sequencing is the standard sequence to which all the human mtDNA are compared [8]. Any variation in the mtDNA genome, from the Cambridge sequencing is named as polymorphisms.

Control region of mtDNA is called D-loop which is highly polymorphic and hence is used for forensic purpose in criminal investigations. The length of this loci is 1100 bp and has two regions called (hypervariable regions) HVR-I and HVR-II [9]. Mitochondrial have been used as a tool for forensic identification since 1993 [10]. Mitochondria contain 2-10 copies of mtDNA, and there can be as many as 1000 mitochondria per somatic cell. In common, blood epithelial cells are preferentially used in forensic casework [11]; as a result, detection becomes extremely sensitive even in low amount samples. Some regions of the mtDNA genome appear to evolve at a rate of 5–10 times higher than that of single-copy nuclear genes. These regions are of interest for human identity testing because of their hyper-variability consequent of the higher mutation rate [12].

Overview of Mitotyping Protocol

Most laboratories providing mtDNA service have very similar protocols [13]. All samples including DNA abundant blood reference samples are handled very carefully from the beginning of sample collection to sequencing. The primary reason for the approach is to avoid cross contamination between samples. The analysis is mainly based upon the strategy of polymerase chain reaction (PCR) amplifications that focuses upon control hyper variable regions HVR-I and HVR-II. The various steps of the mtDNA analysis include primary visual analysis, sample preparation, DNA extraction, PCR amplification, post-amplification, quantification, purification and automated DNA sequencing and data analysis. The case samples require individualize attention during PCR and sequencing phase either because of damaged DNA or because of sequence specific variation, such as length heteroplasmy or more specifically rare site heteroplasmy. Previous report suggested that handling of single sample is much easier than batch samples analysis [14].

Identification of Biological Samples from Crime Scene

It is very important to observe the crime scene with optimal attention and the specimen related to biological samples. It is the first priority to follow the crime scene guidelines for sample collection which determines if the samples are subjected to mtDNA analysis or not. If the samples are blood or body fluids, sample needs to be examined with corresponding reference human specimen to decide the samples are human origin. Again, if the samples are degraded skeleton, teeth, bones or skull remains; forensic anthropologist needs to examine the samples for comparing with human reference samples for confirmation of the origin [15].

Sample Collection and Preservation

Sample collection from the crime scene is followed by standard operating protocol (SOP) but inherent rigidity in case of mass disaster and bioterrorism, SOP for crime scene processing could be unwieldy and impractical. Therefore, consultation among different entities involved in a response should offer best practice options. The plan can be modified after scrutiny of the crime scene or information obtained during the process of investigation. The goal of a hazardous material crime scene investigator is to obtain sufficient biological agent and associated materials, when available, to support a meaningful analytical investigation for species and strain identification or for toxin identification. The biological agent itself, however, is not the only forensic evidence to consider. Related chemical and physical signatures, including by-products, and traditional forensic evidence, such as fingerprints, computer records, and trace evidence, can provide clues to the identity of the individual (s) who committed the crime.

Place of sample, the method of sampling, and how many samples to collect are important considerations to obtain the right type, quantity, and quality of data to support attribution. Furthermore, it is essential to maintain the integrity of the evidence, to the extent possible, from the time of collection and during subsequent storage; otherwise, crucial and reliable forensic information can be lost [16]. Perhaps the best and most widely accepted approach for development of a sampling strategy in a particular case is to follow established guiding principles (for crime scene investigations) in combination with expert knowledge, including chemical and biochemical knowledge, investigative experience, and common sense. The results quality largely depends on the process of sample collection, transportation and storage. Once the samples are confirmed as human origin, they are collected into different container or plastic bags with numbering as per the number of crime scene.

If the crime scene is remote area where refrigeration and drying is not possible, samples need to be air dried and make moisture free before sending to forensic laboratory. Samples are dried and placed into zip locked plastic bags with serial numbers. Blood that is in liquid pools should be picked up on a gauze pad allowed to air dry at room temperature and wrap the item in clear paper or in a brown paper bag without scrubbing the stain. The tissue samples can be asked from the pathologist to collect a section of liver, bone, and/or deep muscle tissue and freeze for typing. Transfer of evidence to a secure place with proper identity, and detailed information for sampling can be conducted in a controlled laboratory environment under appropriate sterile conditions.

Sample Preparation

Once the samples are received by forensic scientist, cleaning is the first procedure to reduce exogenous human DNA samples. Blood and tissue samples are kept -20°C until analysis. Bone and teeth samples are sanded to remove any attached unwanted materials. Hair sample needs to be washed and sonicated for half an hour to remove dust, microbes and any other fine particles [17]. Using forceps and a scalpel cut a 2-3 cm region of the hair or hair shaft. A picture of the cutting should be taken at this time. If the hair is also to be tested for nuclear DNA, the mitochondrial DNA cutting should be away from the root. Place the unused portion of the hair onto the backing of a post-it note and return to the packaging.

Nomenclature of Forensic Samples

Samples re-extracted for the purposes of duplication (new cutting): The suffix “dup” will be added to the sample name to identify separately the re-extraction sample from the original, and this suffix will be applied to these duplication samples throughout the second processing. Samples reamplified in order to improve on the quality of the results or for other purposes suffix “reamp” will be added to the sample name. If multiple reamplifications are necessary, the numeral 1, 2, 3, etc. will be added to the suffix.

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MtDNA Extraction

The prepared sample is extracted by phenol/chloroform or alkaline chemicals that separate the DNA from other biological materials, such as proteins, cofactors, ions etc [18]. Then it is centrifuged, sedimented and filtered to obtain purified DNA sample. For quick extraction, commercial extraction is well validated in almost all forensic laboratories. This sample is further subjected to polymerase chain reaction with HVR-I and HVR-II primers, where the sample is amplified, quantified and sequenced.

Sequencing of mtDNA

Several methods have been adopted for mtDNA sequencing depending on the laboratory facilities. Direct sequencing is normally done using ABI 310/3130/3730/3730xl or Next Generation Sequencer (NGS). Distinct Laboratories were asked to sequence mtDNA HVR-1 region (16024–16365) from three bloodstains, proceeding in accordance with the protocol and strategies currently used in each individual laboratory by European DNA profiling group (EDNAP). The sequences are compared to determine if they match with maternal lineage or with FBI database. The analysts assess the results of the analysis and determine if any portions of it needs to be repeated. Finally, in the event of an inclusion or match, the Scientific Working Group of DNA Analysis Method (SWGDAM) mtDNA database, which is maintained by the FBI, is searched for the mitochondrial sequence that has been observed for the samples. The analysts then reported the number of observations of this type based on the nucleotide positions that have been read.

Despite the diversity of methodologies used, all the laboratories reported the same results [19]. The successful result of this exercise shows that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories [18]. With the advancement of methodologies, Applied Biosystem's SOLiD sequencing by ligation is also popular and practiced by some laboratories [20]. The method is used for whole mtDNA sequencing and also for targeted resequencing. Method includes sample preparation, emulsion PCR and substrate preparation, ligation chemistry, imaging and data analysis. Solid sequencing is 2 base encoding systems and provides 99.94% accuracy [21]. Solid system increases the flexibility of running two independent flow cells and increasing the research productivity. Solid system is good enough for large number of samples to detect SNPs and also for database enrichment for forensic laboratory. For the last ten years, Next Generation Sequencing (NGS) by Illumine has become popular. NGS delivers a single platform and workflow to overcome the wide range of difficult samples encountered in missing person.

Genetic analysis of mass fatality evidence with NGS let scientists to extract as much information as possible from highly compromised samples. It is possible to get clue of mutations and SNPs from the whole genome sequencing where several suspects and victims are involved. Population database is suggested to be used to convey information about the mtDNA profile's rarity in case of evidence and reference sample ambiguity. The databases tend to represent the general major population groups of the potential contributors of evidence. The relevance and representativeness of these databases should be considered for forensic applications. Pair wise comparison of haplo types and genetic diversity has been used to assess the relevance and representativeness of these databases [22]. Currently, a fast and highly accurate mass spectrometer-based process for detecting the presence of a particular nucleic acid in a biological sample for diagnostic purposes is also being attracted to many scientists [23]. Mass spectrometry provides detailed information about the molecules being analyzed, including high mass accuracy. It is also a process that can be easily automated.

Direct sequencing has several advantages; it is faster and produces more discrete results. The sequencing can be performed on formalin fixed tissues, on blood which was exposed to normal temperature, on cadaveric tissue and on hair samples. In compare to any other technique attempted in forensic laboratory, the multiplex mini sequencing of mtDNA provided high success rates [24]. Heteroplasmy is a problem for forensic investigators since a sample from a crime scene can differ from a sample from a suspect by one base pair and this difference may be interpreted as sufficient evidence to eliminate that individual as the suspect. Hair samples from a single individual can contain heteroplasmic mutations at vastly different concentrations and even the root and shaft of a single hair can differ. The detection methods currently available to molecular biologists cannot detect low levels of heteroplasmy. Furthermore, if present, length heteroplasmy will adversely affect sequencing runs by resulting in an out-of-frame sequence that cannot be interpreted [25,26].

Conclusion

It has been long years that scientific community has been trying to develop, validate and rationalize the use of mtDNA in forensic case study, thereby minimize the debate that arises in the courtroom. Rapidly developing biotechnologies offer an almost perfect tool for law enforcement agencies. The advantage of molecular genetics typing over any other methodology is in continuation and should not be considered with uncertainty. Although many of the quality assurance, quality control and interpretational guidelines used for PCR based DNA analyses apply to mtDNA analysis, there are some features of mtDNA that requires specific consideration. In majority of forensic samples, involving analysis of HVR-I and HVR-II of mtDNA genome, heteroplasmy is not observed. Heteroplasmy at more than one site may occur but at very lower frequency.

The fact that heteroplasmy occurs more often than originally observed and the mechanism and rate of heteroplasmy are not well defined is often raised in acceptability challenges in an

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attempt to exclude mtDNA evidence. But with careful evaluation, one can avoid erroneous interpretations. The forensic lab increasingly will be concerned about amplification competition between authentic DNA, contaminating modern DNA, and the occasional damaged postmortem DNA template. What is clear is that the small amplicon approach is highly successful at capturing degraded but abundant mtDNA from challenging samples and should be part of the testing repertoire for all missing persons and mass disaster programs. With the aid of all modern technologies, it is possible to analyze mtDNA for forensic laboratory with careful consideration of sample collection.

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