DNA FORENSICS: A POPULATION GENETIC AND BIOLOGICAL ANTHROPOLOGICAL PERSPECTIVE

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Keywords: convenient sampling, DNA forensics, DNA mixture, exclusion probability, gene diversity, kinship, likelihood ratio, match probability, mitochondrial DNA, population substructure, sample size, short tandem repeat (STR) loci, transfer evidence, Y-chromosome STR.

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Summary

DNA forensics is a discipline in which genetic variations at DNA level is used to aid in forensic investigations to attribute the source of biological samples collected in the context of investigations. Knowledge of human genome diversity as well as operational biological characteristics of DNA markers are essential in selecting genetic markers that are useful in DNA forensics, and in assessing the statistical strength of DNA evidence. For the latter in particular, biological diversity within and between anthropologically defined populations plays a critical role in formulating protocols for evaluating statistical strength. This general thesis is presented in this chapter, with an introduction to the subject of DNA forensics and its brief historical development. For the different generic types of investigative cases handled in DNA forensics, commonly asked
questions of legal relevance are enumerated. It is argued that use of population genetic models of DNA variation, and empirical data on world-wide diversity in anthropologically defined populations, are being used to provide conservative assessment of DNA-based evidence which is helpful for source attribution of forensic specimens. The increasing popularity of DNA forensics demands attention to the future directions in which DNA forensics may be used for human identification, particularly in complex cases arising from mass disasters and natural calamities. The chapter ends with noting the research questions that are to be addressed in enhancing the power of applying the knowledge of human genome diversity in DNA forensics.

1. Introduction

Forensic investigations involving applications of recombinant DNA technology have been described as the most important tool for human identification, since Francis Galton invented the use of fingerprints for such a purpose (NRC 1996). In this chapter, our objective is to present a general overview of the current status of DNA forensics as it is used in criminal as well as in civil proceedings of legal investigations, to exemplify how understanding of anthropological diversity of human populations is used in this area, particularly to assess the statistical strength of forensic DNA evidence. We will begin with a brief history of DNA forensics; outline the generic problems handled in DNA forensics; describe the genetic markers used; and show that anthropological considerations of genetic diversity within and between populations play a critical role in assessing the statistical strength of DNA evidence findings in forensic investigations.

2. A Brief History of DNA Forensics

Jeffreys (1985a,b,c) is credited as the inventor of “DNA Fingerprinting”. In these publications he and his colleagues described the technique of DNA profiling using DNA hybridization probes comprised of tandem repeats of core nucleotide sequences detecting multiple variable human DNA fragments by Southern blot hybridization. As the composite profile of genetic variation of such multiple genetic markers resemble bar codes, and creates virtually individualized patterns of each person tested, the term “DNA Fingerprinting” appeared legitimate. Even before that Wyman and White (1980) documented the existence of variable number of tandem repeats (VNTRs) at specific locations of the human genome, suggesting that such repeat variations of core sequences can generate genetic variation several orders larger than that detected by classical serological and biochemical markers.

Though the commercialization of DNA testing by Jeffreys’ multilocus probes was started by Cellmark and Lifecodes in 1986, for technical reasons, in 1988 the Federal Bureau of Investigation (FBI) adopted the use of single locus probes to score multiple VNTR loci through repeated re-hybridization by Southern blotting (Butler 2005). In the same year, the UK Home Office and Foreign and Commonwealth Office ratified the use of DNA fingerprinting for resolving family relationships and verification of relationships in immigration cases (Home Office 1988). Used in parentage testing and forensics, this technology soon came under scrutiny during court proceedings for questions regarding procedural and scientific validity (Lander 1989), resulting in a bigger controversy with regard to the statistical strength of DNA evidence for positive

Invention of the polymerase chain reaction (PCR) technique of DNA amplification (Saiki et al 1988) paved the way of addressing the technical limitations of the Southern blot RFLP analysis of VNTR loci, when PCR-based DNA typing methods were introduced in DNA forensics (e.g. HLA-DQA1 typing method, Saiki et al 1986). Interestingly, at the same time the National Research Council (NRC 1992) issued the first report, part of which criticized the use of the RFLP method of DNA fingerprinting. Ironically, this report of NRC (called NRC-I) generated even more controversy, as alternatives of RFLP method of DNA typing became popular, and their suggestions for statistical interpretation of DNA evidence were flawed from the viewpoint of population genetic principles. In addition, their recommendations were vague enough to be misused in legal applications. Research and development work in this area, in the meantime, characterized over a dozen of genetic markers, at which genetic variations were ubiquitous (though not as great as those of the VNTR loci, individually), but operationally advantageous in the sense that they each produced discrete alleles, and could be typed with automation and ease of multiplexed PCR methods (Edwards et al 1992; Hammond et al 1994). Called short tandem repeat (STR), the first commercial kit of PCR-based STR typing was introduced in 1993.

The US Congress DNA Act of 1994 resulted in establishing a governing body (US National DNA Advisory Board) to delineate guidelines, quality control and quality assurance protocols, and adherence of standard operating procedures of DNA typing for forensic use. This body completed its chartered tasks in 2000, resulting in objective sets of criteria to be used in DNA forensics. The so-called “DNA war” lost its fury with the issue of a second National Research Council report, NRC-II (NRC 1996). Even before this, in UK a DNA database was established under the guidance of the Forensic Science Services (FSS) office. In USA, the FBI launched the combined DNA index System (CODIS) in 1998, by which time the PCR-based STR loci (13 of them, along with the sex-typing amelogenin locus) became the major platform of DNA typing for forensic use. With wide acceptance of DNA evidence, nationally as well as internationally, mitochondrial sequencing (of the hypervariable control region) and Y-chromosome linked STR loci were also added to the battery of forensic markers to assist in typing and interpretation of old or degraded evidence samples, and handle DNA mixture with further ease. Two more penta-nucleotide autosomal markers (Penta-D and Penta-E) were added to the battery to autosomal STR kits (Krenke et al 2002), and use of DNA forensics became widely popular worldwide, not only to solve criminal and civil cases of human identification on an individual basis, but also for victim identification of mass disaster cases (Beisecker et al 2005).

3. Generic Problems Handled in DNA Forensics

The DNA forensic problems related to human identification can be broadly classified in three groups: transfer evidence, DNA mixture analysis, and kinship determination. Transfer evidence relates to scenarios in which DNA profile of an evidence sample (from a crime scene) shows signatures of being DNA from a single source, and the problem is to identify the source of this DNA through comparison of the DNA profile of the evidence sample against those of one or more known persons tested. Three possible
outcomes of such comparisons may arise:

1. Exclusion; i.e. evidence sample profile does not match the profiles of the known persons tested, which results in exclusion of the tested persons as being the source of DNA in the evidence sample;
2. Inconclusive; i.e. due to compromised nature of the evidence samples, their DNA profiles are ambiguous, but neither exclusion nor definitive inclusionary inference can be made; and
3. DNA Match; i.e. at the typed loci, the DNA profile of the evidence sample is indistinguishable from the one found in one of the known persons tested.

The obvious inference drawn under this third scenario is: the tested person cannot be excluded as the contributor of DNA found in the evidence sample. Statistical strength of the evidence is crucial in this third event, since the rarity of the profile would argue against any such coincidental match, should the known person be implicated wrongly in the case.

As most forensic evidence sample are gathered from compromised conditions, by nature, in a great majority of cases, they show signatures of having DNA from multiple individuals. These are termed as DNA mixture, in which, like the transfer evidence scenarios, the results are of three types:

1. Exclusion, occurring when the alleles present in the profiles of known persons are clearly absent in the mixture DNA of the evidence sample;
2. Inconclusive; due to ambiguity of the definitive allele determinations in the mixture DNA; and
3. Inclusion; implying that the known persons cannot be excluded as part contributors of DNA in the mixture sample.

As before, this third alternative observation demands statistical assessment of the evidence. Because of the complexity of the mixture DNA profile, however, the nature of statistical evaluation of DNA mixtures is different (even though the principles are the same) from that of the transfer evidence, which we will discuss later.

Kinship determination, the third type of DNA forensic problem, relates to comparisons of DNA profiles from evidence samples with the ones from one or more individuals biologically or affinally related. The objective is to determine whether or not the evidence sample could belong to a family member of the tested persons. The most popular cases of parentage analyses are indeed special cases of kinship determination, in which through contrasts of alleged father’s DNA profile against those of a mother-child pair, one attempts to determine whether or not the questioned person fathered the child. Kinship determination can also attempt to establish stated relationship between evidence sample and that of a family member, or to determine whether or not it represents the DNA of missing offspring of a tested married couple. While all three types of outcomes could result from this type of cases as well, statistics are needed to evaluate the strength of the inclusionary observations. Identification of victims of mass murders, man-made or natural disasters (like the terrorists’ attack of 11 September 2001, or the tsunami in South-east Asia in 2004) also fall under this category of DNA forensic issues.
4. Desired Characteristics of DNA Forensic Markers

Though the desirable characteristics of genetic markers that increase their efficiency for application in DNA forensics are related with intra- and inter-population variation, a separate discussion of these features is useful. This is so because some operational features are also relevant as desired characteristics of DNA forensic markers. Historically, genetic markers assayed from coded gene products have been in use for kinship determination and parentage analyses. Blood groups, serum protein and enzyme, and immunological markers have been widely used in parentage analyses since the 1930s. While the efficiencies of such classical genetic markers in ascertaining biological relatedness, individually as well as collectively, have been discussed extensively in the genetic literature, one operational drawback of such markers is the fact that the evidence sample tested should provide uncompromised gene products, suitable enough to type the markers used. Further, barring the immunological markers (e.g. HLA and Gm factors), few of these classical markers have enough segregating alleles to provide high power of discrimination for distinguishing genotypic profiles from different individuals. Relatively, abundance of polymorphic DNA markers in the genome, together with ability to type DNA variation without the gene products, and stability of DNA under compromised condition, offer operational advantages of DNA typing for forensic applications. Further, as DNA can be extracted from a wide variety of biological materials (e.g. blood, saliva, body fluids, tissues, bones, teeth, etc.—in fact any material that contains nucleated cells), applications of genetic markers, which can be assayed with DNA technology, have a much broader scope in comparison to that using the classical genetic markers, such as blood groups, serum proteins and enzymes.

Though the above-mentioned operational advantages are attainable by using several alternative platforms of DNA typing (e.g. Southern blot RFLP method, PCR-based oligo-nucleotide-specific hybridization technique, or PCR-based gel/capillary electrophoresis method), the markers amenable for PCR-based approaches are more advantageous, in the sense that a lower quantity of DNA is enough (e.g. 0.1-1ng versus 50-500ng), degraded DNA is also typable (amplicon size of 500 bp is enough), genotyping reagents are non-isotopic, typing methods are rapid and can be automated, and the allele designations are discrete.

<table>
<thead>
<tr>
<th>Locus Name</th>
<th>Chromosomal Location</th>
<th>Repeat Motif</th>
<th>Allele Range</th>
<th>Number of Alleles Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>5q33.3-34</td>
<td>TAGA</td>
<td>6-16</td>
<td>15</td>
</tr>
<tr>
<td>FGA</td>
<td>4q28</td>
<td>CTTT</td>
<td>15-51.2</td>
<td>69</td>
</tr>
<tr>
<td>TH01</td>
<td>11p15.5</td>
<td>TCAT</td>
<td>3-14</td>
<td>20</td>
</tr>
<tr>
<td>TPOX</td>
<td>2p23-pter</td>
<td>GAAT</td>
<td>6-13</td>
<td>10</td>
</tr>
<tr>
<td>VWA</td>
<td>12p12-pter</td>
<td>[TCTG][TCTA]</td>
<td>10-24</td>
<td>28</td>
</tr>
<tr>
<td>D13S1358</td>
<td>3p</td>
<td>[TCTG][TCTA]</td>
<td>9-20</td>
<td>20</td>
</tr>
<tr>
<td>D5S818</td>
<td>5q21-31</td>
<td>AGAT</td>
<td>7-16</td>
<td>10</td>
</tr>
<tr>
<td>D7S820</td>
<td>7q11.21-22</td>
<td>GATA</td>
<td>6-15</td>
<td>22</td>
</tr>
<tr>
<td>D8S1179</td>
<td>8</td>
<td>[TCTA][TCTG]</td>
<td>8-19</td>
<td>13</td>
</tr>
<tr>
<td>D13S317</td>
<td>13q22-31</td>
<td>TATC</td>
<td>5-15</td>
<td>14</td>
</tr>
<tr>
<td>D16S539</td>
<td>16q24-pter</td>
<td>GATA</td>
<td>5-15</td>
<td>10</td>
</tr>
<tr>
<td>D18S51</td>
<td>18q21.3</td>
<td>AGAA</td>
<td>7-27</td>
<td>43</td>
</tr>
<tr>
<td>D21S11</td>
<td>21q21</td>
<td>[TCTA][TCTG]</td>
<td>24-38</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 1. Characteristics of forensic STR loci

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chromosome</th>
<th>Allele</th>
<th>Size</th>
<th>Min. Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penta-D</td>
<td>21q</td>
<td>AAAGA</td>
<td>2.2-17</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Penta-E</td>
<td>15q</td>
<td>AAAGA</td>
<td>5-24</td>
<td>20</td>
</tr>
</tbody>
</table>

Further, together the 13 or 15 short tandem repeat loci (see Table 1 for their list and other biological characteristics) currently used in the US forensic community, has been studied well enough in worldwide populations to address the initial population genetic criticisms which pertain to the relevance of biological and anthropological diversity in DNA forensics.

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describing methods of analyzing different types of genetic data].


**Biographical Sketches**

**Ranajit Chakraborty** is the Director of the Center for Genome Information and Robert A. Kehoe Professor at the Department of Environmental Health of the University of Cincinnati College of Medicine in Cincinnati, Ohio. Professor Chakraborty’s research and teaching activities are related in the general area of complex disease genomics and development of design of such studies and data analysis methods. Another focus of his research is the use of recombinant DNA technology in human DNA forensics and microbial forensics of pathogen detection and identification. Inventors of several new design strategies of complex disease gene mapping (such as Mapping by Admixture Linkage Disequilibrium, MALD), Chakraborty has also worked extensively on formulating population genetic methods for assessing strength of DNA forensic evidence for human and pathogen identification. Raised in a family of religious background, Professor Chakraborty is also active in local Indian community serving in spiritual activities.

**Ranjan Deka** is a Professor at the Center for Genome Information in the Department of Environmental Health of the University of Cincinnati College of Medicine, Cincinnati, Ohio. He is also the Director of the Core Genotyping Laboratory at this Center. Trained as a physical anthropologist, Dr. Deka moved into the field of complex disease research. His primary interest is identification of genetic variants associated with type 2 diabetes, obesity and metabolic syndrome. His research involves utilization of isolated populations in understanding the genetic basis of these complex diseases.