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# 20 DNA Methods to Identify Missing Persons

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## Abstract

Since its discovery in 1985 by Alec Jeffreys, forensic DNA profiling has emerged as an immensely powerful technology. In this chapter, the development of genetic approaches to forensic human identification will be discussed in a variety of contexts, including the analysis of skeletal remains and other trace evidence. The use of autosomal, X and Y chromosome genetic loci and maternally-inherited mitochondrial DNA in relationship analysis will be briefly reviewed. More recent advances in the application of single nucleotide polymorphisms (SNPs) and next-generation sequencing (NGS) to human identification, particularly in the development of ancestry informative markers (AIMS) and externally visible characteristics (EVCs) will also be introduced, with related socio-ethical issues. A range of case studies are used to illustrate application of these technologies. Forensic genetics has a range of roles in missing person cases, including homicides and human rights related investigations. It is also important in the investigation of living missing persons, including trafficked children and persons displaced due to conflict and migration.

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**Keywords**

Missing persons, human identification, DNA profiling, kinship analysis, STR, ChrX, ChrY, mtDNA

## 20.1 Introduction

Human identification by DNA analysis in missing person cases typically involves comparison of two categories of sample: a reference sample, which could be obtained from intimate items of the person in question or from family members, and the questioned sample from the unknown person—usually derived from the bones, teeth, or soft tissues of human remains. Exceptions include the analysis of archived tissues, such as those held by hospital pathology departments, and the analysis of samples relating to missing, but living persons. DNA is extracted from the questioned and reference samples and well characterized regions of the genetic code are amplified from each source using the Polymerase Chain Reaction (PCR), which generates sufficient copies of the target region for visualization and comparison of the genetic sequences obtained from each sample. If the DNA sequences of the questioned and reference samples differ, this is normally sufficient for the questioned DNA to be excluded as having come from the same source as the individual for whom the reference sample was provided. If the sequences are identical, statistical analysis is necessary to derive the probability that the match is a consequence of the questioned sequence coming from the same individual who provided the reference sample or from some other randomly-occurring individual in the general population. Match probabilities that are currently achievable are frequently greater than 1 in 1 billion, allowing identity to be assigned with considerable confidence in many cases.

The genetic analysis may interrogate all major classes of genetic material, including autosomal (non-sex-chromosome), X-chromosome, and Y-chromosome targets in the nuclear genome, as well as from the mitochondrial genome. Statistical analysis is underpinned by theoretical and empirical research in population genetics, which allows the frequency of DNA sequences from these particular regions of the genome to be estimated with confidence for various populations. Where reference samples arise

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from family members, a further level of complexity and inference is required. Nevertheless, statistical random match probabilities that are highly discriminating can still be obtained.

In this chapter, the development of genetic approaches to forensic human identification will be discussed in a variety of contexts, including the analysis of skeletal remains and other trace evidence. The use of autosomal, X and Y chromosome genetic loci and maternally-inherited mitochondrial DNA in relationship analysis will be briefly reviewed. More recent advances in the application of single nucleotide polymorphisms (SNPs) and next-generation sequencing (NGS) to human identification, particularly in the development of ancestry informative markers (AIMS) and externally visible characteristics (EVCs) will also be introduced, with related socio-ethical issues. A range of case studies are used to illustrate application of these technologies.

**20.2 DNA extraction, purification and characterization**

Forensic DNA analysis requires the extraction and purification of DNA from both reference and questioned samples. These are intended to release the double-stranded helical DNA molecule from the cellular and biomolecular structures within which it is normally constrained—or from their break-down products—and to accumulate it in sufficient quantity and purity that will allow sequences within it to be characterized.

Methods for extraction and purification of DNA from biological samples collected in vivo are well established and routine (Lee and Ladd 2001). Sources may include blood, hair, hair roots, saliva, semen and so forth. Samples collected from post mortem remains may also be analyzed, although they may sometimes be problematic. DNA break-down or diagenesis following death may advance rapidly in a time and environment dependent process. In many cases, collection of peripheral blood samples is impaired, and viable material may be restricted to the soft and hard tissues. In hot and humid climates, skeletonization can occur in is little as a few days. DNA may be fragmented and chemically modified. In such conditions, ‘low-template’ DNA analysis may be necessary.

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The quantity of DNA recovered varies according to the source. In peripheral blood, for example, 20,000 to 40,000 ng/ml of DNA may be present and in semen 150,000 to 300,000 ng/ml. As sperm cells contain haploid DNA—having unpaired chromosomes, semen samples generally contain half the number of copies of a particular genetic target compared with their diploid counterparts found most other cells of the body.

Hair and bone contain much smaller amounts of DNA. Plucked hair routes may yield 750 ng DNA, whereas naturally shed roots may yield only 1 to 12 ng. Bone may yield 3 to 10 ng/mg of DNA depending on the bone condition, which under a range of conditions may be sufficiently poor that little or no DNA may be detectable—typically this amount will be below a threshold of about 1 ng of DNA.

In forensic investigations, DNA may be recovered from any of these sources and from biological material deposited at a scene of crime. Pathology specimens in the form of formalin fixed paraffin embedded (FFPE) tissues are preserved for decades and are sometimes an excellent source of DNA, if sometimes fragmented (Funabashi et al. 2012).

Characterization of the purified DNA is targeted at well understood regions of the nuclear and mitochondrial genomes known to vary sufficiently in populations that they may permit individuals to be distinguished one-from-another. A powerfully discriminating method of human identification from DNA was first developed by Alec Jeffreys (1985) and was based on the observation of minisatellites—highly varying regions of the human genome that could have a unique pattern for each individual—that could be used as ‘DNA fingerprints’. At the same time, Kary Mullis (Saiki et al. 1986, 1988) developed a method of amplifying DNA by enzyme catalyzed molecular copying of a target region in a process referred to as the Polymerase Chain Reaction (PCR). Combining the two methods permitted DNA fingerprints to be amplified from scene of crime trace evidence with the aim

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of comparison with the same regions in the suspect, a breakthrough of enormous significance for forensic science and the justice system.

Following Jeffreys' discovery, other highly varying regions—microsatellites, specifically, short tandem repeats (STRs)—have been adopted as the target of choice in DNA profiling. As the name suggests, these consist of short sequences of DNA in which pairs of the four bases—Adenine, Guanine, Cytosine and Thymine—from which the molecule is constructed are repeated a number times in tandem—for example, ATATATATATAT or GCGCGCGCGCGCGC. STR loci can easily be amplified and distinguished by capillary electrophoresis (CE) which allows them to be separated on the basis of their molecular weight—which differs according to the base composition and repeat number of the STR allele concerned. STR loci have been chosen from different autosomes (non-sex-chromosomes) as this means loci are not linked as they would be in neighboring regions of the same chromosome and the product rule can be used in statistical estimates of frequencies in populations (Evett and Weir 1998). STRs have also been characterized and applied in analysis of the X and Y chromosomes (X- and Y-STRs, respectively). In each case, the surrounding DNA sequences are sufficiently well understood that known short flanking sequences can be used as templates for the DNA primers essential to initiate copying of the target region in PCR.

### 20.3 DNA Profiling using Amelogenin and STR loci

Current routine approaches to human identification using DNA profiling are largely based on STRs, supported by analysis of the amelogenin locus (see Butler 2005, 2012). Amelogenin a protein found in dental enamel. The amelogenin gene is located on the sex chromosomes and shows DNA sequence length differences between the X and Y chromosome analogues, which can be used to assign the sex of the individual. The X allele contains a deletion of 6 base pairs and produces a smaller fragment when amplified by PCR. When the PCR products are analyzed, the female individual with two X chromosomes will show a single band while the male will show two bands, equating to the X and Y chromosome products, respectively. Primers for amelogenin PCR are chosen to complement

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templates that flank short sequences within the locus that will contain the 6 base pair deletion if present—for example, products of 106/112 or 212/218 base pairs—providing an advantage with respect to degraded DNA samples or when limited quantities of substrate are available. STR loci chosen are typically within a range of 120 to 350 base pairs. The simultaneous amplification or 'multiplexing' of several different loci in a single PCR tube makes the approach economical with respect to time and material, and—importantly—efficient use of the available sample.

In 1999, Chackraborty et al. regarded thirteen STR loci as adequate for most problems related to human identification and rightly predicated STRs would continue to be used as part of the DNA profiling and data banking projects that were growing around the world. The thirteen-locus combined DNA Index System (CODIS) emerged as a standard in the USA and several other jurisdictions. Multiplexes have continued to grow, however, due to their greater discriminating power. Various systems constructed around a shared set of core STR markers have arisen permitting some compatibility in databasing and statistical analysis (Butler 2015). In the UK, the ten-locus Second Generation Multiplex Plus (SGM Plus) system is currently being superseded by DNA-17 in England and Wales and DNA-20 in Scotland. These systems are highly optimized for reliability and efficiency in PCR amplification, and tend to target smaller substrate sequences than their precursors—increasing their sensitivity and utility, especially in the analysis of trace evidence. The systems incorporate selective fluorescent dye labelling of PCR products facilitating laser-based allele characterization and quantification—visualized as a peak on an electropherogram graph—and automated allele assignment during CE. Routine DNA profiling is now a largely automated process. Statistical match probabilities frequently exceed 1 in 1 billion, making human identification assignments—essentially, judicial rather than scientific decisions—straightforward, in criminal and civil cases, including those involving missing persons.

**20.4 Relationship analysis**

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Human identification can rely on comparison with DNA sequences amplified from near relatives, rather than from a directly-derived reference sample. In the case of a missing child, for example, the reference samples may arise from the father and mother. Because of the use of DNA analysis in paternity testing, procedures for relationship analysis are routine.

The basis of paternity and maternity testing is the fact that in the absence of a mutation, the child receives from each of the parents one allele of each locus analyzed. The assignment of paternity or maternity is based on the observation of alleles shared and not-shared between the child and the known parent and—in the event the questioned individual cannot be excluded—on statistical calculations: the most common test used being the 'paternity index'. Occasionally, the genetic relationship analysis is complicated by the absence a reference sample from of one of the 'trio' of the mother, son and supposed father. Although uncertainties may arise, the analyses can achieve high levels of resolution. Adding profiles of other relatives of the first degree—such as grandmothers or grandfathers, or brothers of the alleged father—can increase the statistical reliability in these cases (Evett and Weir 1998).

Relationship analysis can be applied to the identification of a person—living or deceased—by inference based on the known DNA profiles of near relatives. Lineage markers, such as the STRs of the X and Y chromosome may also be used.

### 20.4.1 Y-STR analysis

In forensic laboratory analyzes, the polymorphic STR markers of the Y chromosome are used because of their male specificity (Henke et al. 2001, Gill et al. 2001, Bosch et al. 2002). Initial studies with Y-chromosome DNA used a set of 7 to 9 STR loci, which has increased to approximately 17 (see Martin et al. 2004 and Chemale et al. 2014 for recent studies) with a three-multiplex reaction available that amplifies 19 loci (Bosch et al. 2002).

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These targets are particularly useful in cases of rape or sexual assault and in paternity investigations where the child is male. In the latter case, as a supplement—or, for example, in the absence of a reference sample from the father—inference may be made using other paternal lineage relatives, such as brothers or cousins, and so on. In missing person investigations, paternal lineage relationships may be assessed in a similar way.

### 20.4.2 X-STR analysis

The STR markers of chromosome X may efficiently complement the analysis of autosomal and Y chromosome STRs. Relative to autosomal markers, X-STR markers are characterized by higher values of the mean exclusion chance (MEC), the statistic used to estimate the power of exclusion. In other words, they have a higher exclusion capacity in some situations, such as in analyses involving blood relatives in the absence of a profile from the alleged father, among other scenarios.

The X-STR loci applied in human identification tests (see Szibor et al. 2003) are located in regions where recombination between the X and Y chromosomes does not occur, but where recombination does occur between the paired X chromosomes of women, ensuring variability in the population of the markers concerned. Chosen X-STR regions recombine in the same way as the autosomes, permitting a model that allows to the haplotype to be directly determined. X-STR analysis can be applied the investigation of maternity and—in some situations—paternity.

#### 20.4.2.1 X-STR analysis in maternity testing

In maternity testing, X-STR analysis can be applied in cases where kinship between a woman and supposed son is questioned. For males, the X chromosome is identical to one of the two X chromosomes possessed by the mother. For example, a case was reported in China (Li et al. 2012), where a woman wanted to adopt a child and was asked to undertake to a maternity test to see if there were chances that the woman could be the child's biological mother. A comparative test of forty-six

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autosomal STRs was carried out and it was concluded that the woman and the boy shared at least one allele at all 46 loci tested. Nevertheless, actual motherhood could not be confirmed. Further tests were carried out using X-STR and maternally-inherited mitochondrial DNA (see below), essential to exclude motherhood. The X-STR loci were analyzed and maternity was excluded. Certain population groups appear relatively homogenous in X-STR variation, including the Chinese (Li et al. 2012). In contrast, recent studies by Ribeiro-Rodrigues et al. (2011) reveal heterogeneity of X-STR variation among Brazilian samples.

**20.4.2.2 X-STR analysis in paternity testing**

In paternity testing involving blood relatives, X-STR markers are more efficient than autosomal. An illustrative example is the case where suspicion about the paternity of a child involves both the father and son. That is, both the father and son are suspected of being a girl's father. Father and son have X-chromosomes of different maternal origin, as their X chromosomes are from different women. Differences in X-STR markers when compared to the child will allow identification of the real father from the two candidates. Another illustrative example is a case where the putative parent may be one of two brothers. In this case, the chance of inheriting a single reference allele X is 0.5 (50%), which is equal to the probability of two alleles of the same locus on an autosome, reducing the advantage of using X-STR loci to that of autosomal STRs.

In certain cases of rape and incest, the woman may choose to opt for abortion. In this context, it is possible to undertake fetal paternity testing using X-STRs. There is no advantage to using X-STRs if the fetus is male. If the fetus is female, there are methodological complications with regard to the collection of samples from the fetus. In the 6 to 8 week period, fetal tissue may be difficult to exclusively identify, and dissection may yield a mixture of maternal blood and other tissues. The dissection of chorionic villi is usually undertaken, yielding samples consisting of a mixture of fetal and maternal DNA. To overcome these difficulties, both autosomal and X-STR analysis can be used for comparison with the suspect sample. The markers of the X have the greatest power of exclusion

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under these circumstances. Although, in a case of incest, little can be expected from the analysis of X-STR as all fetal alleles correspond to maternal alleles.

So-called 'deficient' paternity cases show the greatest advantage for X-STRs (Trindade-Filho et al. 2013). In the absence of a biological sample from the alleged father, DNA from relatives becomes a necessary prerequisite for X-STR analysis. The approach is based on the fact that sisters of the same father have the same paternal X chromosome. The investigation of two sisters or half-sisters X-STRs has the power of paternity exclusion when four different alleles or haplotypes are detected. That is, four alleles present on the X chromosome that are divergent from those present in the profiles inferred from relatives are sufficient to exclude the individual for whom there is no reference sample to be the biological father. In this scenario, the alleged paternal grandmother's profile is helpful as all contributing alleles can be determined by investigating X-STRs and the MEC can be calculated in the same manner as that used for autosomal markers. If the grandmother's profile is not available, the alleged father's X-STR genotype can be reconstructed to some extent from offspring, if they include women. Better information can also be obtained if the alleged father's brothers' reference profiles are available and the grandmother is heterozygous for X-STR loci studied. This is because the brothers of the alleged father may carry different alleles. On the other hand, if siblings share the same alleles, the mother can be both homozygous as heterozygous for the corresponding locus.

The discriminatory power of X-STR analysis depends on the sexes concerned and is normally of equal value to autosomal STRs when female samples are compared to others that are also female. However, when male individuals are investigated in comparison with reference samples that are also male, the discriminatory power of the X-STR markers is usually lower than that of autosomal STRs. This is due to the fact that the male X-chromosome analysis can rely on only one allele at each STR locus.

It is important to note that despite current understanding of the genetics of X-STR markers and their considerable potential in complex cases of identification from relatives, they are not commonly in

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routine use, but because X-STRs are especially efficient in cases where desired reference samples of supposed parents are unavailable, a growing demand for their use can be anticipated. X-STR markers also offer utility in efforts to reunite families separated in the context of war or migration.

### 20.4.3 Mitochondrial DNA analysis

Mitochondrial DNA (mtDNA) sequences are obtained from the mitochondria—energy producing organelles present in most human cells. Each mitochondrion contains a circular DNA molecule of 16,569 base pairs, including a non-coding region—the D-loop, which is highly variable between individuals who do not share a maternal relative. As mtDNA is maternally inherited, sequences are essentially identical in individuals of the same maternal lineage. The first full mtDNA sequence was established from a single individual of European descent by Anderson et al. (1981), and is known as the Anderson sequence or Cambridge Reference Sequence (CRS). With improvements in DNA sequencing technology in subsequent decades, the original material evaluated by Anderson and co-workers was re-sequenced to enable robust understanding of the reference sequence (Andrews et al. 1999).

About 1000 mitochondria are present in most cells, meaning that mtDNA sequences are present in two or three orders of magnitude more than their nuclear—autosomal, X-chromosome and Y-chromosome—counterparts. This simple distinction means that they can more readily be recovered from highly degraded forensic and archaeological specimens. MtDNA analysis is a powerful tool for identifying individuals as a supplement to the analysis of nuclear DNA or when analysis of nuclear DNA is not possible.

MtDNA analysis has been applied to DNA samples of archaeological age, showing that significant amounts of genetic information can survive for long periods in bone (Hagelberg and Clegg 1991). MtDNA analysis of bone fragments of 26 skeletons belonging to the Goeldi Museum collection in Pará, Brazil, for example, resulted in successful typing in 18 of the total of 26 individual Pre-

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Colombian Amerindians dated to 500 to 4000 years ago (Ribeiro-dos-Santos et al. 1996). In Sicily, both mtDNA and amelogenin sequences were analyzed to identify the remains of the family of Prince Branciforte Barresi—who lived in the sixteenth and seventeenth century. Molecular genetic analysis was consistent with historical expectations, although it was not possible to demonstrate directly that they were indeed the prince's remains, due to the unavailability of living maternal relatives. Bone microstructure showed evidence of good preservation (Rickards et al. 2001)

In forensic investigations, human skeletal remains belonging to the US servicemen who were missing from the Vietnam War were identified by analysis of mitochondrial DNA (Holland et al. 1993). In Argentina, skeletons of individuals killed during the military dictatorship of 1976 to 1983 were recovered and having proved resistant to routine STR analysis, were more successfully analyzed using mtDNA (Corach et al. 1997).

**20.5 Single Nucleotide Polymorphisms**

A further class of genetic markers known as Single Nucleotide Polymorphisms (SNPs) have been described, which opening up further possibilities in forensic human identification (Daniel et al. 2015, Eduardoff et al. 2015). As their name implies, SNPs are normally the result of a single base difference and since each SNP locus typically has only two alleles, a greater number of targets are needed to achieve the discriminating power of STR loci—which have multiple alleles. Computer analysis has shown that an average of 25 to 45 SNPs loci would be required to produce a random match probability similar to the 13 core STR loci. The actual number may vary in practice, as many SNP loci have variable frequencies in different populations. A 50 to 100 SNP panel may be needed to achieve the same power of discrimination and problem solving ability that can be obtained with 10 to 16 STR loci (Kidd et al. 2006). The prospects for typing 200 to 300 SNPs in a single reaction are promising, however, and are achievable using Next Generation Sequencing (NGS) systems (Seo et al. 2013).

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SNPs are abundant in the human genome and have been used in linkage studies of genetic diseases. PCR products of SNPs may have a size of less than 100 base pairs, making them most suitable for analysis of degraded samples, in which fragments of the sizes needed for STR PCR are not present. SNPs have the potential to be used in multiplexes and sample processing and data analysis can be automated, a process facilitated by the absence of extraneous 'artifacts' sometimes found in STRs.

Furthermore, SNPs offer further potential for use in the prediction of the ethnic origin of the subject and of certain physical characteristics.

**20.6 Next Generation Sequencing (NGS)**

Next Generation Sequencing (NGS) is a technology via which amplified DNA may be rapidly and extensively sequenced, without the need for relatively time-consuming CE and with the advantage that the DNA sequence is analyzed directly rather than from the fragment size inferred from the peak position detected on an electropherogram. NGS-based genotyping has a range of potential applications in forensic science (Yang et al. 2014). It has been applied to forensic STR (Bornman et al. 2012) and mtDNA analysis (Parson et al. 2013), and offers the potential for analysis of whole mitochondrial genomes. Forensic NGS analysis of ChrY may offer greater resolution than routinely achievable using Y-STRs (Van Geystelen et al. 2013). NGS is more readily suited to SNP analysis as it can easily detect polymorphisms in sequences. NGS-based forensic systems may attempt to incorporate traditional STR assignment, however, due to the legacy of STR-based systems and databases, which are world-wide.

**20.7 Ancestry Informative Markers (AIMS) and Externally Visible Characteristics (EVCs)**

Recent and current research in forensic genetics offers the possibility of estimation of biogeographic ancestry and characteristics of physical appearance from trace evidence. It has been known for many years that gene frequencies vary in populations (Cavalli-Sforza et al. 1994), offering the potential for

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localizing the source population from any particular profile (Novembre et al. 2008). Certain polymorphisms appear to be restricted to individual populations and are sometimes referred to as 'private polymorphisms'. A number of difficulties arise with ancestry estimation, however. Private polymorphisms, for example, tend by their very nature to be so rare that they are unlikely to have wide utility in forensic investigations, whereas more frequently occurring polymorphisms may lack sufficient resolution to any particular population to make an unequivocal assignment. Despite such drawbacks (Rosenberg et al. 2003), a number of studies have identified potential ancestry informative marker sets, including those based on SNPs (Phillips et al. 2014, Huckins et al. 2014, Kidd et al. 2011, Lao et al. 2006).

The potential for estimation of characteristics of physical appearance have centered on research on melanin pigmentation, where polymorphism in the genes of melanogenesis has been investigated with the aim of identifying SNPs that can be used to estimate hair and iris colour (see Maroñas et al. 2015 for a recent review). The genetics of face shape has also been studied—again with the aim of identifying candidate SNPs affecting facial appearance (Claes et al. 2014; Liu et al. 2012).

A number of problems are likely to persist in the forensic application of AIMS and EVCs. Complex genetics means that—with certain exceptions, as may apply in the case of red hair (Valverde et al. 1995)—assignment of colour or ancestry can only be made with limited resolution. Migration and admixture may confound investigative value, and ancestry can be based at least as much on culturally or cognitively held beliefs as on a genetic pedigree. The presumption of a relationship between pigmentation and genetic ancestry (Shriver et al. 2003) and perceived ancestry may be valid, but has the potential to profoundly confound understanding. In Brazil, for example, admixture has been such that assignments of ancestry on the basis of colour, STR, X-STR and mtDNA diversity are frequently contradictory (Soares-Vieira et al. 2008, Ribeiro-Rodrigues et al. 2011, Godoy et al. 2011). Ancestry can be perceived and self-perceived differently by different individuals, and investigators and forensic scientists are likely to have to negotiate the issue of 'racism', whether real or apparent (M'charek 2013).

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## 20.8 Low-template DNA analysis

Forensic specimens encountered in missing person investigations arise predominantly from skeletal material and trace evidence, which is frequently a challenging target for DNA analysis due to post mortem diagenesis, which reduces the quantity and quality of DNA available for analysis. The DNA molecule is frequently fragmented and chemically modified, and is found in the presence of other molecules which impede extraction and purification, and inhibit the PCR reaction.

A good deal of research has been dedicated to overcoming problems of DNA recovery and amplification from skeletal remains and trace evidence (Davoren et al. 2007, Evison et al. 1997). Extended-cycle PCR, widely used in ancient DNA studies, was adopted in forensic science in the 1990s where it is typically described as 'low-template' or 'low copy number' DNA analysis (Gill et al. 2000). Careful optimisation of the parameters of the PCR reaction is accompanied by additional cycles, which theoretically double the quantity of DNA generated with each reiteration.

Understanding of patterns of DNA diagenesis in different parts of the skeleton and in different environments may offer a route to improved low-template DNA analysis from challenging specimens. While histological studies of bone indicate that diagenesis is promoted by fungi and other microorganisms (Collins et al. 2002, Hackett 1981), and that these processes may advance rapidly in hot and humid climates (Iwamura et al. 2005), there is also evidence for DNA survival (Iwamura et al. 2005, 2004), which may be greater in certain parts of the skeleton (see Callaway 2015). Progress has also been made on the recovery of DNA from historical FFPE specimens (Gillio-Tos et al. 2007).

## 20.9 Standards and regulation

In the United States, the old 'RFLP' technology was replaced with STRs in 1997, increasing efficiency in the order of four times (Schneider 1997). The Forensic Science Division of the FBI

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selected 13 STRs loci of the CODIS system for inclusion in the American convicted individuals database. The thirteen STR loci that are part of this system are: VWA, FGA, D8S1179, D21S11, D18S51, D13S317, D7S820, D16S539, D3S1358, D5S818, TH01, TPOX, and CSF1PO. If there is a match between two samples at the 13 CODIS loci, statistical calculations typically indicate that these samples are from the same individual or from a random individual who is present in the population at a frequency of only one in several hundred million or more (Ban 2001). Use of standardized kits and instrumentation frequently improves efficiency in the analytical processes. Automation may permit analysis of 10,000 to 20,000 samples per year, with high levels of efficiency and quality, and with low costs and a minimal response or 'turn around' time (Steinlechner and Parson 2001).

Recognition of a need for criteria and standards for DNA typing within the forensic community in the United States resulted in the formation of a national group of forensic scientists in the late 1990s, called TWGDAM (Technical Working Group on DNA Analysis Methods). This group published a series of standards for forensic DNA typing, including the Guidelines for Quality Assurance Program for DNA Analysis (NRC 1992, 1996). From October 1998, conformity with the national guidelines for forensic DNA testing in public laboratories—also known as the FBI standards or national standards—became mandatory for certification and accreditation. These standards include national quality assurance programs encompassing laboratory organization and administration, facilities, sample handling control of evidence, validation of laboratory methods and analytical procedures, calibration and maintenance, proficiency testing, and standards for evidential statements and standards of suppliers. The European DNA Profile Group (EDNAP) began in 1989 as an informal association of European laboratories, normally police organizations and University laboratories who performed forensic work. This group represents the European Community and non-member countries of Eastern Europe. Its main objective is the standardization of DNA typing, achieved by performing exercises among members of the group to ensure comparability of genotyping results (Schneider 1997). A Spanish and Portuguese Group (GEP) promotes standards via the International Society for Forensic Genetics (ISFG) and Latin American countries host a Latin American Working Group, as well as promoting national standards (CNP 2001). In Brazil, the Brazilian Society of Legal Medicine

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established the first guidelines for paternity testing in 1999 (Bydlowski et al. 1999). The Integrated Network of Genetic Profile Banks (RIBPG) was formalized through the Law Decree No. 7950 of 12 March 2013. The RIBPG is intended to support criminal investigation and the identification of missing persons, and facilitate the exchange of genetic profiles obtained in official laboratories in the interests of justice (MDJ 2014).

A number of publically available online databases have been established containing reference population data for autosomal STRs (NIST 2016a), X-STRs (ChrX 2016), Y-STRs (YHRD 2016, Y-STR 2016), SNPs (NIST 2016b) and mtDNA (MITOMAP 2016). The X-STR database (ChrX 2016), for example, contains X-STR data for use in forensic practice, anthropological studies and other genetic research. In the database, one can find various information such as genetic and physical location, repeat structure, nomenclature, allelic mutation rate and population frequency of the STRs. Population data are classified according to one of seven metapopulations—Europe, Asia, Latin America, North America, Africa, Oceania-Australia and Arctic—and imply information indicating ancestry. In addition, the site hosts software that calculates various statistical parameters of interest in forensic investigation.

## 20.10 Case studies

### 20.10.1 Idenifications of Joseph Mengele and the Romanov Family

One of the earliest applications of forensic DNA fingerprinting analysis in the identification of missing persons was in the notorious case of Dr Josef Mengele of the Auschwitz death camp. Bones found in a grave in Embu, São Paulo, were examined by a Brazilian team[1], who completed a biographic profile—estimating age, sex, ancestry, possible cause of death and so on—from the remains. They noted a conspicuous injury to the right acetabulum, corresponding to a fracture of the

[1] Dr Daniel Munoz, Dr Marcos de Almeida and Dr Moacir da Silva

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superior margin which had ossified to form a spur. This injury was consistent with a motorcycle accident that Mengele was known to have been involved in. It was confirmed by orthopedic surgeons and pathologists to have been caused by indirect injury due to a violent impact to the knee having been projected up through the femur to its socket in the pelvis. The resulting fracture is typical of those encountered following impact to the flexed knee while in a sitting position riding a motorcycle. Histopathological analysis of a cavity observed in the right maxilla indicated inflammatory osteological reactions typical of a dental abscess. Repeated mixed agglutination tests showed the blood to type to be ABO-A, corresponding to the classification in Mengele's SS records (Dr. Marcos de Almeida personal communication). DNA was extracted from the femoral shaft of remains exhumed in Brazil by Jeffreys et al. (1992). Comparison with reference samples from Mengele's wife and son indicated a pattern consistent with paternity at 10 microsatellite loci with a random match probability in unrelated Caucasian individuals of 1 in 1800. The authors noted that their method was successful in overcoming the presence of strong inhibitors of PCR.

In 1994, Gill et al. reported on the genetic analysis of remains recovered in Ekaterinburg believed to be those of the Russian royal family, the Romanovs. In this analysis, both autosomal STR and mtDNA analysis were undertaken allowing the results compared with reference sequences obtained from a living maternal relative. The findings were consistent with the remains being those of the Tsar, Tsarina and three of their five children. In a subsequent analysis of remains later found nearby, Coble et al. (2009) reported the probable identification of the two other missing Romanov children using mtDNA, and autosomal and Y-STR analysis.

### 20.10.2 Missing, presumed dead—a fraudulent insurance claim

The family of an adult white male (DLF) notified the police of their son's disappearance. After a few weeks, a corpse that presented characteristics similar to those of DLF was found in advanced stages of decay and was identified by the family as being DLF. The family then filed a claim for the life insurance that DLF had taken out just before he disappeared. Suspicions were raised about the identity

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of the corpse, because identification had been done only visually, and because the insurance policy had been taken out just prior to DLF's disappearance. The insurance company requested a post mortem examination for identification. As the corpse had been cremated immediately after identification by the family, biological material that was encrusted on two projectiles removed from the body was used for analysis (see Figure 1). The dried blood provided enough genomic DNA for PCR-based typing of HLA-DQA1, D1S80, HUMCSF1PO, HUMTPOX, HUMTH01, D3S1744, D12S1090, D18S849, and amelogenin (Soares-Vieira et al. 2000, 2001). Results of genotyping from the corpse presumed to be that of DLF were then compared with that of his alleged biological parents, revealing genetic incompatibility (Figure 1).

### 20.10.3 X-STR analysis in the case of a missing child

Relationship analysis relies heavily on the understanding of genetic variation in the relevant population. In Brazil, issues of historical admixture require collection of data for local comparison—such as for X-STRs (Auler-Bittencourt et al. 2015).

Tables 1 and 2 illustrate a relationship analysis conducted using a 15 autosomal STR analysis with an additional 12 X-STR multiplex, offering further statistical resolution. Table 1 shows the results of autosomal STR analysis of a the remains of a body believed to be a missing child, her paternal half-sister SMIP, her mother and the reconstructed profile of the alleged father. Table 2 shows the results of X-STR analysis in the same case.

### 20.10.4 Deficiency paternity testing in a suspected homicide where no body has been found

Barbaro et al. (2006) report on the case of a girl who had been missing for several years in a case of deficient paternity testing—that is, when no reference sample is available for the supposed father. In this case, the authors used X-STR analysis to compare the profile of a sample of hair believed to have come from the girl with the profiles of her mother and sister. Analysis of the latter profiles allowed

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the X alleles of the father to be reconstructed, and the X-STR profile obtained from the hair—recovered from the premises of an individual implicated in another homicide—was found to share the same paternal X alleles, confirming identity in a case of 'special reverse paternity' testing.

### 20.10.5 Missing persons and humanitarian investigations

The latter part of the twentieth century saw a number of humanitarian investigations of alleged homicides committed during war or civil strife.

In 1999, Spain commenced official implementation of a program to try to identify human remains from the Civil War of 1936 to 1939 that could not be identified by the use of traditional forensic methods. Recognizing the importance of accurate recording and retention of information, the Phoenix Program used two independent sources of mtDNA data that could automatically be compared and cross-matched at identical or similar sequences. Comparison was facilitated by the use of a reference database populated with sequences of mtDNA from volunteers who were maternal relatives of missing persons and a questioned database populated with mtDNA sequences obtained from the remains of unknown individuals (Lorente et al. 2001, 2002).

In Yugoslavia, 30,000 people are believed missing as a result of the conflicts of the 1990s. In 2000, the International Commission on Missing Persons (ICMP) was established in an attempt to carry out human identification through a network of agencies in the former Yugoslavia. DNA laboratories in Bosnia and Herzegovina, Sarajevo and Banja Luka initially focused on blood typing from reference samples using multiplex STR and mtDNA systems using a 'dot-blot' method developed by Roche (Huffine et al. 2001).

In Brazil, as in other Latin American countries, investigations have begun into alleged human rights abuses of the military governments of 1964 to 1985. In December 1995, President Fernando Henrique Cardoso signed Law 9140, providing for "the recognition of persons missing as a result of

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participation or alleged participation in political activities in the period from September 2, 1961 to August 15, 1979". In the intervening 20 years, numerous investigations have been undertaken including some supported by forensic DNA analysis. This has led so far to the identification of only two individuals among 437 to 475 formally acknowledged to be missing persons—the true number may be much higher—leading relatives of victims, lawyers and other commentators to suggest that the Brazilian government is disingenuous in its claims to wish to offer justice to victims as part of the transition to democracy (Guimarães et al. 2016a, b).

Also undertaken in Brazil is the Projeto Caminho de Volta (Pathway Home Project), directed at the identification of missing children and adolescents, many of whom have become homeless and involved in crime (CDV 2016). This programme also uses a database approach, in which an online system can be used to register the details of missing persons and their relatives, supported by DNA databases of profiles derived from reference. Although positive outcomes have so far been limited, the Brazilian integrated DNA database RIBPG (Rede Integrada de Bancos de Perfis Genéticos) is now being uploaded with CODIS profiles of missing persons by Brazilian Federal Police, which may be more promising for effective identification in future.

## 20.11 Conclusion

Applications of DNA technology in human identification and relationship analysis have grown rapidly since the work of Alec Jeffreys in 1985. Substantial efforts have been made to identify human remains following homicides and natural deaths, as well as following wars, internal conflict, mass disasters and other cases of widespread fatality. In many cases, DNA analysis can provide a near-definite answer to questions of identification. Forensic genetics has a range of roles in missing person cases, including homicides and human rights related investigations. It is also important in the investigation of living missing persons cases, including trafficked children and persons displaced due to conflict and migration. The forensic science processes do not occur in a vacuum, however, and their success is influenced by many political and socio-economic factors.

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Table 1. Results of autosomal STR analysis

| Locus | Mother of SMIP | | SMIP | | Reconstructed profile of alleged father | | Remains of supposed missing child | |
|---|---|---|---|---|---|---|---|---|
| D8S1179 | 10 | 13 | 12 | 13 | 12 | | 13 | 14 |
| D21S11 | 28 | 31 | 29 | 31 | 29 | | 29 | 29 |
| D7S820 | 9 | 12 | 9 | 12 | 9 / 12 | | 9 | 12 |
| CSF1PO | 11 | 12 | 12 | 12 | 12 | | 11 | 11 |
| D3S1358 | 15 | 15 | 14 | 15 | 14 | | 14 | 16 |
| THO1 | 6 | 7 | 7 | 10 | 10 | | 9.3 | 10 |
| D13S317 | 12 | 13 | 8 | 13 | 8 | | 8 | 13 |
| D16S539 | 12 | 14 | 9 | 14 | 9 | | 9 | 14 |
| D2S1338 | 19 | 20 | 19 | 22 | 22 | | 19 | 20 |
| D19S433 | 13 | 14 | 13 | 14 | 13 / 14 | | 13 | 14 |
| vWA | 15 | 18 | 16 | 18 | 16 | | 16 | 17 |
| TPOX | 8 | 11 | 8 | 8 | 8 | | 8 | 8 |
| D18S51 | 13 | 17 | 15 | 17 | 15 | | 15 | 17 |
| D5S818 | 11 | 11 | 11 | 11 | 11 | | 11 | 12 |
| FGA | 19 | 23 | 19 | 23 | 19 / 23 | | 20 | 22 |
| Penta E | 5 | 7 | 7 | 13 | 13 | | 5 | 13 |
| Penta D | 10 | 10 | 10 | 22 | 22 | | 12 | 22 |
| AMEL | X | X | X | X | X | Y | X | X |

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Table 2. Results of X-STR analysis

| Locus | Mother of SMIP | | SMIP | | Reconstructed profile of alleged father | Remains of supposed missing child | |
|---|---|---|---|---|---|---|---|
| DXS7132 | 13 | 15 | 11 | 15 | 11 | 11 | 12 |
| DXS7423 | 15 | 15 | 15 | 15 | 15 | 15 | 16 |
| DXS7133 | 10 | 13 | 9 | 10 | 9 | 9 | 11 |
| GATA172D05 | 10 | 10 | 10 | 11 | 11 | 10 | 11 |
| DXS7130 | 12 | 14.3 | 12 | 15.3 | 15.3 | 11 | 15.3 |
| DXS6800 | 18 | 20 | 16 | 20 | 16 | 16 | 20 |
| GATA31E08 | 10 | 12 | 9 | 12 | 9 | 9 | 10 |
| HPRTB | 13 | 14 | 12 | 13 | 12 | 11 | 12 |
| DXS6789 | 21 | 21 | 21 | 21 | 21 | 20 | 21 |
| DXS9898 | 8.3 | 11 | 8.3 | 12 | 12 | 11 | 12 |
| DXS9895 | 13 | 15 | 14 | 15 | 14 | 14 | 16 |
| DXS10011 | 39 | 39 | 39 | 42 | 42 | 36 | 42 |

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Figure 1. a) Two projectiles removed from the body of unknown identity; b) D1S80 analysis showing the profile of the DNA extracted from blood sample encrusted in the projectiles (lanes 2 and 3); c) the comparison of the DNA samples from the mother of DLF (lane 2), the father of DLF (lane 3) and one of the two projectiles (lane 4).