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DNA vs RNA in Forensic Applications: Regulations-Fallacies

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Abstract

Human DNA is the genetic material (blueprint) found in every cell except erythrocytes. Traces are found in body fluids (saliva, blood, semen, vaginal secretion, bones, teeth, hair, perspiration). It is unique to every individual and DNA typing methodologies are continuously subjected to scientific and legal scrutiny. Most of these typing methods are dedicated to nuclear DNA. It has been used as unique investigative material for forensic purpose since after Sir Alec Jeffrey's who first introduced RFLP in 1985 which has been improved with the discovery of PCR in the mid-1980's. RNA has been also studied as a better alternative to DNA. Regulations to the use of DNA has since been studied involving either its use in clinical research or in crime settings. 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. Finally, fallacies always exist either due to sample size detected or due to multiplicity of subjects involved in the same sample.

Keywords: DNA vs RNA; DNA Applications; RNA Applications; Regulations of DNA use in Clinical Research; Fallacies of DNA

Abbreviations: Body Fluid Identification; CODIS: Combined DNA Index System; DH: Department of Health; GMC: General Medical Council; HRF: Health Related Findings; HT Act: Human Tissue Act; HTA: Human Tissue Authority; LCN: Low Copy Number; MPS: Massive Parallel Sequencing; MRC: Medical Research Counselling; NDNAD: National DNA Database; NHS: National Health Services; PCR: Polymerase Chain Reaction; Pm: Match Probability; PM: Postmortem; PMI: Postmortem Interval; RFLP: Restriction Fragment Length Polymorphisms; SNPs: Single Nucleotide Polymorphism; STR: Short Tandem Repeats.

Introduction

Human DNA is the genetic material (blueprint) found in every cell except erythrocytes. Traces are found in body fluids (saliva, blood, semen, vaginal secretion, bones, teeth, hair, perspiration). It is unique to every individual and DNA typing methodologies are continuously subjected to scientific and legal scrutiny. Most of these typing methods are dedicated to nuclear DNA. It has been used as unique investigation material for forensic purpose since after Sir Alec Jeffrey's who first introduced Restriction Fragment Length Polymorphism (RFLP) in 1985 to identify the unique markers in the genetic material. The method has been improved with the discovery of Polymerase Chain Reaction (PCR) in the mid-1980's, a critical molecular biology technique. 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 [1,2].

DNA vs RNA in Forensic

Both DNA and RNA contain ribose sugar (a carbon ring surrounded by oxygen and hydrogen). While RNA contains a complete ribose sugar, DNA contains a ribose without

one oxygen and one hydrogen atoms. The extra oxygen and hydrogen atoms in RNA leave it prone to hydrolysis, a chemical reaction that breaks the molecule in half. Under normal cellular conditions, RNA undergoes hydrolysis 100X faster than DNA [3]. Due to its stability, DNA is used to identify the subject from the oldest of remains. However, it is important to know not only the source, but also from which body fluid or tissue the DNA profile originated. To date biological samples at a crime scene before sample collection, RNA would be required since it is less stable. RNA is also present in greater abundance within a sample than DNA. Each nucleated cell contains two DNA copies encoding for the gene, whereas 100s-1000s of RNA copies may be present [4]. Expression of specific mRNA varies among cell types; therefore, analysis of these markers can be used to determine the presence of specific biological fluid within a sample [5]. Tissue-specific mRNA detection offers crucial advantages due to:

- High sensitivity due to the possibility of PCR amplification.
- High specificity due to the pattern of gene expression.
- Unique for the functional status of cells and organs.
- Simultaneous DNA isolation without material loss.
- Co-extraction methods that isolate both RNA and DNA from the same stain extract.
- Messenger RNA stability in forensic stains. In this way, analysis of mRNA extracts will yield information regarding stain origin, and that of DNA extracts will reveal the donor's identity [4].

DNA Application in Forensics

Analysis of autosomal Short Tandem Repeats (STRs) profiling

These are short, repeated DNA sequences (2–6 bp) that account for 3% of human genome. Number of repeat units is highly variable among individuals offering a high discriminatory power when analyzed for identification purposes. They are non-coding in nature and are therefore not implicated in gene expression. The first STR markers used in forensic casework were a quadruplex amplification system consisting of four tetranucleotide STRs (TH01, vWA, FES/FPS, and F13A1) which were suitable for PCR amplification due to their simple repeat sequences and their propensity to display regularly spaced alleles differing by four bases [6]. The Federal Bureau of Investigation (FBI) nominated 17 autosomal STR loci to form the core of the Combined DNA Index System (CODIS), a database consisting of profiles contributed by federal, state, and local forensic laboratories. It is bound by stringent privacy protection protocols, in that stored DNA samples and subsequent analyses be used strictly for law enforcement identification purposes. The DNA Analysis Backlog Elimination Act of 2000 reaffirms that markers used for forensic applications were specifically selected because they are not known to be associated with any known physical traits or medical characteristics [7].

Analysis of the Y-chromosome

The male-specific part of the human Y-chromosome is widely used in forensic DNA analysis, particularly in cases where standard autosomal DNA profiling is uninformative. Haplotypes composed of Y-chromosomal STR polymorphisms (Y-STRs) are used to characterize paternal lineages of unknown male-trace donors, especially when males and females have contributed to the same trace (sexual assaults). They can (1) exclude male suspects from a crime, (2) identify paternal lineage of male perpetrators, (3) highlight multiple male contributors to a trace, (4) provide investigative leads for finding unknown male perpetrators, (5) in paternity disputes of male offspring and other types of paternal kinship testing, including historical cases, (6) in special cases of missing persons and disaster victim identification (DVI) involving men [8].

Analysis of mitochondrial DNA (mtDNA)

It is located in the mitochondria, outside the nucleus of a cell. It is therefore a very useful tool in a sample that is either degraded or limited in quantity. Its typical sources include hair, bones, teeth, and body fluids (saliva, blood, semen). It is desirable for forensics due to its (1) high copy number, (2) lack of recombination, (3) matrilineal inheritance, (4) heteroplasmy, (5) expression variability, and (6) mitotic segregation. Typing of mtDNA has become routine in forensic biology since mid-1980s and is a last resort for testing highly degraded biological debris. Its high mutation rate became a promising biomarker to differentiate between monozygotic twins with rare SNPs since monozygotic twins cannot be separated by STR profiling. With technology advancement, it is now possible to characterize minor difference of the mtDNA genomes in routine identification of bones of missing persons, DVI, and twin identification (Sultana and Sultan, 2018).

Analysis of autosomal Single Nucleotide Polymorphisms (SNPs)

These are single, base-pair positions at which different sequence alternatives (alleles) exist in normal individuals in some population(s). A vast amount of data is available on different SNPs in the human genome and one of the biggest tasks when applying SNPs to forensic applications is to select the most appropriate SNPs from the overwhelming numbers that are available. The major attraction is that they can provide results from highly degraded when conventional STR profiling has failed. Prediction of the geographical ancestry where the identification of the population group from which

a crime scene sample has come from can be valuable for investigating agencies: was the person Caucasian, Asian, African, mixed ancestry? Panels consisting of mtDNA, SNPs and Y-SNPs were found useful for this purpose but were limited by only providing information on either the maternal or paternal ancestry [9,10].

Epigenetics

It refers to the heritable alterations in an individual's gene expression and phenotypes that result from factors other than changes in their DNA sequence. These epigenetic processes are the response of both acute and chronic environmental influences that lead to gene inactivation. Within forensics, an individual's epigenetic fingerprint is achieved by DNA profiling (STRs or SNPs). Significant efforts were made to investigate epigenetic variation by measuring DNA methylation differences between CpG sites. Applications include (1) determination of body fluids and tissues, (2) determination of age, and (3) differentiation between monozygotic twins [11-15].

Massive Parallel Sequencing (MPS)

As technology advanced, MPS provided an alternative due to the flexibility and scalability of loci and sample multiplexing. It has been investigated as a tool for DNA profiling, as it allows simultaneous typing of many STR and SNP markers, with high throughput of multiple samples together in one run with reduced analysis time. Its use is very promising; not only a DNA profile with STR markers can be obtained, but also ancestry and phenotype can be determined using SNPs. Besides autosomal DNA, also mtDNA or DNA methylation state can be analyzed. In case samples are low in quantity and quality, significantly more information can be obtained with MPS than with conventional techniques. High cost of machines and kits render MSP techniques difficult to implement in forensic laboratories [16-21,9].

RNA Application in Forensics

Analysis of Messenger RNA (mRNA)

Development in forensic genetics demonstrated that mRNA is useful in forensic identification where it can be used in the (1) identification of body fluids (buccal and nasal mucosa, vaginal and menstrual blood, semen), (2) determination of time since deposition of biological material, (3) determination of age from biological stains, and (4) in PMI estimation by quantification of degraded RNA (RNA degeneration and/or loss of certain RNA transcripts are more susceptible than DNA, in terms of rapidity and temporal correlation after death of the organism) [22-29]. Its use in forensic investigations increased after discovering that it is sufficiently stable in vitro. Moreover, the differential expression provides the point of difference between DNA and RNA: while all cells from one individual have same DNA for STR analysis, they differ in their RNA expression pattern. However, due to their susceptibility to degradation, they are not suitable for all forensically relevant samples, especially those exposed to harsh environments. Moreover, samples are often minute traces, and any test that results in sample consumption poses the risk of not having enough sample remaining after testing to obtain a DNA profile. The total nucleic acid content, however, can be extracted from a sample by means of a co-extraction, producing two fractions: the DNA content and the total RNA component. This allows efficient processing of forensic samples, with body fluid identification (BFID) and DNA profiling occurring in parallel [30-32].

Analysis of MicroRNA (miRNA)

These are short (18-22bp), single-stranded, noncoding RNA molecules. Being smaller in size, miRNAs contain a wealth of information and are significantly more robust and more stable to degradation conditions than their mRNA counterparts. They play critical roles in biological and pathological processes, tissue-specific and provide a signature of disease. In 2009, miRNAs application was suggested due to their potential for BFID. In addition, miRNA profiling can be performed in cold case investigations where only DNA extracts remain from the original evidence submitted. MicroRNA markers are also used for the identification of brain, kidney, liver, lung, skin, heart muscle, and skeletal muscle [33-41].

Regulations of DNA Fingerprinting

The Human Tissue Act 2004 regulates the removal, storage and use of human tissue which is defined as material that has come from a human body and consists of, or includes, human cells. It also created an offence of DNA 'theft' where it is unlawful to have human tissue with the intention of its DNA being analyzed, without the consent of the person from whom the tissue came [42].

Regulations for use in Research Projects

The Human Tissue [42] set out a legal framework for regulating the storage and use of human tissue from the living, and removal, storage and use of tissue from the deceased, for research in connection with disorders, or the functioning of the human body. The Human Tissue Authority (HTA) produced several Codes of Practice and Standards [42].

Consent

Appropriate consent is based on the principle that individuals are to choose freely whether to participate in research and should be given appropriate information to be able to

make this choice. As an additional safeguard, it should be complemented by independent ethical review of the consent process and proposed protocol [43]. Participants should be (1) properly informed, (2) have capacity to decide under no pressure, (3) understand the right to withdraw from the research at any time without giving a reason, and (4) in case of patients, without their future medical care being affected. Information should include (1) the process involved in obtaining samples, (2) any significant associated risks, (3) what the samples will be used for, (4) how the results of the research might affect their interests, (5) intentions for future storage, (6) future use of samples, and (7) possible sharing of samples with others [44,45].

Living Adults with Capacity to Consent

Consent should be obtained from the person concerned in line with the HT Act and any other relevant legislation.

Living Adults without Capacity to Consent

According to the Mental Capacity Act (2005; 2016) the following should be considered:

1. Research shall not be carried out on any incapacitated adult in relation to a decision about participation unless a similar research cannot be carried out on a capable adult in relation to such a decision.
2. The purpose of the research is obtaining knowledge of the cause, diagnosis, treatment, or care of the adult's incapacity.
3. The following conditions should be fulfilled:

- The research produces real and direct benefit to the adult.
- The adult is willing to participate in the research.
- The research was approved by the Ethics Committee.
- The research imposes no or minimal foreseeable risk to the adult.
- The research imposes no or minimal discomfort on the adult.

Living Children

According to the MRC Ethics Guide: Medical Research Involving Children (2007), it is extremely important to consider the following:

1. When a choice of age is possible, older is better, although some research questions are specific to younger children and babies.
2. Pressure attempts should be avoided that might lead the child to volunteer for research or that might lead the parents to volunteer their children with the expectation of direct benefit (therapeutic or financial).
3. No financial benefit should be offered.
4. Research in which children are submitted to more than minimal risk with only slight, uncertain or no benefits to themselves requires serious ethical consideration Consent should be written when possible (and always when legally required). If the person giving consent is unable to write or is giving verbal consent, this should be clearly documented, including when consent was given and for what purposes. Consent should ideally be witnessed, normally by the researcher, signed by the witness and kept for future reference. Members of some ethnic or religious groups might find some types of research, or donation of certain types of human material, unacceptable [45].

Broad and Enduring Consent (Generic Consent)

When obtaining consent for sample use in research, it is important to consider the value of these samples for future research. Therefore, broad and enduring consent (consent which is broad in both scope and time) should be sought whenever possible. This allows (1) efficient sample use, (2) fosters trust with donors, (3) avoids the need to obtain further consent, or (4) use samples without consent [46]. Participants should be informed that samples may be used in future research, and it should be made clear that possible future use could include areas viewed as 'sensitive' (reveal clinically relevant findings, identify participants). They should be notified that any future research will conform to relevant legal, governance and ethical requirements and if the participant has concerns about future use, then the consent should not be used [47,48]. One way of managing broad and enduring consent is to adopt a two-part consent process. The participant is first asked to consent for the planned research, and then to consent for storage and future use of samples in another research [46].

Tiered Consent

The participant is able to consent to some, but not all, future uses of their sample (some categories of the research could be excluded) which is challenging to manage and requires robust systems that need to be maintained for as long as the sample is held in order to avoid the risk that the donor's wishes are not respected [49].

The Human Tissue Act and DNA Testing

It is an offence to have human tissue (hair, nail, gametes), with the intention of its DNA being analyzed without the consent of the individual from whom the tissue came or of those close to them if they have died. Section 45 of the HT Act has a relevance to paternity testing as it covers the non-consensual DNA analysis. It stipulates that "A person commits an offence if he has any bodily material intending that":

• DNA in the material be analyzed without qualifying consent.

• Results of the analysis be used otherwise than for an excepted purpose.

Qualifying Consent

It is only used within Section 45 of the HT Act. It is fundamentally the same as any other consent for research. The only difference lies in who can give it. The requirements differ depending on whether the person is deceased or living, an adult or child. If consent for research has previously been obtained and it is later decided to include DNA analysis in the research, as long as the consent does not rule-out DNA analysis, then the original consent will be a 'qualifying consent'. But if, when seeking consent, DNA analysis was intended to be done in the future, then it should be made clear to donors during the consent process [50]. As stated by the HT Act [51], qualifying consent is not legally required if the results of DNA analyses are to be used for an 'excepted purpose' such as

- Medical diagnosis or treatment of the person whose body made the DNA.
- Samples are from a living person and used for clinical audit, education or training relating to human health, performance assessment, public health monitoring, or quality assurance. • Samples are an 'existing holding' (held prior to the 1st September 2006).
- Samples are from living, non-identifiable, to be used for research with ethical approval.
- Where another legal framework applies (research involving adults who lack capacity to consent in very specific circumstances).
- Samples are from bodies dead for over 100 years.

According to the HT Act [42], the MRC Regulatory Support Centre: Human Tissue Legislation Summaries: Consent, (2004) and the MRC Ethics Guide: Medical research involving adults who cannot consent [52,50], the following can give a qualifying consent:

1. Living adults with and without capacity to consent as well as from living children are dealt with in earlier sections.
2. Deceased adults

▪ Given from the individual themselves, if given while alive and with capacity to consent.

▪ If the individual did not consent nor specifically refuse before death, then it can be given by anyone who stood in a 'qualifying relationship' with the deceased adult immediately before their death.

3. If the deceased appointed a 'nominated representative' then their consent will be valid if that person was in a 'qualifying relationship' with the deceased.
4. Deceased children

▪ Qualifying consent for DNA analysis is valid from a competent child if given whilst alive (see Living children).

▪ If the child did not decide whilst alive or was not considered competent, qualifying consent should come from a person with parental responsibility. If there is no such person, consent can be sought from someone in a 'qualifying relationship'. Those in a 'qualifying relationship' are (1) spouse or partner, (2) parent or child (a child of any age), (3) brother or sister, (4) grandparent or grandchild, (5) niece or nephew, (6) stepfather or stepmother, (7) half-brother or half-sister, or (8) friend of long-standing. The person giving consent should discuss the decision with other family members.

Handling Health-Related Findings (HRFs)

Any research involving human tissue has the potential to reveal significant HRFs (reveal a family genetic condition, relevant not only to the individual themselves, but also to their immediate family, or future persons). The consent process plays an important role in managing participants' expectations about individual feedback. The consent process should include information on the feedback of HRFs, to enable participants to decide about taking part in the research and, if relevant, to allow participants to make a choice about whether 11 they want HRFs to be fed back to them [46,53,54].

Licensing

Relevant material is any sample containing human cells and it requires a license from the HTA to be stored for further research. Therefore, most 'bodily material' need be held under a license, but DNA and RNA do not [52].

Regulations for use in Crime Settings

Police access to DNA records is a problem where it can be seen as a violation of privacy. It is also no more invasive than taking a fingerprint, which police do regularly for individuals arrested but not convicted of a crime [55]. If arrested, a person does not have the right to deny giving a DNA sample. Like fingerprinting, it is mandatory. If not arrested for a crime, then standard state laws regarding DNA testing apply, and police must get the person's permission (written consent). This is applicable if you are an accessory, witness, or a victim of a crime. Additionally, parents must give consent for minor children to have their DNA tested [55]. If the person was not arrested due to lack of probable cause, and if police are investigating whether the person is guilty or not, they cannot ask for a DNA profile and include that evidence in their investigation. Additionally, if the DNA profile appears to implicate the donor in a crime but it is the only piece of evidence, it is not a proof of guilt and still requires other evidence. DNA testing can only be used to conclusively prove innocence or a non-match [55].

Protection of Freedoms Act 2012

It implements that only people convicted of an offence will have their fingerprint records and DNA profiles retained indefinitely. The court ruled that blanket retention of DNA

profiles taken from innocent people posed a disproportionate interference with the right to private life, in violation of Article 8 of the European Convention on Human Rights.

DNA Samples

The Act requires all DNA samples to be destroyed within 6 months of being taken. This allows sufficient time for sample analysis and a DNA profile to be produced for use on the database. Exception occurs only if the sample is required for use as evidence in court, then it may be retained for duration of the proceedings [31].

DNA Profiles

A DNA profile is stored on the National DNA Database (NDNAD) and allows the person to be identified if they leave their DNA at a crime scene. Retention periods differ according to the offence. Where the retention framework requires the deletion of a person's DNA profile, the Act first allows a speculative search of their DNA against that obtained from crime scenes which are stored on the NDNAD. Once the search is completed, the profile is deleted unless there is a match in which case it will be retained for the duration of any investigation and thereafter in accordance with the retention framework [31].

Extensions

Extensions to given retention periods for DNA profiles occur if deemed necessary for prevention or detection of crime.

Forensic Evidence Admissibility and Expert Witnesses

The Frye Standard (Scientific Evidence - Principle of General Acceptance)

Under the Frye standard of evidence law, scientific evidence into a court case must be regarded as technically sound by the majority of experts in the field. The Frye test doesn't just apply to physical evidence, but it also applies to expert testimony, in which professionals in a certain scientific field explain and support the scientific methods behind the submitted evidence. In a courtroom setting, the expert is asked to defend the procedure in question. If his or her credentials are challenged (as in cross-examination), or the explanations are not satisfactory, the court could deem the expert's testimony inadmissible. The Frye standard is applied whenever new or questionable scientific procedures are introduced into evidence such as fingerprinting and DNA evidence [56].

Federal Rules of Evidence, Rule 702

Federal courts included rules on expert testimony; their alternative to the Frye Standard was used more broadly because it did not strictly require general acceptance and was seen to be more flexible. A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if:

- The expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue.
- The testimony is based on sufficient facts or data.
- The testimony is the product of reliable principles and methods.
- The expert has reliably applied the principles and methods to the facts of the case.

The Daubert Standard (Court Acceptance of Expert Testimony)

The Daubert standard is a test used by the court to determine whether an expert witness presenting a scientific opinion should be admitted as evidence and whether the expert uses scientific reasoning appropriate for the case. The objective is to prevent parties from introducing "pseudoscientific" evidence or "junk science" in court. The party introducing the expert evidence has the burden of proof to demonstrate that the expert witness's testimony is considered as "scientific knowledge" and follows a methodology or technique that is generally accepted in the scientific community.

Limitations

The entire process of DNA profiling takes around 1–2 days. Automation greatly reduced analysis time. Similarly, mRNA profiling requires more laboratory steps and kits, which makes it more time consuming, or be completed by untrained individuals. The entire process, from RNA extraction to analysis of results, may take 2–3 days. Nucleic acid extraction involves several tube transfers and with sexual assault samples; a differential extraction method is required to separate spermatozoa and epithelial cells. Loss of 20-90% of DNA in a sample due to wash steps and use of multiple tubes was reported. Quantity of DNA present for profiling directly impacts the quality and completeness of the resulting profile(s), which is of special consideration when the amount of starting material is low. Loss of DNA and RNA at each step of extraction and multiple steps increase the likelihood of contamination. Nucleic acids have been found to degrade, lose conformation, and lose function over time. These processes are accelerated when exposed to various environmental insults such as
(1) prolonged UV light,
(2) excessive heat,
(3) water/moisture, and
(4) chemical/enzymatic damage [57,33,41].

Fallacies

The evidential weight of a match between crime stain profile and suspect is quantified by the match probability (Pm); the chance of two unrelated people sharing a profile. However, situations in which Pm can be increased include (1) partial profile due to degradation, reducing the number of informative loci; (2) both suspect and perpetrator share many alleles by descent (brothers); (3) both suspect and perpetrator originate from the same subpopulation (population structure can cause frequencies of alleles, hence profiles to vary between subpopulations) [58]. Factors that can complicate DNA profile interpretation include mixed samples and the availability of minute DNA which is analyzed by 'low-copy number' (LCN) methods that may lead to allele DROP-OUT that can increase contamination probability. Alternatively, contamination can add 1-2 alleles/DNA profile (allele DROP-IN); therefore, the DNA profile will be either matching or not matching a suspect. Anomalous profiles can also arise from (1) mutations in PCR primer target region that can cause allele drop-out, (2) an STR can be duplicated and segregated in a normal Mendelian fashion (3) a somatic STR mutation occurring early in development can lead to a three-peak profile, (4) discordant results in amelogenin sex test observed in rare individuals such as cases of sex-reversal (XX males and XY females), (5) a true mixed profile can originate from a single individual as in cases of bone marrow transplants ≥5 years previously, in buccal and fingernail samples → mixed profile, in blood → recipient's own profile had been completely replaced by the donor's, in hair → unmixed recipient's profile [59].

According to Olson [59], complexity of a mixture is determined by 3 main factors: 1. How many people contributed DNA to the mixture? More contributors make a mixture more complex, and therefore, more difficult to interpret. 2. How much DNA did each person contribute? Even if a mixture contains plenty of DNA overall, one or several people might have contributed only a tiny amount. The lower those amounts, the more complex the mixture. 3. How degraded is the DNA? DNA degrades over time and with exposure to the elements. This can also increase complexity. Most large labs are capable of obtaining usable DNA from the smallest of samples, like so-called touch DNA (a smeared invisible thumbprint on a window), and of identifying individual DNA profiles in complex mixtures, which include genetic material from multiple contributors. DNA profile is like a stack of transparency films, the analyst must determine how many contributors are involved, and which alleles belong to whom. If the sample is very small or degraded, alleles might drop out in some locations or appear to exist where they do not [6]. In the operation of crime laboratories, subjectivity can still be observed: standards vary, training levels vary, quality varies. DNA transfer which is the migration of cells from person to person, and between people and objects, is inevitable. A study showed that sperm cells from a single stain made their way onto every other item of clothing in the washer. Since we all shed different amounts of cells, the strongest DNA profile on an object does not always correspond to the person who most recently touched it. Given rates of transfer, the mere presence of DNA at a crime scene should not be enough for a prosecutor to obtain a conviction [55,59]. A new technique LCN analysis can derive a full DNA profile from as little as 10 trillionths of a gram of genetic material which not only carries a higher risk of sample contamination and allele dropout but could also implicate someone who never came close to the crime scene [60,55]. The growing potential for mistakes in DNA testing inspired a solution fitting for the digital age: automation, or the "complete removal of humans from doing any subjective decision making. A software was designed that could take some of the guesswork out of DNA profiling and could also process results much faster. The idea was to correctly differentiate individual DNA profiles found at crime scenes [55,59,61-69].

Conclusion

DNA typing of biological traces or micro-traces containing nucleated cells is possible if not destroyed. It is an important tool in solving caseworks in forensic medicine (establishing custody of a child through paternity or maternity tests; identifying victims from disasters or crimes; exonerating innocent people convicted to prison). Recent advances in molecular genetics proposed other biomarkers to be used in forensic such as mRNA, miRNA, and DNA methylation. Analysis of PM RNA degradation is a great potential concerning PMI estimation. It is crucial that researchers work toward developing techniques for use in forensic investigations that produce results in record time, providing higher powers of discrimination, and that are sufficiently robust to gain acceptance by both the scientific community and, of course, the courtroom. The problem with all DNA profiling is that there is not skepticism, and just because further steps are done does not mean mistakes are not still being made.

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