Recent Advances in Forensic DNA Analysis

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Abstract

Forensic DNA (deoxyribonucleic acid) analysis or DNA profiling has played a major role in the criminal justice system. New techniques and technologies for DNA profiling continue to evolve every year. This paper reviews the literature reported during January 2011 through June 2013 in the field of forensic DNA analysis. Recent advances in almost all aspects of DNA analysis – which include sample collection, storage, and pretreatment, DNA extraction, DNA quantitation, quality assurance of DNA testing, and DNA databases are discussed. New developments now enable new kinds of forensically relevant information to be extracted from limited quantities of biological samples, and offer unprecedented potential for high-throughput DNA analysis.

Introduction

DNA is the chemical code that is found in every cell of an individual's body. Although approximately 99.9 percent of human DNA sequences are the same in every person, forensic scientists are only interested in the 0.1 percent of the DNA that is unique to each individual. As a matter of fact, the likelihood of two unrelated individuals having the exact same DNA profile is $\sim 10^{-15}$, or about 1 in 594 trillion individuals [1]. Typically, the following steps are performed during forensic DNA analysis: 1) Sample preparation: crime-scene evidence is collected, stored, and transported to an accredited DNA laboratory; 2) DNA extraction: DNA is isolated from the unknown crime-scene evidence (and/or any bodily fluids from the suspect); 3) DNA amplification: certain regions of DNA are replicated exponentially in order to generate detectable amounts of DNA samples for subsequent analysis; 4) DNA quantitation: DNA fragments of different sizes are separated and detected spectrophotometrically; and 5) DNA profile matching: the profile obtained from the crime-scene evidence is either entered into a DNA database for comparison to locate a possible person of interest, or is compared directly with that from the suspect to determine whether the suspect contributed the DNA at the crime scene.

The increasing public visibility of state-of-the-art forensic methods (e.g., DNA profiling) has been fueled by the growing popularity of several TV shows such as CSI: Crime Scene Investigation, Bones, and Forensic Files etc., and is reflected by a steady increase in enrollment in many forensic science degree programs across the country, since CSI first aired in 2000 [2]. While the general public may regard DNA analysis as a quick and simple process, which can provide infallible evidence for forensic investigations, the reality of forensic science is far less clear and certain than what is portrayed on television [1-3]. Forensic DNA analysis has played an increasingly vital role in the criminal justice system. But as this role expands, many social, legal, and ethical concerns are raised [1]. Among these, how to prevent individual DNA profiles from unauthorized use is, perhaps, of paramount concern. Another important concern is the reliability and robustness of DNA testing itself, as many possible errors exist during the various steps of DNA analysis including evidence collection, sample storage, and DNA extraction etc., which can lead to accusation of the wrong persons.

As DNA analysis becomes more the norm for forensic procedures, other previous techniques have begun to be re-evaluated for effectiveness. In the past 2.5 years (January 2011- June 2013), new technologies for every step of forensic DNA analysis continued to emerge. In this review, recent developments with particular emphasis on new techniques for manipulating and analyzing DNA are summarized.

Collection and Quantification of DNA Samples

Sample preparation

Forensic scientists continue to evaluate the effectiveness of sample collection methods, the integrity of DNA samples, and storage of samples to ensure accurate and reliable collecting of DNA for further analysis. Before the sample collection process is started, it is valuable to know if even the DNA is intact. One approach for determining if the biological origin of an unknown sample could be blood, saliva, semen, vaginal fluid, feces or urine is the use of mass spectrometry. Trypsin can be used to digest the sample to obtain the peptides that are present and then the peptides be injected into a mass spectrometer. Biomarkers can be used to identify what type of sample is being analyzed. This technique doesn't have the destructive nature of other biochemical tests, such as benzidine, and is not specific to one sample type. In addition the mass spectrometer test can be added to the chelex step of DNA extraction, making it easily integrated into an already existing procedure [4]. Another possibility for determining the biological origin of samples is the use of Fourier transform infrared (FTIR) spectroscopy. In particular, FTIR can be used as indirect screening for DNA integrity when dealing with bone samples [5]. Since the DNA in bone can be damaged due to excessive heat, FTIR spectroscopyis able to evaluate the bonds of collagen, which correlate with similar hydrogen and covalent bonds in DNA. In addition, this technique is inexpensive, facile, and requires little preparation of sample, making it an ideal screening tool for viable DNA [5]. Extracellular or cell free DNA has been found to

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exist in many biological media such as blood, saliva, semen and urine., which can easily be found in the supernatant of DNA samples during the extraction process, especially if the procedure involves the use of Chelex. Saving this DNA resource could increase overall DNA yield and be a source for additional alleles [6].

Typical sample collection involves the use of touch DNA and cotton swabs. Touch DNA incorporates the use of skin cells that an individual has left behind on objects he or she has touched. These cells can then be evaluated using DNA analysis techniques. Touch DNA has been shown to provide more forensic evidence than fingerprinting but this does not necessarily mean that more individualized identifications are made [7]. Collecting sample cells can also be done with a cotton swab. One approach is to use sterile water which is used to moisten the tip of the swab which is then wiped across the specimen area, followed by a dry sterile swab that collects the water and cells. Other solutions have also been tested in place of sterile water, including detergents such as Triton X-100 and sodium dodecyl sulfate (SDS). The use of a detergent has been shown to increase DNA yields from fingerprints in comparison to using sterile water on cotton swab for cell retrieval [8]. The amphiphilic characteristics of detergents allow for cells to be more readily attracted to the detergent. A 1-2% solution of SDS is the recommended concentration due to higher concentrations precipitating. In certain situations, such as extracting DNA from a fire arm, it was found appropriate to use multiple swabs and to combine these swabs to increase DNA yield to get interpretable results. However, combining swabs also increases the chance of contamination [9]. In the extraction process of sexual assault evidence, cotton tipped swabs are found to have a slightly better yield of DNA than polyester swabs, which is most likely due to spermatozoa being retained better on cotton [10]. In addition, testing of methods to collect cells should be done with not only samples seeded with semen but also with more accurate and real samples. For example, due to the intricate processes of the human body, methods only tested with "seeded samples" will often perform poorly when used in real post-coital samples [11].

Aside from collecting DNA with swabs, many other inanimate items from the crime scene can contain DNA, such as adhesive tape or even sticky seals of envelopes that have been licked. In particular, the stronger the glue of the adhesive tape, the more likely epidermal cells will stick to the tape providing a source of DNA. However, adhesive tape used for gagging and immobilization tends to have mostly victim's DNA and is a poor resource for perpetrator DNA [12]. DNA that can also be found under fingernails can be complicated due to the influence of the donor DNA present. In most situations, the DNA under the fingernails is analyzed when there is a close physical contact or struggle between the donor and the assailant. Foreign DNA in the form of blood, saliva, and semen under the fingernails is more likely to establish a DNA profile then skin cells alone. Since there are so many factors that can influence the amount of DNA recovered from under a fingernail, including the activity of the victim, length of contact, presence of body fluid, amount of DNA recovered and the influence of donor DNA, analysis of DNA under fingernails can be complicated but can provide a viable DNA profile [13].
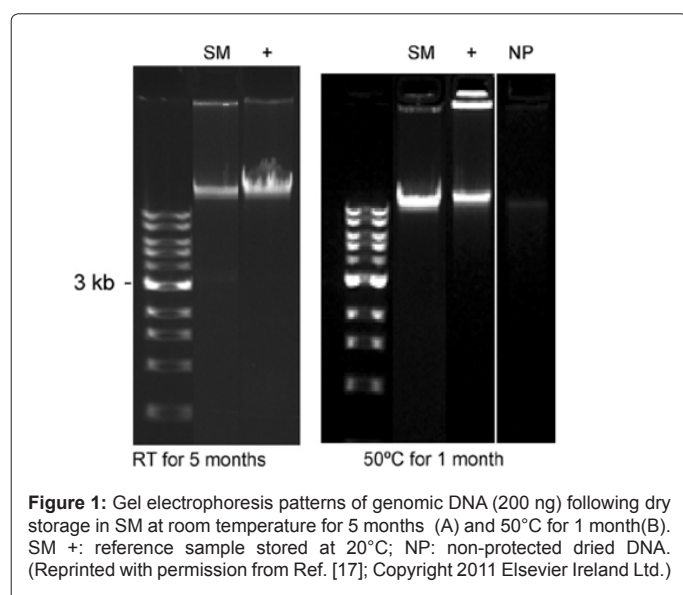
Not all DNA sources are clearly visible to the naked eye in natural light. Alternative techniques are used to help identify and collect possible sources of DNA. Ninhydrin can be used but has the disadvantage of working best with material that can provide a contrast to the dye. 5-Methylthioninhydrin (5-MTN) paired with zinc chloride ($ZnCl_2$) improved visibility of evidence that could not easily be seen. In addition, 5-MTN has not been shown to affect or influence further

DNA analysis [14]. In particular, blood can often be diluted enough that it is not readily seen. Presumptive tests are able to detect the presence of blood even if it cannot be seen with the naked eye. The drawback of presumptive tests is that they can also cause damage to the DNA and prevent further analysis. Common presumptive tests include commercial luminol formations (such as Lumiscene, Bluestar, and benzidine [15,16]. Two of the most common formulations of luminol include the sodium carbonate and sodium perborate combination and the other is the sodium hydroxide and hydrogen peroxide combination. Bluestar and Lumiscene are common commercial luminol products which have the advantage of being easier to prepare. Luminol is often used for blood detection because it does not have a positive reaction with other substances such as urine, semen, saliva and perspiration. Reduced luminescence has been observed in cases where the sample was more concentrated, possibly due to the quenching effect that the iron in blood has with chemiluminescent oxidation of luminol. Porous substrates that can absorb blood decrease the sensitivity to luminol. Overall, luminol and its commercial equivalents are all encouraged to be used within 24 hours of mixing (or else the formulations showed a reduction in performance after 24 h, especially on diluted stains) and have established the best results within four hours of mixing [15]. In addition, studies have shown that the use of the reagent benzidine can prevent the human antiglobulin inhibition test and greatly degrade DNA within 48 hours. However, there is a contradiction of findings of whether luminescent presumptive tests degrade the amount of DNA within a few months. As the degradation of DNA by luminol and Bluestar doesn't necessarily mean that a complete genetic profile cannot be acquired and depends on how low the level of DNA is in the sample [16].

Once the samples are collected, the storage process must also be taken seriously due to the possibility of future DNA tests. Storage of DNA samples is important especially when the sample cannot be immediately analyzed or needs to be reanalyzed at a later time. One approach for DNA storage is based on the idea that nucleic acids are stable when they are dried. DNA can be dehydrated via spray drying, spray freeze drying, air drying or lyophilization. Additives, such as trehalose (a disaccharide), can also be added to a dry DNA sample. Sample matrix, another additive that acts similarly to trehalose, is a room temperature storage medium that allows DNA to be stored dry. Compared to DNA stored in frozen liquid, the dry medium has a higher DNA recovery in long term storage testing. In addition, samples do not need to be purified again after rehydration [17]. As shown in Figure 1, no substantial differences exist between the quality of samples stored frozen in liquid and those samples maintained dry at ambient temperatures protected in a room temperature DNA storage medium, SampleMatrixTM (SM; Biomatrica, Inc., San Diego, CA) [17].In summary, ways of sample collection, determination ofutilizable DNA for collection, and proper storage of crime-scene samples continue to be reevaluated, improved techniques are being established to ensure the accuracy and reliability of the DNA samples for downstream analysis.

DNA extraction methods

DNA extraction methods have become increasingly more effective with regard to being able to obtain purified DNA from samples of biological origin. These extraction methods include organic extraction, ion-exchange, solid-phase extraction, and laser capture micro dissection (LCM) etc. New generations of these types of extractions have evolved over time and some new versions are continuing to be explored. Currently, one of the most often used methods of DNA extraction is organic extraction. This method utilizes SDS and proteinase K to

Figure 1: Gel electrophoresis patterns of genomic DNA (200 ng) following dry storage in SM at room temperature for 5 months (A) and 50°C for 1 month(B). SM +: reference sample stored at 20°C; NP: non-protected dried DNA. (Reprinted with permission from Ref. [17]; Copyright 2011 Elsevier Ireland Ltd.)

breakdown the cell membrane and proteolytic digestion, whereby the addition of proteinase K rapidly inactivates nucleases (e.g., DNases and RNases) that might otherwise degrade DNA during extraction. After lysing, the DNA is purified by mixing it with phenol-chloroform solution, centrifuged, and then the DNA is precipitated using ethanol and then resuspended in a low-salt buffer [18]. The phenol-chloroform method is considered to be most effective when extracting high molecular weight DNA [19]. Alternatives to the precipitation step have been also employed, switching to a filtration technique that could involve Centricon, Microcon, and Amicon filter devices that allow for increased DNA recovery and purification. However, the drawback is that these filtration devices also require multiple transfers, more time, and are best suited for double-stranded high molecular weight DNA [20]. In addition, the elevated purity that could be gained through the use of centrifuge filters could also be at the loss of overall DNA quantity [21]. A modified version of the organic method can be used when trying to selectively separate female and male DNA in sexual assault cases. In the modified version, a reducing agent, dithiothreitol (DTT), is used to lyse sperm cells. In this procedure, after the first lysis and centrifugation, the washed pellet of sperm cells is lysed by SDS, proteinase K, and DTT. The sperm DNA is then found in the supernatant of the second lysis [20].

Another commonly used method of DNA extraction is the use of chelating resins that are based on an ion-exchange approach. Most often the samples are added to a 5% solution of Chelex and then boiled for several minutes. The resins are able to bind to Ca^{2+} and Mg^{2+}, deactivating unwanted nucleases, and therefore preventing the cleavage of DNA. Non-polar nuclear DNA and RNA become denatured and stay in solution while polar components bind to the polar resin. The sample is then centrifuged with DNA present in the supernatant. The process of boiling the sample denatures the DNA and one is left with single stranded DNA, which results in having to use a PCR-based method to analyzed DNA. In addition, the purity of the DNA is not as good as compared to the traditional organic extraction or solid-phase method [20]. Another drawback to the manual Chelex method is an increased number of PCR inhibitory components that are also extracted with the DNA. The PCR inhibitors cause problems in the subsequent quantification and short tandem repeat (STR) reactions used in DNA profiling [18,19].

An extraction process that continues to gain popularity in DNA extraction is the use of solid-phase extractions, which employs silica in the presence of chaotropic salts. The salts include thiocyanate, sodium iodide, and guanidinium hydrochloride. Often the cells are lysed with proteinase K first, and then presented to chaotropic salt buffer to allow for the binding of DNA to silica. Once DNA is bound to silica, impurities such as proteins and other contaminants can be washed away. DNA can then thereafter be eluted [20]. Silica can be presented in a column fashion or as paramagnetic beads. The use of silica column does require a centrifugation step. Silica magnetic beads allows for a facile purification procedure that can result in high throughput extraction using robotic platforms. In addition, the magnetic beads can be used with many different sample types, including blood, saliva, and sperm, with little cross contamination. However, highly degraded DNA may have difficulty binding to a silica surface in silica column extractions more so than in silica magnetic bead extraction [20,22,23]. Automated DNA extraction methods that use magnetic beads have been demonstrated to have lower levels of PCR inhibitors co-extracted with DNA in comparison to a manual organic method [18]. In addition, the yield of DNA was found to be more consistent with samples extracted with automated magnetic beads in comparison to the manual organic extraction method [24]. Solid-phase extraction techniques have also been integrated into microfluidic systems. The reversible binding sites and increased surface areas of the silica monolith in the microfluidic device increased the recovery of DNA and did not require the use of additional carrier molecules such as poly-A carrier RNA. The microfluidic system was also shown to deliver results with samples that had less than 15 ng of DNA [25].

Another method often used in mixed samples is LCM. This method allows for cells to be selected and collected by cell type, therefore resulting in less cellular material needed. This method tends to work better with mixed samples where there is a minor contributor and a major contributor, such as when there is a large number of female epithelial cells in comparison to sperm cells in the sample. It also decreases the chances of mixed DNA profile results and the interference of PCR inhibitors [20,26,27]. LCM methods are separated into ultraviolet (UV) cutting and infrared (IR) capture systems. The UV system can capture cells by photovolatilization. The IR capture system allows visualization of cells via microscope and then cells are isolated by laser energy to a thermolabile polymer. The isolated cells, in both methods, are placed in a vial for DNA extraction [20]. Variations of the LCM approach include the use of an alkaline differential extraction method. The alkaline method allows for rapid purified sperm fraction lysates with minimal steps to prevent the possibility of contamination and faster results. The procedure includes heating the substrate in a mild alkaline solution of 0.1 N NaOH, neutralizing it, and then using enzymes to remove residual non-sperm DNA. The sperm fraction lysates are heated in a 1 N NaOH and then concentrated and purified with the use of silica columns. Results with the alkaline differential extraction method could yield up to 96 sexual assault samples within a four hour period and a robotic system was not necessary [10]. Another variation of the LCM method includes an extraction based on the organic extraction method, using phenol-chloroform. However, the addition of proteinase K to the organic procedure hasbeen shown to have better results [27].

Bone and teeth undergo different extraction procedures. All bone and teeth DNA extractions involve two main steps, pulverization in liquid nitrogen and incubation with ethylenediaminetetraacetic acid (EDTA). The hydroxyapatite matrix is demineralized by the EDTA, allowing the osteocytes or odontocytes to be susceptible tolysis. Once

the cells are lysed, DNA can be extracted using organic extraction [20]. This organic method using phenol-chloroform and the silica solid-phase extraction have been compared to each other with results indicating that the phenol-chloroform solution was able to capture more complete DNA profiles than silica based extraction [28]. Unfortunately, the DNA preserved in bones can vary greatly from one cadaver to the next due to environmental conditions, animals, insects, and microbes, leaving the remaining DNA limited in quantityor degraded [29]. However, the teeth and jaws are very resistant to extreme heat and become valuable identification tools in situations where the body has been exposed to fire [30]. The cells inside molar and pre-molar teeth with high molecular weight DNA can remain intact for long lengths of time even if a body is in a high decomposition state, making teeth a valuable DNA source [31].

Additional DNA extraction methods continue to be developed, addressing the issues of time, storage, and getting DNA from insoluble samples. Fast Technology for Analysis of nucleic acids (FTA) can be used in DNA extraction, especially when the sample is blood or saliva. FTA can reduce extraction time, provide a way to store samples at ambient temperature and can be incorporated into automated systems. A cellulose-based matrix is treated with a chelating agent, weak base, a detergent or anionic surfactant and a urate salt or uric acid. Cells are lysed by the chemicals on the cards and at the same time, the DNA is immobilized [20]. Processing of tissue samples can often become time-consuming during the extraction process due to the need to create a soluble sample to work with for PCR. An alternative to this lengthy process is the use of heated stainless steel wires that could be used to stab tissue and blood samples to get DNA. Wires can be cut and shaped to get the needed amount of DNA, samples can be easily stored and directly loaded into the PCR [32].

Quality assurance and validation

Protocols must be taken into consideration and adhered to when dealing with DNA samples. Many factors that can easily cause contamination of samples can include inappropriate handling of the sample, instruments that are not sterile and faulty storage containers. The more steps and individuals involved in the DNA collection, extraction and amplification process, the higher probability of contamination [33]. In fact there is a protocol called the "Guidelines for forensic science laboratories" that was issued by the International Laboratory Accreditation Cooperation (ILAC). Overall the steps involved in a forensic DNA laboratory can be separated into the pre-laboratory, laboratory, and post laboratory. The pre-laboratory involves case assessment. The laboratory includes inspections, DNA extraction, DNA quantification, DNA amplification, electrophoresis and typing. Then the interpretation of results, data basing, and statement reporting are considered the post laboratory step. These steps are executed in different areas to avoid contamination and make a smooth transition from one step to another [34]. Even when technical analysis of DNA is done with the upmost accuracy, situations that involve mixed samples of DNA bring the possibility of being skewed due to the examiners interpretation of results [35].

Polymerase chain reaction (PCR) amplification

In forensics, repetitive DNA regions, which are located outside the coding regions of DNA, are used to further analyze DNA. These regions are different for each individual and can be used for identification of one person as well as a group of people, such as a group of family members [36]. Developed by Kary Mullis in 1983, PCR continues to be a valuable tool in forensic DNA analysis. PCR is able to replicate specific nucleotide sequences from low levels of DNA or degraded DNA. The primers in PCR are specific to human DNA and results are not affected by bacterial DNA if it is present. The DNA sequence is amplified after it is denatured and the single strands are separated. Amplification involves the addition of DNA primers, nucleotides, and DNA polymerases, which are then taken through a series of temperature changes. Products of amplification, or amplicons, are then separated using electrophoresis. The amplification process continues to be used in more advanced techniques, including being able to amplify just a sequence-specific region or a whole genome [37]. The detection of DNA is often further evaluated through the use of fluorescence, which uses fluorescent dyes that attach to PCR primers in the amplicons. Reagents used in the PCR process often consist of dimethyl sulfoxide (DMSO), glycerol, formamide, single stranded DNA binding proteins and betaine [38].

Quantification of DNA can be greatly affected by PCR inhibitors, which can easily be obtained with a sample during DNA extraction. PCR inhibitors can then prevent the amplification process. Common inhibitors found in forensic samples include hematin, indigo, melanin, collagen, tannic acid, humic acid, and calcium phosphate [39,40]. Inhibitors can not only interfere with PCR amplification, giving false negatives, but also cause problems with STR amplification. STR results become unstable and have off-scale or split peaks due to increased inhibitors. In addition, as the concentration of inhibitors increase, DNA quantification values become underestimated [39].

Forensic scientists have been using the slot-blot hybridization approach to target the D17Z1 locus, a highly repetitive alphoid primate-specific sequence, for DNA quantification in forensic casework. However, this methodology is tedious, time-consuming, and does not have the necessary sensitivity especially for low-copy number forensic DNA samples. The development of real-time PCR human DNA quantification kits has contributed to the popular use of real-time PCR in forensic genetics. Real-Time PCR, also known as quantitative PCR or qPCR was developed. One of the positive features of a qPCR quantification assay is that it is able to establish the presence and quantity of male DNA in a mixed sample, usually from sexual assault cases. Knowing the quality and quantity of male DNA can determine which STR amplification kit to use in further analysis of the DNA, saving time and decreasing costs [41]. Variations of qPCR continue to be developed due to the need to know the quantity of DNA, which can determine the use of what type of commercial STR profiling kit to use, such as a mini STR. A DNA quantification assay that uses *Alu*, a highly repetitive DNA sequence, has been able to detect down to 4pg during DNA quantification. Specifically, the degradation detection assay uses a common forward primer and two reverse primers to generate two *Alu* amplicons (63 and 246 bp). Two different fluorophores (Quasar 670 and FAM) are placed on 63 bp and 246 bp reverse primers, respectively. The intensity in fluorescence for each fluorophoreis then monitored during the real-time PCR. The premise of this assay is that amplification of the long *Alu* amplicon is possible only in non degraded or slightly degraded DNA whereas the short amplicon can be amplified even in highly degraded DNA. The ratio of the concentrations of the two PCR products offers a good estimate of degradation state. The concentration of DNA measured by the long amplicon can be used to calculate input DNA for STR analysis and the ratio predicts the amplification of high molecular weight alleles observed in STR analysis [40]. This *Alu* sequence has also been used to label quantum dots that resulted in a detection limit of 2.5 fg of DNA. Overall the use of an Alu sequence can provide a more sensitive and human-specific quantification tool for DNA analysis [42].

Additional variations of the PCR method have been developed to be more precise, able to work with trace amounts of DNA and able to amplify and quantify more than one specific DNA target. In particular, digital PCR (dPCR) offers a higher degree of precision. In combination with duplex reactions, where two targets are analysed per reaction, dPCR can provide a more precise measurement. Particularly in cell free DNA analysis, duplexing dPCR can reduce the number of individual PCR reactions [37]. Other quantitative methods that are able to detect picogram levels of DNA include the hybridization method and the threshold method. The hybridization method uses either radioactive isotopes or chemiluminescent compounds, whereas the threshold method is mediated by antibodies. These above-mentioned quantitative methods can also be labor-intensive and time-consuming for analysis [20]. One of the limitations of PCR is that it cannot amplify and quantify more than one specific target DNA. It also cannot amplify the whole DNA content, which means that whole DNA is only estimated based on quantity of a specific DNA amplified. To overcome this limitation, a Real-Time degenerate oligonucleotide primed PCR (DOP-PCR) was designed. DOP-PCR can amplify the whole genome regardless of DNA size. It is also independent of DNA sequence and can be used for many different species, giving it a universal property. The primers of DOP-PCR are placed at the 3' end, randomly in the middle, and at the 5' end. This method has been successfully demonstrated in the determination of the human placental DNA ranging from 80fg to 8ng [43].

Advanced and Emerging Techniques and Methodologies of DNA Analysis

Since the advent of forensic DNA analysis in the 1980s, it has gone through several stages of development [1]. The first generation of DNA analysis -restriction fragment length polymorphism (RFLP) profiling is no longer used by the forensic community, as it requires relatively large amounts of DNA and degraded samples could not be analyzed with accuracy. The second generation of DNA analysis was based on PCR and mainly involved dot-blot techniques. However, it is not suitable in the analysis of longer strands of DNA. The third generation of DNA analysis or the current method of choice is short tandem repeat or STR analysis. Despite its undoubted advantages (which are elaborated below), it does not work well for highly degraded DNA samples, such as in cases of mass disaster situations or accidents where an individual is too badly damaged to identify. More effective, faster and cheaper DNA analysis techniques are continually being developed, which are addressing different targets for forensic applications (Table 1). This section highlights some of the recent progresses made in the analysis of STPs, SNPs, low-template DNA, mitochondrial DNA, and DNA methylation, and illustrates how microfluidic devices and nanotechnology can be integrated to develop a new generation of DNA analysis.

Short tandem repeat (STR) analysis

The PCR technique can be used to amplify STR typing with highly polymorphic DNA sequences of repeating 2-7 base pairs. These STR loci are considered polymorphic due to being unique to each individual. In particular, 5-10 alleles of particular STRs are often the focus of forensic profiling [19,44,45]. The amplification of STR, via PCR, starts with targeting loci by sequence-specific primers. Electrophoresis is used to separate the DNA fragments. The STR markers used in human identification need to exhibit the highest variability among individuals and are measured by the lengths of the different alleles [36]. STRs are generally classified by the length of their repeat: mono-, di-, tri-, tetra-, penta- and hexa- nucleotides. Tetranucleotides are the most often used

Target of DNA Analysis	Definition	Major Limitations of Analysis
Short Tandem Repeats	Short sequences of DNA that contains short segments consisting of 2-7repeating base pairs	• Unsuitable for the analysis of highly degraded or low copy number DNA samples • Inconsistency of number of STR markers in different DNA databases
Single Nucleotide Polymorphism	DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.	• Less informative per locus than STR • Unsuitable for DNA mixtures
Low Copy Number or Low-Template DNA	Less than 200 picogram of DNA found in a sample	• Ease of contamination and amplification of contaminants • Mixed profiles being produced and wrongful accusations
Mitochondrial DNA	A circular molecule of DNA 16,569 base pairs in size, obtained from the mitochondrion organelle found within cells	• Less discriminatory (maternal inheritance) • Relatively time consuming and expensive
DNA Methylation	Addition of a methyl group to the cytosine or adenine DNA nucleotides	• Large amount of samples needed • Labor-intensive and time-consuming

Table 1: Definition of DNA analysis targets and major limitations.

in STR analysis due to the fact that they have a smaller probability of stutter products, amplicons that are one repeat less than the true allele. PCR of STRs also allows for multiplexing, which enables the analysis of several different loci at the same time [45]. STR loci can even be obtained from maggots removed from a corpse [46]. STR detection at this time involves the use of fluorescence with a gel or capillary electrophoresis (CE) and ABI gel-based DNA sequences. STRs are most informative with samples that involve well-preserved soft tissue and bone [19].

ABO blood group recognition is also an important aspect of forensics in human identification. The differences in nucleotide sequences of ABO alleles have become a powerful basis for ABO genotyping. A multiple system has been developed to simultaneously obtain ABO and STR genotypes in a rapid and cost-effective procedure. It involves a single reaction and needs only cells containing a nucleus that can be obtained from hair, cartilage, blood, semen, bones, etc. The ABO amplicons can be used with already existing STR kits. More specifically PCR with sequence-specific primers (PCR-SSP) has been paired with 15 STR genotypes to reduce time and costs in identifying DNA in forensic investigations [47].

The size of DNA can also cause problems with identifying STR loci, which has led to a different version of STR amplification. Higher molecular weight STR loci are difficult to amplify and result in a DNA profile that is incomplete. Moving the PCR primers closer to the STR region has been able to overcome some of these difficulties. These reduced-size STR amplicons are sometimes referred to as 'miniSTR' assays and are able to obtain information from degraded DNA [19,36]. In fact conventional STR kits have been found to be somewhat ineffective for collecting genetic information from degraded ancient DNA (aDNA), whereas the miniSTR assays are able to determine STR loci from degraded aDNA [33]. However, even with the increase

in sensitivity, miniSTR typing cannot overcome all levels of DNA degradation [48]. Another situation that poses a problem to STR typing is when samples have DNA from various individuals, especially if the different DNAs are at different amounts [49].

Single nucleotide polymorphism (SNP) analysis

The great variability of DNA polymorphisms has made it possible to offer strong evidence for concluding that DNA from a suspect and from the crime scene are indeed from the same person. However, one of the dilemmas that occur with low level DNA or degraded DNA is the inability to create complete STR typing. The result is low template (LT) or degraded DNA amplification that includes allele drop-outs and allele drop-ins [48-50]. Allele drop-outs result in lack of an allele and can be overcome by increasing the sensitivity of the assay via increased PCR cycles, adding more PCR products, and post purification of PCR products. However, increasing sensitivity also increases likelihood of allele drop-ins, which is an allele that was not part of the original DNA. Alternatives to increased PCR sensitivity of STRs are to use SNPs and insertion/deletions (indels). SNPs used in LT DNA can result in fewer allele drop-ins [51].

SNPs offer an advantage over STRs due the fact that heavily degraded DNA fragments can be analyzed with SNPs. The SNPs are base substitutions, insertions or deletions and occur only at one position of a genome. It is the biallelic nature of SNPs that can help in DNA profiling but also make them not as informative per locus as STR and difficult in identification when working with DNA mixtures. Single SNPs have less information than STRs, but this can be overcome by increasing the number of SNP markers analyzed. The varied heterozygosity level of the genome is one of the most valuable characteristics of SNPs. Another positive feature of SNPs is that size-based separation is not needed, making multiplexing and automation more accessible than STR analysis. In addition, SNPs have a low mutation rate making them more stable as genetic markers [19,36,52,53]. Determining the likelihood for a genotype data, algorithms are used that can evaluate genetic linkage and linkage disequilibrium (LD) [52]. To develop a way for an individual identification, a universal panel of 92 SNPs was created. Of the 86 SNPs that have no significant pairwise linkage disequilibrium, 45 can be used to determine individual identification [54]. ABO genotyping used with SNP can be used to determine the ABH antigen expression that can be found on the surface of red blood cells. ABO genotyping has been used as a rapid screening tool before STR profiling. Using a fast PCR instrument, polymerase, a multiplex allele-specific primer set based on compatibility with alleles of SNPs, ABO genotyping can also be used for rapid direct genotyping of SNPs [55].

The amelogenin gene has been most widely studied in humans, where it is a single copy gene, located on the X and Y chromosomes at Xp22.1-Xp22.3 and Yp 11.2. Variations of the SNP detection have included the use of four ABO loci and an amelogenin gender marker, so that individual identification and paternity testing can be done simultaneously [54]. In addition, SNPs that are more likely to be in the nucleosome forming regions have also been considered preferred due to their ability to resist   common degradation processes [53]. DNA chips have also become a tool used for analyzing SNPs. Combining the use of DNA chip technology and ABO genotyping can allow for the simultaneous analysis of multiple DNA samples, detecting the major ABO allele-specific SNPs. However, it can also be limited by sensitivity and the presence of inhibitors [56].

Analysis of degraded or low-template (LT) DNA

LT or degraded DNA has been successfully amplified for STR genetic profiling using whole genome amplification (WGA). In particular, WGA can be used to amplify highly degraded or LT DNA so that it can be further analyzed in the PCR method. Among the variations of WGA, there are degenerate oligonucleotide-primed PCR (DOP-PCR), primer extension preamplification (PEP), multiple displacement amplification (MDA), blunt-end ligation-mediated WGA (BL-WGA), rolling circle amplification (RCA), and restriction and circularization-aided rolling circle amplification (RCA-RCA). PEP is found to work well with highly-degraded DNA or low copy number DNA [57].

Some of the problems that occur during WGA are allele drop-outs, allele drop-ins, increased stutter, and heterozygous peak imbalance. However the use of rolling circle amplification (RCA) and multiple displacement amplification (MDA) can improve STR genotyping results. These two types of WGA use random primers, a DNA polymerase and 3'-5' exonuclease. The specialty of the random primers is that they can recognize any genomic sequence, even if the DNA is badly degraded. They also use a DNA polymerase that has strong strand displacement activity and the exonuclease that possesses proofreading activity. In particular, RCA amplifies circular DNA. With the use of ssDNA ligase the circularization of individual ss and dsDNA can be created for RCA. It has been shown that the addition of single-stranded DNA binding protein (SSB) during WGA increased the yield of DNA [58].

In situations where DNA samples are a mixture of sources or LT DNA, statistics begins to play an important role. Likelihood ratios (LR) are used to help to determine the identification of DNA profiles [19,50]. Interpreting the results of LT DNA becomes difficult due to increased number of allelic drop-outs, or alleles from true contributors not present. These allelic drop-outs are represented by low peak heights in profiles and can cause ambiguity in DNA interpretations. These low peaks are evaluated by minimum distinguishable signal (MDS), which attempts to differentiate their reliability from noise. This MDS is also considered a stochastic, or analytical, threshold, commonly referred to as relative fluorescence units (RFU). Unlike with substantial amounts of DNA where baseline noise is increased, the use of negatives should be used in determining RFUs for LT DNA and these RFUs should be validated for each allele post-PCR [59]. However, a pair of heterozygous alleles from original contributor can fall below the detection threshold. In the presence of allelic drop-outs, probabilistic reasoning becomes more prominent and the results are often approached with LRs to help interpret the data, especially with mixed sample DNA and LT DNA [60,61]. Probabilistic reasoning can also include calculating the random man not excluded (RMNE) probability or its cumulative probability of inclusion (CPI) and a random match probability (RMP). In particular, RMNE works well in situations where there are two prominent contributors to a sample and a third trace contributor. Both RMP and LR are preferred in situations where there are allelic dropouts but no non-concordant alleles, or an allele present in a person of interest but not in the electropherogram. Unlike RMP, LR can also be used when non-concordant alleles are present [62]. The LR and RMP are often helpful in binary methods and use similar data measurements to draw conclusions. CPI can often overlook information and be less informative [63].

An LR approach can be binary, semi-continuous, or fully continuous. Binary method includes alleles that are present or absent while the semi-continuous method includes a probability of dropout

or non-dropout and present or absent alleles. The probability of drop-outs based on peak heights defines the fully continuous method. Modifications of binary, semi-continuous, and fully continuous methods have been made to increase reliability and accuracy with mixed and LT DNA samples [62,64]. For example, the binary method has been shown to have better results with multiple data samples examined simultaneously then individual analysis of samples in isolation [60]. Joint likelihood ratios have also been explored as statistical analytical approaches. As shown in Figure 2, it is evident that by computing with a joint likelihood function, in combination of DNA evidence, much more identification information can be inferred [65].

Further analysis of LT DNA can be done through replication of the amplification. Several methods have been explored to interpret the replicated DNA profiles which include choosing the profile that is the most informative, a consensus approach, a composite approach, the Bayesian approach, and a continuous model. Choosing the most informative profile can come under scrutiny for being biased. The consensus profile involves an allele that appears in at least two of the replications. Composite profiles are considered the least conservative but do include all alleles in replication. As long as the samples are simple, the consensus profile method is comparable to a Bayesian approach in acceptability [66]. Variations and additions of these afore-mentioned methods include the N/2 consensus and the pool method. N/2 consensus method and a pool method have attempted to interpret complex LT DNA mixtures due to their ability to detect more alleles and rarely have drop-ins. The N/2 method is similar to the above mentioned consensus method and the pool method involves the injection of PCR products onto a CE machine [49].

Mitochondrial DNA (mtDNA) analysis

Mt DNA is found in the mitochondria, which are tiny organelles in the cell, not associated with the nuclear chromosomes. Aside from the advances for LT DNA that SNPs and WGA have made, alternative sources and methods continue to be looked into including mtDNA and singe cell analysis. MtDNA remains as a viable source because of its quantity. MtDNA is often used in LT DNA due to the higher proportional amount of mtDNA to nuclear DNA and its ability to be

Figure 2: (A) A handgun swabbed in four locations (marked as 3, 4, 5 and 6 in circles), each amplified twice (stars). (B) Likelihood function, showing mixture data and genotype model. (C) DNA mixture weights for the four swabs. (D) DNA log (LR) match information, increasing as more data are combined together. (Reprinted with permission from Ref. [65]; Copyright 2011 Elsevier Ireland Ltd.)

less prone to degradation. The hypervariable (HV) regions of mtDNA are used for analysis due to their polymorphic characteristics and are valuable sources for analysis of degraded DNA including bone samples. More specifically, the hypervariable regions I and II (HVI and HVII) are found to have the most variations [19,36,67-70]. qPCR assay offers a high specificity that can be used in the analysis of mtDNA. This assay can utilize a synthetic DNA that can further assure the quality of DNA. In addition this technique can also indicate the presence of PCR inhibitors and indicate the need for further purification [70].
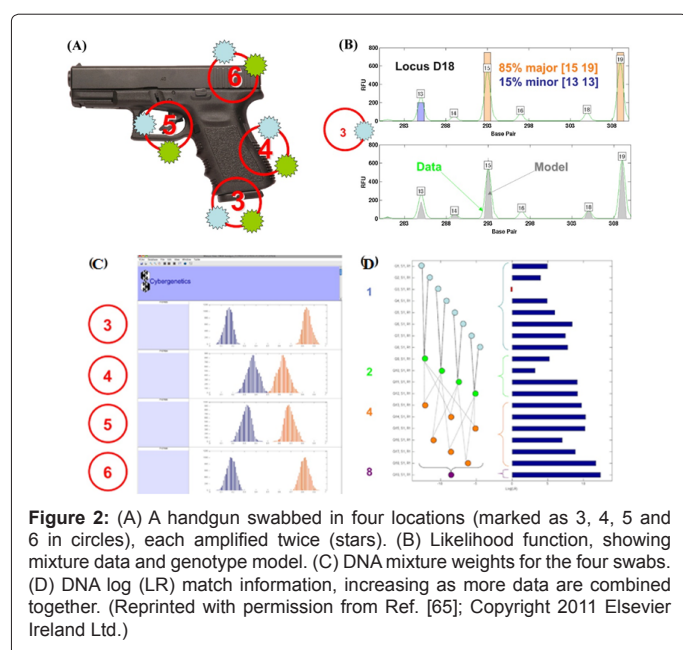
In order to analyze more than just fragments of mtDNA, modified multiplex PCR systems can be used to produce small overlapping amplicons that can be used to determine the sequence of mtDNA [69]. The analysis of mtDNA can be paired with the PCR to be further amplified. The traditional Sanger method is often used to sequence the amplicons, which involves fluorescent dideoxynucleotide and cycle sequencing. An alternative to the Sanger method is the use of electrospray ionization mass spectrometry (ESI-MS). Comparatively, the ESI-MS method provides similar accuracy and sensitivity to Sanger method, but ESI-MS has lower costs, requires less time, and is able to quantify DNA. However, both methods are limited in analyzing mixed samples due to chimeric mtDNA products forming during analysis [71].
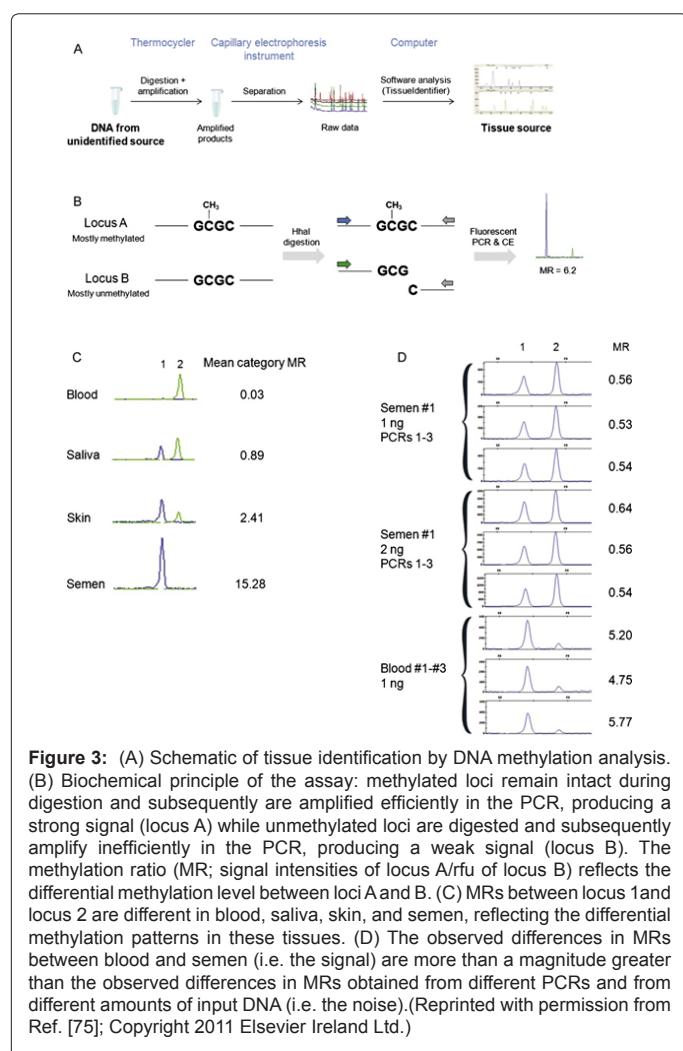
MtDNA analysis has also been paired with SNP-based screening methods providing a higher discrimination [68]. Aside from WGA and the use of mtDNA, the use of single cells has been explored as another option to get LT DNA for analysis. Single cell analysis has the potential to eliminate other steps in the analysis process, making it a growing focus in forensic analysis. In particular, micro globes can be used as carriers and micro tweezers can be used to transfer cells to receptacles. An advantage to this approach is the ability to separate mixed samples into individual samples and to be able to lift cells from glass, metal, and plastic and being able to directly transfer to reaction tube [72]. Another advantage to detecting single molecules is that enzymatic amplification can be avoided, which removes problems such as artifacts and PCR inhibitors [73].

DNA methylation analysis

DNA analysis is able to determine the identification of the individual that the sample came from; however, tests are still being developed to help determine what type of source the DNA came from, including sperm, saliva, vaginal fluid, and blood. Current techniques involved the use of mRNA, micro RNA, immune-based assays and DNA methylation. DNA methylation appears to be the best suited for body fluid identification at this time, due to its high specificity and compatibility with current STR typing protocols. In CpG dinucleotides there is a DNA methylation at C5 of cytosine residues. This is a modification that occurs in mammals and is involved in cellular differentiation. In particular, there are tissue-specific differential methylated regions (tDMRs) that occur in the genome of mammals. PCR methods that are methylation specific are able to identify body fluid identification and could possibly be multiplexed with existing STR typing protocols [74-76]. DNA methylation also has the potential to be used to help determine an estimated age [77].

A scheme of tissue identification by DNA methylation analysis is shown in Figure 3A. This assay uses a set panel of loci that aredifferentially methylated between tissues to determine the most probable source tissue of an unknown DNA sample [75]. Typically, approximately 1 ng of DNA from the unknown sample will be digested with the HhaI methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence GCGC only if it is unmethylated.

Figure 3: (A) Schematic of tissue identification by DNA methylation analysis. (B) Biochemical principle of the assay: methylated loci remain intact during digestion and subsequently are amplified efficiently in the PCR, producing a strong signal (locus A) while unmethylated loci are digested and subsequently amplify inefficiently in the PCR, producing a weak signal (locus B). The methylation ratio (MR; signal intensities of locus A/rfu of locus B) reflects the differential methylation level between loci A and B. (C) MRs between locus 1 and locus 2 are different in blood, saliva, skin, and semen, reflecting the differential methylation patterns in these tissues. (D) The observed differences in MRs between blood and semen (i.e. the signal) are more than a magnitude greater than the observed differences in MRs obtained from different PCRs and from different amounts of input DNA (i.e. the noise).(Reprinted with permission from Ref. [75]; Copyright 2011 Elsevier Ireland Ltd.)

Loci with higher methylation levels are amplified more effectively, producing a relatively strong signal in the electropherogram (Figure 3B, locus A). Conversely, loci with a lower methylation level are amplified poorly, resulting in a relatively weak signal (Figure 3B, locus B).A more definitive tissue identification can then be made by using the methylation ratio values, which are generally tissue-specific (Figure 3C and 3D).

Analysis of non-human DNA

It is not always apparent whether the DNA is human or non-human. Genome profiling (GP) can provide a way to multiply DNA fragments using a random primer and electrophoresed gel patterns. The advantages of using GP include results within a shorter time frame, limited technical skill is needed, and no expensive equipment is required. It can distinguish between human DNA and animal DNA without the use of sequencing, PCR and temperature gradient gel electrophoresis (TGGE) analysis. Unfortunately, these results could be unreliable if the samples are contaminated with other genomes [78].

Real time PCR genotyping, involving the detection of a human-specific nuclear gene target, forkhead box P2 (FOXP2), can be used to distinguish between human and non-human DNA. FOXP2 can encode a transcription factor that accumulates amino acid changes in the human lineage that are spe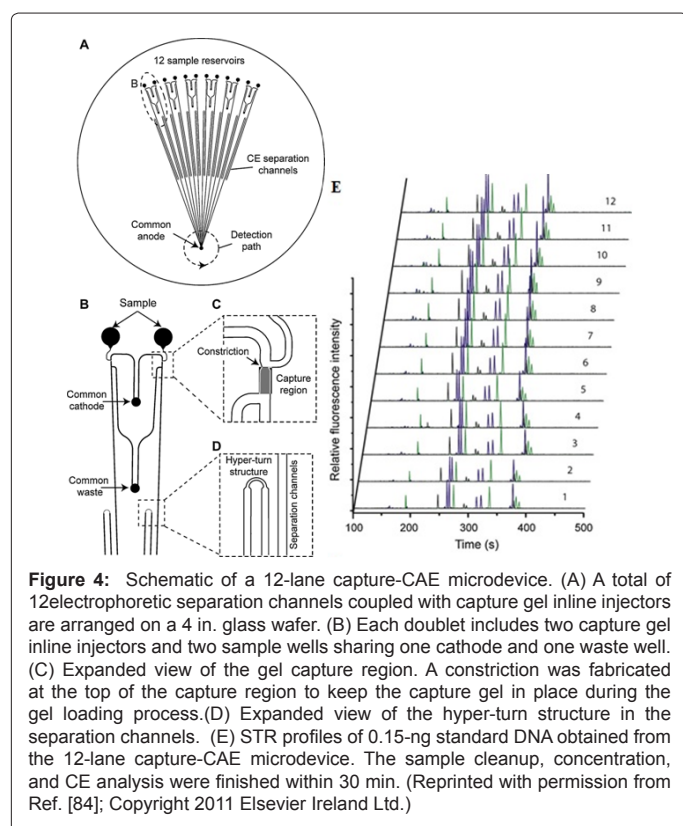cifically involved in speech and language development in humans. The detection of FOXP2 can be used as a quantification method conducted before further STR analysis preventing unneeded analysis of non-human DNA [78]. Just as DNA chips have been shown to be useful in the analysis of SNPs, combining chip technology and ABO genotyping can also be used to identify species [56,79].

Microfluidic systems for DNA analysis

Microfluidic devices have become more popular since the invention of the "Lab-on-a-Chip". A microfluidic system is composed of two or more microdevices, or chips that can perform a single processing step, such as microcapillary electrophoresis. The micro sizes allow for minimal reagent and sample volume. The system also can be completely sealed once the sample is added decreasing the chances of contamination [25]. These micro devices have to be efficient to work with the sample, so constructing the right type of valve, mixer and/or pump on the microdevice continues to be explored [80,81]. Elastomeric membranes, such as dimethylsiloxane (PDMS), are often used as valves because they allow for positive/negative pneumatic or hydraulic actuation and they can be reversible. PCR microdevices are commonly made of glass or from polymeric substrates, such as poly (methy methacrylate) (PMMA). Polymeric substrates continue to gain attention due to being more cost-effective and requiring less time to design and create. Hot embossing and laser ablation can be used instead of hydrofluoric acid to fabricate the microfluidic systems from polymeric substrates. One of the advantages of PDMS valves is that they can be bonded with PMMA devices [82]. External peristaltic pumps or syringes are often large devices outside the microfluidic system and require an additional source of electricity. A micropump made of magnetic shape memory (MSM) elements can be sealed within the microfluidic system and does not need electrical contacts. The MSM elements consist of Ni-Mn-Ga alloys that are able to create magnetic fields but do not interfere with the PCR amplification process [83].High-throughput platforms have been shown to work with as many as 96 samples simultaneously [20]. Other examples of new microsystems are the multi-lane microfabricated capillary array electrophoresis (CAE), which uses a photopolymerized streptavidin-gel capture process [84]. The design of a 12-lane capture-CAE microdevice is illustrated in Figures 4A-D. The same sample was loaded into all 12 sample reservoirs and analyzed simultaneously. As demonstrated in Figure 4E, STR profiles were successfully obtained from all the 12 lanes within 30 min. This platform allows for better sensitivity and an option for low-copy-number analysis [84].

The use of robotics is becoming more and more incorporated into the microfluidic systems. The appeal is the fact that extraction and purification can be automated, reducing cost, time, and the chance of contamination. In particular, the ability to create micro total analysis systems (μTAS) continues to foster many new inventions and methods. One of the major goals of the μTAS is to create a system that is a completely enclosed system for DNA analysis and also has the benefit of being portable. Integrating the extraction process into a microfluidic system can later help in the overall outcome of downstream analysis techniques, such as capillary gel electrophoresis [20,85,86]. For example, the automated DNA extraction platform which uses magnetic-particle chemistry provides a robust, and more cost-effective method of extracting DNA with less chance of contamination, however such a platform is not an optimal choice for samples with low concentrations of DNA [87].

Many purification protocols have been adapted to work with a microfluidic system, including silica-based, solid-phase methods and the use of an organic polymeric monolith and ion-exchange resin.

Figure 4: Schematic of a 12-lane capture-CAE microdevice. (A) A total of 12 electrophoretic separation channels coupled with capture gel inline injectors are arranged on a 4 in. glass wafer. (B) Each doublet includes two capture gel inline injectors and two sample wells sharing one cathode and one waste well. (C) Expanded view of the gel capture region. A constriction was fabricated at the top of the capture region to keep the capture gel in place during the gel loading process. (D) Expanded view of the hyper-turn structure in the separation channels. (E) STR profiles of 0.15-ng standard DNA obtained from the 12-lane capture-CAE microdevice. The sample cleanup, concentration, and CE analysis were finished within 30 min. (Reprinted with permission from Ref. [84]; Copyright 2011 Elsevier Ireland Ltd.)

One technique used to integrate the extraction and purification in a microfluidic system is the use of surface-charge switchable magnetic particles, which help with the flow of nucleic acid through the system. Another is the use of a concentrated PCR reagent mixture added to the eluted DNA from a solid-phase extraction in a separate purification chamber. In order to create the flow of the system, external syringe pumps or electro-osmotic pumping (EOP) can be fitted to the microfluidic platforms to ensure fluidic control. Further methods continue to be developed, coupling previous methods with new ones, for instance the use of an agarose gel that is already pre-loaded with reagents and is able to extract and purify. In addition, a bi-functional silica monolith can work as a solid-phase DNA extractor and as a pump for electro-osmotic pumping, further integrating extraction and purification in one microfluidic platform [85].

Low-frequency electric fields can provide an alternative to the reagents that are often used for the fluorescent detection and extraction step of microfluidic systems. DNA will aggregate, or concentrate in an area, when exposed to low-frequency electric fields. Wavelet analysis of the concentrated DNA can then be used to semi-quantify and help determine the amount of DNA amplification that can then occur. Overall, the use of low-frequency electric fields incorporated into microfluidic systems provides a label-free method to detect DNA especially in long DNA molecules [87,88]. Variations of the use of electric fields for DNA extraction include the use of nanotip concentrators made of SiC nanowires and single walled carbon nanotubes. DNA can be captured by nanotip concentrators in a single step and then be directly eluted into a PCR tube, reducing preparation time for further downstream analysis [89]. The use of a four-ferrocene modified oligonuleotide has also been investigated as a probe for DNA detection and then used in a gold electrode microsystem. An electrochemical biochip has the advantage of being more convenient than the traditional fluorescent DNA detection [90].

In a microfluidic system, amplification is often performed in a single chamber that is cycled through a sequential heating process. In three steps, thermal denaturation, annealing and extension can occur, reducing reagents and chance of contamination. Some of the sources of heat include hot air cycling, infrared radiation and a Peltier-based method. In particular, the Peltier-based method allows for better contact and more thermal instrumentation control [91]. Rapid thermal cycling has also been evaluated for more rapid results in DNA amplification. By using rapid PCR enzymes and fast thermal cycles, steps of the PCR method can be shortened. Microfluidic systems have been modified to integrate steps, including the use of an on-chip extraction paired with a multiplexed STR amplification [92]. Even in cases where improvised explosive disposals (IEDs) have thought to destroy most DNA evidence or limit the amount of DNA that can be extracted, improved multiplexes have been able to identify STR profiles [93].

The integration of CE has also made improvements in the micro-total analysis system (µTAS) system. Inline injection methods have been found to overcome the dilemma with poor efficiency of cross-injector for CE separation. In one integrated approach encompassing extraction, purification, and analysis, streptavidin-coated magnetic beads are used. DNA is able to bind to magnetic beads, which are then transferred to the PCR reactor, and then the products are electrophoretically injected into a gel which is streptavidin-modified. The concentrated and purified plug from the gel is then injected into a CE column. Altogether, it creates a fully integrated microfluidic system [86].

Other variations of the microfluidic system include the use of various enzymes. For example, Antarctic Bacillus is an enzyme that can be used in a liquid-base DNA extraction on a microfluidic platform. This enzyme is a neutral proteinase that can be used to lyse cells. It can also degrade proteins and nucleases without harming the nucleic acids in a PCR-compatible buffer. The enzyme-based microfluidic platform extraction can produce viable results with buccal swabs, whole blood, and blood found on denim or cotton without having to centrifuge or transfer the sample [81]. One of the drawbacks to a microdevice is that it often does not quantify the DNA. An engineered Pyrococcus-like proofreading DNA polymerase can also amplify reactions, is inhibitor tolerant, and allows for the omission of the final soaking step in PCR. Paired with rapid cycling and direct analysis of the sample, the proofreading heat stable polymerase can be effective in defining a complete DNA profile for high level DNA in single source samples [92].

Nanotechnology for DNA analysis

Nanoparticles have begun to be incorporated into the process of PCR amplification due to their unique ability to create physical and chemical properties based on what may be on their surface. For example, gold nanoparticles (AuNPs) can improve specificity and increase PCR efficiency. Carbon nanotubes (CNTs), nanometer-sized polymers and silver nanoparticles (AgNPs) have also been able to enhance specificity of PCR. It is theorized that AuNPs are able to work in the same fashion as SSBs, however, the actually mechanism is still under investigation [38]. In particular, microtechnology and nanotechnology have become a method of interest due to the ability to create devices that can prepare, manipulate, and analyze at a very small levels [20].

An example of how nanoparticles have aided in DNA extraction and amplification is in situations where urine is the source of DNA. Conventional genomic DNA from urine has involved the use of a centrifuge or filtration system, large amounts of sample, and use of toxic organic reagents. A beneficial alternative in DNA extraction from urine is the use of carboxylated magnetic nanoparticles, working as

solid phase adsorbents, which have been found to isolate intact DNA for PCR amplification [94]. Nanoparticles are also starting to take a role in the use of DNA biosensors. DNA field-effect device (FED) biosensors can be covered with gold nanoparticles (AuNPs) which attract the charges of DNA sequences to the sensor surface, allowing for low level DNA detection [95]. In addition, AuNPs have been shown to enhance electrochemiluminescence (ECL). This technique is combined with the isothermal reaction of polymerase and nicking endonuclease. Overall, the AuNP ECL approach has shown to be sensitive enough to detect approximately 5 attomolar of DNA [96].

Providing a technique for DNA detection that is portable, quantitative and available to the public continues to make strides in the shadows of traditional methods like PCR and DNA microarrays that are expensive and require instruments and operations that are not simple. One of the growing methods is the use of personal glucose meters (PGMs) to quantify DNA. PGMs are found to be able to quantify organic molecules, proteins and metal ions linked to functional DNA sensors [97]. As demonstrated in Figure 5, the immobilized DNA-invertase conjugates catalyze the hydrolysis of PGM-inert sucrose to yield PGM-detectable glucose, and pM~nM level of target DNA could produce mM level of glucose required for the readout in a PGM [22].
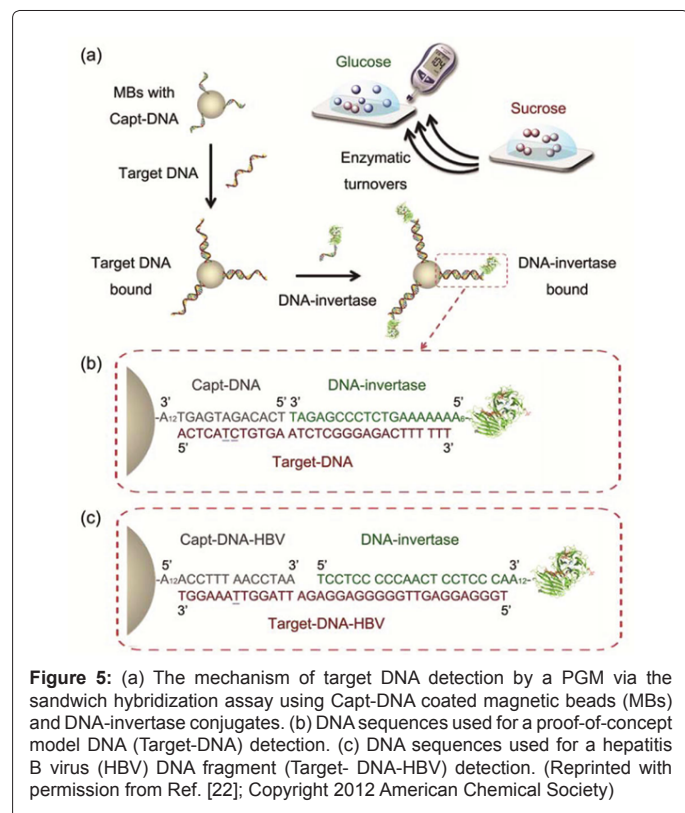
DNA databases

A set of standard DNA markers have been determined as criteria for a DNA profiling system called the combined DNA index system (CODIS). These markers are developed from DNA that has been identified using STR loci. The CODIS include 13 core loci and was developed in the United States. Sever loci were encouraged to be the standard by the European Network of Forensic Science Institutes (ENFSI) and Council of the European Union for a European standard. In the United Kingdom, there is another profiling system called Second

Figure 5: (a) The mechanism of target DNA detection by a PGM via the sandwich hybridization assay using Capt-DNA coated magnetic beads (MBs) and DNA-invertase conjugates. (b) DNA sequences used for a proof-of-concept model DNA (Target-DNA) detection. (c) DNA sequences used for a hepatitis B virus (HBV) DNA fragment (Target- DNA-HBV) detection. (Reprinted with permission from Ref. [22]; Copyright 2012 American Chemical Society)

Generation Multiplex DNA profiling (SGM+) system, containing eight loci from CODIS standard loci. These databases have become efficient tools in providing information during forensic investigations. The amount of defined STR loci for admittance into database varies from county to country. Since there is not an overall consensus on the standard STR loci used in the different databases, more and more advocates are pushing for the use of STR loci that have a high degree of polymorphism [19,36,44,45,98]. European Network of Forensic Sciences Institutes (ENFSI) and European Standard Set of Loci (EDNAP) have recommended the addition of five loci to their standard of DNA submitted into their databases. This increase of markers allows for better discrimination and useful in DNA profile sharing among countries [99]. This goal to have better discrimination has led to further developments of multiplex systems that can exceed the current STR loci detection number, including multiplex systems that can detect up to 17 STR loci [100]. A 20-locus multiplex system has been developed in China, so that a universal system could be put in place that can include their already existing STR loci databases [98].

Conclusions

In this review, a brief overview of the major developments in the field of forensic DNA analysis during the past 2.5 years is given. New approaches continued to be explored for more effectiveness. However even before DNA can be isolated, it is important to verify the true identity of the forensic samples. Current techniques for determining the biological origin of samples include luminescence-based presumptive tests, mass spectrometry, FTIR spectroscopy, and DNA methylation-specific PCR. The efficiency of different DNA extraction methods is dependent upon the exact nature of the sample (such as blood, semen, saliva, urine, sweat, and bone etc.). There is a tradeoff between purity and quantity for each DNA extraction method. For example, one strategy for high throughput DNA extraction was made possible by integrating silica magnetic beads with an automated robotic platform. The method was successfully applied to a variety of samples such as blood, saliva, and sperm; however it is not an optimal choice for samples with low concentrations of DNA. Fast Technology for Analysis of nucleic acids was shown to reduce extraction time especially for blood or saliva. An enzyme-based microfluidic platform can extract DNA directly from buccal swabs, whole blood, and blood found on denim or cotton.

Time continues to be a negative factor in forensic analysis. As DNA tends to degrade under ambient conditions, how to retain the integrity of DNA over an indefinite time of storage becomes a challenge. It has been demonstrated that DNA can be either dehydrated or kept in a specially-designed medium for long term storage. In order to reduce DNA analysis time, which could also save in costs, scientists continue to look at new approaches. By using real-time PCR, it is possible to identify the presence and further determine male DNA in a mixed sample, usually from sexual assault cases. Another variation of the PCR method - digital PCR can dramatically reduce the number of individual PCR reactions in cell free DNA analysis. By moving PCR primers closer to the STR region, miniSTR assay is able to determine STR loci from highly-degraded ancient DNA. The analysis of mtDNA is greatly enhanced by the pairing with modified multiplex PCR systems and with SNP-based screening methods. Low-frequency electric fields provide a green alternative to the reagents that are often used in the extraction step, the use of nanotip concentrators made of SiC nanowires and SWCNs makes it possible to capture DNA in a single step, as a result, the preparation time for further downstream analysis is largely reduced. More recently, personal glucose meters have been shown to

be able to quantify DNA in a point-of-care setting. This development suggests that forensic DNA analysis may be conducted more promptly near the actual crime scene in the future.

A significant portion of recent efforts has been devoted to investigating statistical analytical approaches to improve selectivity when dealing with DNA samples from a mixture of sources, or LT DNA. There is a growing consensus that better discrimination can be achieved by adding more standard DNA markers (STR loci) to the existing DNA databases around the world. In order to promote data sharing across a wide number of jurisdictions, a universal standard is yet to be agreed upon by all countries.

It is worth noting that although numerous scientific improvements are sure to come, the current methods are reliable and valid. Arguments continue to be made whether SNP markers or even DNA methylation will eventually surpass STR loci as the future main target of forensic DNA analysis. The great potential of automated microfluidic devices coupled with nanotechnology for high throughput DNA analysis is yet to be completely fulfilled. Many more exciting scientific and technological advances are still on the horizon, there is no doubt that the future landscape of forensic DNA analysis will look very different from what we see today.

Acknowledgments

References

1. Gaensslen RE, Harris HA, Lee HC (2007) Introduction to Forensics & Criminalistics. McGraw-Hill Companies, Inc.

2. Ley BL, Jankowski N, Brewer PR (2012) Investigating CSI: portrayals of DNA testing on a forensic crime show and their potential effects. Public Underst Sci 21: 51-67.

3. Machado H (2012) Prisoners' views of CSI's portrayal of forensic identification technologies: a grounded assessment. New Genet Soc 31: 271-284.

4. Van Steendam K, De Ceuleneer M, Dhaenens M, Van Hoofstat D, Deforce D (2013) Mass spectrometry-based proteomics as a tool to identify biological matrices in forensic science. Int J Legal Med 127: 287-298.

5. Fredericks JD, Bennett P, Williams A, Rogers KD (2012) FTIR spectroscopy: A new diagnostic tool to aid DNA analysis from heated bone. Forensic Sci Int Genet 6: 375-380.

6. Vandewoestyne M, Van Hoofstat D, Franssen A, Van Nieuwerburgh F, Deforce D (2013) Presence and potential of cell free DNA in different types of forensic samples. Forensic Sci Int Genet 7: 316-320.

7. Nunn S (2013) Touch DNA collection versus firearm fingerprinting: comparing evidence production and identification outcomes. J Forensic Sci 58: 601-608.

8. Thomasma SM, Foran DR (2012) The Influence of Swabbing Solutions on DNA Recovery from Touch Samples(,). J Forensic Sci .

9. Richert NJ (2011) Swabbing firearms for handler's DNA. J Forensic Sci 56: 972-975.

10. Hudlow WR, Buoncristiani MR (2012) Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence. Forensic Sci Int Genet 6: 1-16.

11. Hopwood AJ, Elliott K (2012) Forensic DNA research: keeping it real. Int J Legal Med 126: 343-344.

12. Zech WD, Malik N, Thali M (2012) Applicability of DNA analysis on adhesive tape in forensic casework. J Forensic Sci 57: 1036-1041.

13. Matte M, Williams L, Frappier R, Newman J (2012) Prevalence and persistence of foreign DNA beneath fingernails. Forensic Sci Int Genet 6: 236-243.

14. Schulz MM, Brune V, Maierthaler M, Graw M (2011) Visualization of latent biological traces via 5-methylthioninhydrin (5-MTN) staining for forensic typing. Forensic Sci Int Genet Suppl Series 3: e530–e531.

15. Patel G, Hopwood A (2013) An evaluation of luminol formulations and their effect on DNA profiling. Int J Legal Med 127: 723-729.

16. de Almeida JP, Glesse N, Bonorino C (2011) Effect of presumptive tests reagents on human blood confirmatory tests and DNA analysis using real time polymerase chain reaction. Forensic Sci Int 206: 58-61.

17. Lee SB, Clabaugh KC, Silva B, Odigie KO, Coble MD, et al. (2012) Assessing a novel room temperature DNA storage medium for forensic biological samples. Forensic Sci Int Genet 6: 31-40.

18. Stangegaard M, Hjort BB, Hansen TN, Hoflund A, Mogensen HS, et al. (2013) Automated extraction of DNA from biological stains on fabric from crime cases. A comparison of a manual and three automated methods. Forensic Sci Int Genet 7: 384-388.

19. Ziętkiewicz E, Witt M, Daca P, Zebracka-Gala J, Goniewicz M, et al. (2012) Current genetic methodologies in the identification of disaster victims and in forensic analysis. J Appl Genet 53: 41-60.

20. Alonso A (2013) DNA Extraction and Quantification, in Encyclopedia of Forensic Sciences. (2nd edn), Elsevier Ltd. 214-218.

21. Norén L, Hedell R, Ansell R, Hedman J (2013) Purification of crime scene DNA extracts using centrifugal filter devices. Investig Genet 4: 8.

22. Xiang Y, Lu Y (2012) Using commercially available personal glucose meters for portable quantification of DNA. Anal Chem 84: 1975-1980.

23. Witt S, Neumann J, Zierdt H, Gébel G, Röscheisen C (2012) Establishing a novel automated magnetic bead-based method for the extraction of DNA from a variety of forensic samples. Forensic Sci Int Genet 6: 539-547.

24. Verdon TJ, Mitchell RJ, van Oorschot RAH (2011) Evaluating the efficiency of DNA extraction methods from different substrates. Forensic Sci Int Genet Suppl. Series 3: e93–e94.

25. Kashkary L, Kemp C, Shaw KJ, Greenway GM, Haswell SJ (2012) Improved DNA extraction efficiency from low level cell numbers using a silica monolith based micro fluidic device. Anal Chim Acta 750: 127-131.

26. Vandewoestyne M, Van Nieuwerburgh F, Van Hoofstat D, Deforce D (2012) Evaluation of three DNA extraction protocols for forensic STR typing after laser capture microdissection. Forensic Sci Int Genet 6: 258-262.

27. Meredith M, Bright JA, Cockerton S, Vintiner S (2012) Development of a one-tube extraction and amplification method for DNA analysis of sperm and epithelial cells recovered from forensic samples by laser microdissection. Forensic Sci Int Genet 6: 91-96.

28. Rucinski C, Malaver AL, Yunis EJ, Yunis JJ (2012) Comparison of two methods for isolating DNA from human skeletal remains for STR analysis. J Forensic Sci 57: 706-712.

29. Piglionica M, De Donno A, Baldassarra SL, Santoro V, Scorca A, et al. (2012) Extraction of DNA from bones in cases where expectations for success are low. Am J Forensic Med Pathol 33: 322-327.

30. Manjunath BC, Chandrashekar BR, Mahesh M, Vatchala Rani RM (2011) DNA profiling and forensic dentistry--a review of the recent concepts and trends. J Forensic Leg Med 18: 191-197.

31. Raimann PE, Picanço JB, Silva DS, Albuquerque TC, Paludo FJ, et al. (2012) Procedures to recover DNA from pre-molar and molar teeth of decomposed cadavers with different post-mortem intervals. Arch Oral Biol 57: 1459-1466.

32. Chen T, Catcheside DE, Stephenson A, Hefford C, Kirkbride KP, et al. (2012) A rapid wire-based sampling method for DNA profiling. J Forensic Sci 57: 472-477.

33. Montelius K, Lindblom B (2012) DNA analysis in Disaster Victim Identification. Forensic Sci Med Pathol 8: 140-147.

34. Ricky Ansell (2013) Internal quality control in forensic DNA analysis. Accred Qual Assur 18:279-289.

35. Dror IE, Hampikian G (2011) Subjectivity and bias in forensic DNA mixture interpretation. Sci Justice 51: 204-208.

36. Giardina E, Spinella A, Novelli G (2011) Past, present and future of forensic DNA typing. Nanomedicine (Lond) 6: 257-270.

37. Whale AS, Cowen S, Foy CA, Huggett JF (2013) Methods for applying accurate digital PCR analysis on low copy DNA samples. PLoS One 8: e58177.

38. Pan D, Mi L, Huang Q, Hu J, Fan C (2012) Genetic analysis with nanoPCR. Integr Biol (Camb) 4: 1155-1163.

39. Seo SB, Lee HY, Zhang AH, Kim HY, Shin DH, et al. (2012) Effects of humic acid on DNA quantification with Quantifiler® Human DNA Quantification kit and short tandem repeat amplification efficiency. Int J Legal Med 126: 961-968.

40. Nicklas JA, Noreault-Conti T, Buel E (2012) Development of a real-time method to detect DNA degradation in forensic samples. J Forensic Sci 57: 466-471.

41. da Silva CV, Costa HA, Santos JC, Espinheira RM (2013) Forensic Genetics as a Tool for Peace and Justice: An Overview on DNA Quantification. J Forensic Res S11: 008.

42. Tak YK, Kim WY, Kim MJ, Han E, Han MS, et al. (2012) Highly sensitive polymerase chain reaction-free quantum dot-based quantification of forensic genomic DNA. Anal Chim Acta 721: 85-91.

43. Kang MJ, Yu H, Kim SK, Park SR, Yang I (2011) Quantification of trace-level DNA by real-time whole genome amplification. PLoS One 6: e28661.

44. Butler JM, Hill CR (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. Forensic Sci Rev 24: 15.

45. McDonald J, Lehman DC (2012) Forensic DNA analysis. Clin Lab Sci 25: 109-113.

46. de Lourdes Chávez-Briones M, Hernández-Cortés R, Díaz-Torres P, Niderhauser-García A, Ancer-Rodríguez J, et al. (2013) Identification of human remains by DNA analysis of the gastrointestinal contents of fly larvae. J Forensic Sci 58: 248-250.

47. Jiang X, He J, Jia F, Shen H, Zhao J, et al. (2012) An integrated system of ABO typing and multiplex STR testing for forensic DNA analysis. Forensic Sci Int Genet 6: 785-797.

48. Fattorini P, Marrubini G, Sorçaburu-Cigliero S, Pitacco P, Grignani P, et al. (2011) CE analysis and molecular characterisation of depurinated DNA samples. Electrophoresis 32: 3042-3052.

49. Benschop C, Haned H, Sijen T (2013) Consensus and pool profiles to assist in the analysis and interpretation of complex low template DNA mixtures. Int J Legal Med 127: 11-23.

50. Benschop CC, Haned H, de Blaeij TJ, Meulenbroek AJ, Sijen T (2012) Assessment of mock cases involving complex low template DNA mixtures: A descriptive study. Forensic Sci Int Genet 6: 697-707.

51. Børsting C, Mogensen HS, Morling N (2013) Forensic genetic SNP typing of low-template DNA and highly degraded DNA from crime case samples. Forensic Sci Int Genet 7: 345-352.

52. Kling D, Welander J, Tillmar A, Skare O, Egeland T, et al. (2012) DNA microarray as a tool in establishing genetic relatedness--Current status and future prospects. Forensic Sci Int Genet 6: 322-329.

53. Freire-Aradas A, Fondevila M, Kriegel AK, Phillips C, Gill P, et al. (2012) A new SNP assay for identification of highly degraded human DNA. Forensic Sci Int Genet 6: 341-349.

54. Wei YL, Li CX, Jia J, Hu L, Liu Y (2012) Forensic identification using a multiplex assay of 47 SNPs. J Forensic Sci 57: 1448-1456.

55. Lee HY, Park MJ, Kim NY, Yang WI, Shin KJ (2011) Rapid direct PCR for ABO blood typing. J Forensic Sci 56 Suppl 1: S179-182.

56. Watanabe K, Ikegaya H, Hirayama K, Motani H, Iwase H, et al. (2011) A novel method for ABO genotyping using a DNA chip. J Forensic Sci 56 Suppl 1: S183-187.

57. Maciejewska A, Jakubowska J, Pawłowski R (2013) Whole genome amplification of degraded and nondegraded DNA for forensic purposes. Int J Legal Med 127: 309-319.

58. Tate CM, Nuñez AN, Goldstein CA, Gomes I, Robertson JM, et al. (2012) Evaluation of circular DNA substrates for whole genome amplification prior to forensic analysis. Forensic Sci Int Genet 6: 185-190.

59. Bregu J, Conklin D, Coronado E, Terrill M, Cotton RW, et al. (2013) Analytical thresholds and sensitivity: establishing RFU thresholds for forensic DNA analysis. J Forensic Sci 58: 120-129.

60. Ballantyne J, Hanson EK, Perlin MW (2013) DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. Sci. Justice 53: 103–114.

61. Lohmueller KE, Rudin N (2013) Calculating the weight of evidence in low-template forensic DNA casework. J Forensic Sci 58 Suppl 1: S243-249.

62. Kelly H, Bright JA, Curran J, Buckleton J (2012) The interpretation of low level DNA mixtures. Forensic Sci Int Genet 6: 191-197.

63. Bille T, Bright JA, Buckleton J (2013) Application of Random Match Probability Calculations to Mixed STR Profiles. J Forensic Sci .

64. Haned H, Slooten K, Gill P (2012) Exploratory data analysis for the interpretation of low template DNA mixtures. Forensic Sci Int Genet 6: 762-774.

65. Perlin MW (2011) Combining DNA evidence for greater match information. Forensic Sci Int Genet Suppl Series 3: e510–e511.

66. Bright JA, Gill P, Buckleton J (2012) Composite profiles in DNA analysis. Forensic Sci Int Genet 6: 317-321.

67. Egeland T, Salas A (2011) A statistical framework for the interpretation of mtDNA mixtures: forensic and medical applications. PLoS One 6: e26723.

68. Chemale G, Paneto GG, Menezes MA, de Freitas JM, Jacques GS, et al. (2013) Development and validation of a D-loop mtDNA SNP assay for the screening of specimens in forensic casework. Forensic Sci Int Genet 7: 353-358.

69. Kim NY, Lee HY, Park SJ, Yang WI, Shin KJ (2013) Modified midi- and mini-multiplex PCR systems for mitochondrial DNA control region sequence analysis in degraded samples. J Forensic Sci 58: 738-743.

70. Kavlick MF, Lawrence HS, Merritt RT, Fisher C, Isenberg A, et al. (2011) Quantification of human mitochondrial DNA using synthesized DNA standards. J Forensic Sci 56: 1457-1463.

71. Warshauer DH, King J, Eisenberg AJ, Budowle B (2013) Validation of the PLEX-IDâ,¢ mass spectrometry mitochondrial DNA assay. Int J Legal Med 127: 277-286.

72. Brück S, Evers H, Heidorn F, Müller U, Kilper R, et al. (2011) Single cells for forensic DNA analysis--from evidence material to test tube. J Forensic Sci 56: 176-180.

73. Ranasinghe RT, Brown T (2011) Ultrasensitive fluorescence-based methods for nucleic acid detection: towards amplification-free genetic analysis. Chem Commun (Camb) 47: 3717-3735.

74. Lee HY, Park MJ, Choi A, An JH, Yang WI, et al. (2012) Potential forensic application of DNA methylation profiling to body fluid identification. Int J Legal Med 126: 55-62.

75. Frumkin D, Wasserstrom A, Budowle B, Davidson A (2011) DNA methylation-based forensic tissue identification. Forensic Sci Int Genet 5: 517-524.

76. Gršković B, Zrnec D, Vicković S, Popović M, Mršić G (2013) DNA methylation: the future of crime scene investigation? Mol Biol Rep 40: 4349-4360.

77. An JH, Shin K, Choi A, Yang WI, Lee HY (2013) DNA methylation-based age estimation in the forensic field. Korean J Leg Med 37: 1-8.

78. Suwa N, Ikegaya H, Takasaka T, Nishigaki K, Sakurada K (2012) Human blood identification using the genome profiling method. Leg Med (Tokyo) 14: 121-125.

79. Linacre A, Gusmão L, Hecht W, Hellmann AP, Mayr WR, et al. (2011) ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. Forensic Sci Int Genet 5: 501-505.

80. Njoroge SK, Chen HW, Witek MA, Soper SA (2011) Integrated microfluidic systems for DNA analysis. Top Curr Chem 304: 203-260.

81. Lounsbury JA, Coult N, Miranian DC, Cronk SM, Haverstick DM, et al. (2012) An enzyme-based DNA preparation method for application to forensic biological samples and degraded stains. Forensic Sci Int Genet 6: 607-615.

82. Lounsbury JA, Karlsson A, Miranian DC, Cronk SM, Nelson DA, et al. (2013) From sample to PCR product in under 45 minutes: a polymeric integrated microdevice for clinical and forensic DNA analysis. Lab Chip 13: 1384-1393.

83. Ullakko K, Wendell L, Smith A, P Mullner P, Hampikian G (2012) A magnetic shape memory micropump: contact-free, and compatible with PCR and human DNA profiling. Smart Mater Struct 21: 115020.

84. Liu P, Scherer JR, Greenspoon SA, Chiesl TN, Mathies RA (2011) Integrated sample cleanup and capillary array electrophoresis microchip for forensic short tandem repeat analysis. Forensic Sci Int Genet 5: 484-492.

85. Shaw KJ, Joyce DA, Docker PT, Dyer CE, Greenway GM, et al. (2011) Development of a real-world direct interface for integrated DNA extraction and amplification in a microfluidic device. Lab Chip 11: 443-448.

86. Liu P, Li X, Greenspoon SA, Scherer JR, Mathies RA (2011) Integrated DNA purification, PCR, sample cleanup, and capillary electrophoresis microchip for forensic human identification. Lab Chip 11: 1041-1048.

87. Castella V, Kummer D, Gehrig C, Hall D (2011) DNA extraction using the QIAsymphony: Evaluation of PCR inhibitor removal. Forensic Sci Int Genet Supplement Series 3: e69-e70.

88. Diakité ML, Champ J, Descroix S, Malaquin L, Amblard F, et al. (2012) A low-cost, label-free DNA detection method in lab-on-chip format based on electrohydrodynamic instabilities, with application to long-range PCR. Lab Chip 12: 4738-4747.

89. Kalyanasundaram D, Kim JH, Fotouhi G, Lee HB, Hiraiwa M, et al. (2013) Nanotips for single-step preparation of DNA for qPCR analysis. Analyst 138: 3135-3138.

90. Chatelain G, Ripert M, Farre C, Ansanay-Alex S, Chaix C (2012) A "four-ferrocene" modified stem-loop structure as a probe for sensitive detection and single-base mismatch discrimination of DNA. Electrochim Acta 59: 57- 63.

91. Estes MD, Yang J, Duane B, Smith S, Brooks C, et al. (2012) Optimization of multiplexed PCR on an integrated microfluidic forensic platform for rapid DNA analysis. Analyst 137: 5510-5519.

92. Verheij S, Harteveld J, Sijen T (2012) A protocol for direct and rapid multiplex PCR amplification on forensically relevant samples. Forensic Sci Int Genet 6: 167-175.

93. Berti A, Barni F, Virgili A, Colozza C, Maiorino F, et al. (2011) The recovery of DNA profiles from saliva and touch evidences after postal bomb explosion. Forensic Sci Int Genet Suppl Series 3: e471-e472.

94. Shan Z, Zhou Z, Chen H, Zhang Z, Zhou Y, et al. (2012) PCR-ready human DNA extraction from urine samples using magnetic nanoparticles. J Chromatogr B Analyt Technol Biomed Life Sci 881-882: 63-8.

95. Cherstvy AG (2013) Detection of DNA hybridization by field-effect DNA-based biosensors: mechanisms of signal generation and open questions. Biosens Bioelectron 46: 162-170.

96. Zhou H, Liu J, Xu JJ, Chen HY (2011) Ultrasensitive DNA detection based on Au nanoparticles and isothermal circular double-assisted electrochemiluminescence signal amplification. Chem Commun (Camb) 47: 8358-8360.

97. Xiang Y, Lu Y (2013) An invasive DNA approach toward a general method for portable quantification of metal ions using a personal glucose meter. Chem Commun (Camb) 49: 585-587.

98. Jiang X, Guo F, Jia F, Jin P, Sun Z (2013) Development of a 20-locus fluorescent multiplex system as a valuable tool for national DNA database. Forensic Sci Int Genet 7: 279-289.

99. Hatzer-Grubwieser P, Berger B, Niederwieser D, Steinlechner M (2012) Allele frequencies and concordance study of 16 STR loci--including the new European Standard Set (ESS) loci--in an Austrian population sample. Forensic Sci Int Genet 6: e50-51.

100. Zhang S, Tian H, Wu J, Zhao S, Li C (2013) A new multiplex assay of 17 autosomal STRs and Amelogenin for forensic application. PLoS One 8: e57471.