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Low Template DNA: Tad, Touch and Traces

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Abstract

Small amounts of DNA, typically less than 100 pg, termed Low Template (LT) DNA which is extremely useful in forensic casework. The current advancement of highly sensitive multiplex PCR systems consisting of short tandem repeat (STR) markers have allowed the development of genetic profiles from much lower quantities of DNA, from such samples. However, this sensitivity of the STR profiling systems also leads to issues of secondary transfer of LT DNA such as the implication of innocent parties as a result of background contamination and profiles having artefacts due to stochastic effects. This review explores the varying deposition mechanisms, technological advancements in both collection and analysis, and ultimately evaluates the usefulness of LT DNA considering its admissibility as forensic evidence.

Introduction

Low Template (LT) DNA, also called 'touch DNA', trace DNA or high sensitivity DNA, refers to DNA amount that is typically less than 100 pg [1]. These low levels of DNA may be recovered from as few as seven skin cells which have been deposited on a surface [2]. This review will focus on Low Template Analysis (LTA), which should not be confused with Low Copy Number DNA (LCN), which refers to the specific commercial technique employed by the Forensic Science Service (FFS) for the analysis of trace DNA [3].

The proof of concept for generating DNA profiles from low levels of DNA from touched objects was provided from the analysis of touched objects under controlled conditions [4]. However, a significant setback for the analysis of low level DNA came in 2007 when LCN was suspended across England/Wales whilst it was reviewed by the Crown Prosecution Service. In early 2008, the suspension order was lifted [5] and since then a significant amount of research has been conducted into LTA. The new multiplex PCR assays including kits like Powerplex® ESI 17, NGMSelect, Powerplex® Fusion 6C and GlobalFilerTM are highly sensitive, enabling genetic information to be obtained in instances where DNA degradation or inhibition has taken place [6-8]. It has been shown that an increase in genotypic accuracy can be achieved if the DNA sample is amplified in replicates, reducing the effects of allelic drop in/out [9].

An important consideration when evaluating LT DNA evidence is assessing the varying modes of deposition. One method of deposition the transfer of DNA to a surface through skin contact. However, despite LT DNA being commonly referred to as 'touch' DNA, it is not only touch that may lead to deposition as it may be also expelled orally. Oral DNA can be transferred up to 155 cm during speech and a static speaking individual may contaminate their immediate environment in as little as thirty seconds [10]. DNA may also be deposited on a surface through an intermediary by secondary transfer [5] and this can lead to mixed profile deposits, where the intermediate depositor is another person. Forensically, it is important to be able to conclusively distinguish between the suspect's sample and any background DNA, to allow the courts to draw appropriate inferences.

Whilst LT DNA is lawfully admissible in a UK courtroom, concerns were raised over background DNA affecting its interpretation [1]. The recovery of LT DNA from a scene and its laboratory processing may introduce contamination, with the subsequent genotype profile not accurately reflecting the sample. The term 'admissibility' will be used here, to refer to the general acceptance of LT DNA within scientific communities and its validation as forensic evidence, as opposed to legalized standards.

This review examines the physical recovery of LT DNA, the deposition mechanisms leading to mixed DNA samples and profile analysis using the recent technological advances. This will highlight the concerns over the admissibility of LT DNA and conclude on its effectiveness as modern forensic evidence.

LT DNA in Modern Forensic Science

Since the first introduction of DNA profiling [11] DNA evidence has become a key tool for forensic investigations. However, as forensic technology develops so does the general awareness surrounding the power of DNA evidence, with criminals becoming increasingly more forensically aware. Particularly in major crime, it is likely that a conscious effort could be made by the offender to leave as little DNA evidence as possible. The analysis of lower levels of DNA can therefore prove invaluable, where DNA profiles can be generated from much lower DNA quantities compared to that of thirty years ago.

In 2013, the 1995 murder of Krystal Beslanowitch was solved, and a successful conviction achieved, after re-examination of a rock collected at the scene. DNA analysis conducted on the swabs obtained from the rock in 1995 failed to produce any results however, a major and minor profile was produced after extracting low-level DNA from the rock's surface using the M-Vac® wet-vacuum system [12]. M-Vac® uses sterile water to dislodge cellular material from surfaces and applies a vacuum to collect the water/DNA [13].

Another notable case involving the use of LT DNA was during the 2005 trial of Bradley Murdoch for the murder of Peter Falconio and the abduction of his girlfriend, Joanne Lees. At the trial, low-level DNA evidence was presented, in the Australian Supreme Court Judgment [14]. Surface swabs taken from the gearstick of Falconio's vehicle produced a mixed DNA profile consistent with two different individuals. After further analysis, the mixed profile matched reference samples taken from Falconio and Murdoch, placing Murdoch in the victim's vehicle. This evidence substantially linked Murdoch to the scene and was undoubtable a key element for the prosecution's case [14].

The defence [14] raised the issue of there being no recognized scientific body experienced in the analysis of LT DNA as it was at the forefront of DNA analysis. This was a valid point raised at the time and since then in the UK, further work has been completed to further develop LT DNA analysis. In particular, the need for standardization of DNA interpretation across different scientific laboratories has been expressed, with the interpretation of the results needing to be irrespective of the specific LT DNA processing method [3].

Deposition of LT DNA

Low-template DNA can be deposited on a surface through touch or oral projection [10]. The deposition of DNA can take place in three different modes: primary, secondary and tertiary (Figure 1). Primary transfer is the direct deposition of DNA onto a surface by an individual. Secondary transfer involves the deposition of DNA from an individual to an item, or person, through an intermediary [5]. Where secondary transfer is concerned, there are however three theoretical outcomes for the LT DNA profile generated from the final deposition site: DNA from person A, DNA from person B (intermediate) or a mixed profile of DNA from both persons. Rutty [15] suggested the possibility of tertiary transfer, and this takes place when an additional party's DNA is present. Third party DNA may be deposited on a surface after passing through two intermediates (Figure 1), where person A is the third party and person B either an intermediate person or an object touched.
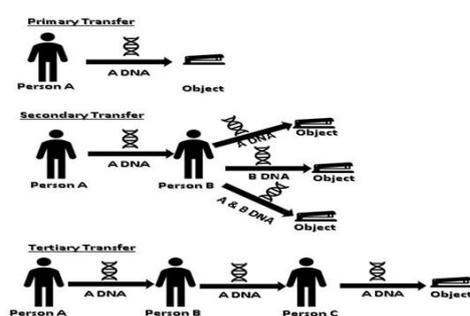

Figure 1: Illustration of primary, secondary and tertiary DNA transfer mechanisms

To contextualize further, the scenario of a door handle being touched can demonstrate the three different modes. A person touches the door handle and their DNA is recovered - primary transfer. A person shakes hands with a friend and the friend touches the door handle, with DNA recovered matching the original person secondary transfer. If an individual touches the door handle and then another person happens to touch the door handle before firing a gun, the DNA recovered might match that of the first individual – tertiary transfer. Here, the door handle and second person acted as intermediaries, enabling the transfer of DNA to the gun. It is important to note that such a scenario could lead to an innocent party, being implicated in firing the gun. In case work, it is vital that contextual information is viewed in conjunction with the LT DNA profile to avoid incorrect interpretations, and the possibility of implicating innocent parties.

In terms of quantifying the DNA recovered, there is likely to be a significant reduction in deposition on the final object as additional transfer takes place. If secondary transfer has taken place, it would be expected that there were lower levels of DNA due to the sample DNA being deposited by the intermediate and not direct from the source as with primary transfer. As tertiary transfer takes place, it is likely that even lower levels of DNA were present on the final surface/object as a gradual reduction in DNA takes place as it passes between the intermediary stages. Having an awareness of the varying DNA quantities over different transfer modes, could prove a useful precursor to determining the mode of deposition. At one extreme, high levels of LTDNA are likely to be due to direct contact with the source whereas at the other end of the spectrum, low level LTDNA is more likely to have arose through intermediary transfer.

The deposition of LT DNA is affected, by and large, due to either variation in the DNA contact or the deposition surface. An alteration in the DNA contact with a surface can have a significant impact on the level of LT DNA collected. A decrease in contact time and increase in both subsequent handling of the object and time since deposition, can inversely affect the physical quantity of DNA recovered [1]. Shedder status can also influence DNA deposits and it refers to the level of DNA that an individual typically deposits. Good shedders deposit a greater level of DNA on a surface compared to poor shedders [16] and therefore increase the possibility of LT DNA recovery.

The quantity of DNA deposited is also thought to be pressure linked [17]. Generally, the amount of DNA deposited on a surface increased with pressure, which is expected. However, the effects of pressure on DNA deposition are individualistic and further experimental research is needed expand on the initial work of Tobias to establish the underlying factors causing these differences [17].

The surface which the DNA is deposited on can also have an impact on the quantity of recoverable LT DNA. Detailed research conducted by Daly [18] investigated how DNA deposition varied over wood, fabric and glass surfaces. Calculation of the average amount of DNA deposited on the surfaces touched enabled a ranking to be established. Wood provided the greatest average quantity of DNA at 5.85 ng followed by fabric 1.23 ng and glass 0.52 ng [18].

Considering the surface types in terms of porosity, the greatest deposition of LT DNA occurred when contact was made with the porous surfaces, wood and linen, compared to non-porous surfaces such as glass. These results seem logical since DNA deposited on a non-porous surface will remain on the surface, as opposed to a porous surface, which would adsorb the DNA during contact.

Considering the porosity of surfaces can therefore prove extremely helpful in forensic casework. Prioritizing, where possible, porous surfaces for LT DNA analysis is likely to provide more successful profile generation as more DNA is likely to have been deposited, and retained, on the surface. This especially useful for cases where multiple surfaces or scenes are involved, enabling a more cost-effective approach to the recovery of LT DNA through the analysis of specific samples.

Technological Advances in LT DNA Collection

The traditional technique used for collecting DNA is the double swabbing technique [19] typically cotton swabs, and is still a widely used method for recovering DNA today. The double swabbing technique involves first using a moistened swab to wet the sample surface, then using a dry swab on the sample area [20]. Distilled water is usually used to moisten the wet swab. The first swab is intended to dislodge the cellular material from the surface, with the second dry swab picking up the cellular material. However, this technique is deployed in the recovery of bodily fluid DNA, for instance saliva, and therefore it should be emphasized that this is not a technique developed specifically for Low Template DNA recovery.

There has been some research to assess the effectiveness of various buffer solutions to recover LT DNA in place of distilled water [21,22] however to date, there is still a lack of clear evidence for its effectiveness and therefore more work is needed in this area. In particular, assessing the effectiveness of buffer solutions across a wide range of surface types and investigating whether the use of buffers has any negative effects for the subsequent analysis of DNA.

Nylon swabs can also be used such as microFLOQ® swabs, designed to have a smaller head compared to traditional fiber swabs therefore reducing sample consumption [23]. Although, it is noted that the flocked design of the swab containing a non-absorbent core may lead to a loss of DNA if the swab is handled extensively [23] therefore impacting profile generation. There are other types of swabs available such as foam swabs, but these generally perform poorly overall for low level DNA recovery [24].

Tape lifting is a standard method used within the forensic laboratories to recover cellular DNA from a range of surfaces and it has been shown to be an effective technique for recovering greater quantities of DNA from ridged surfaces compared to double swabbing [25]. This is likely due to the fact that the tape will be able to follow contours of the ridges whereas a swab has a more rigid form. The tape lifting technique can also be applied to fabric [26,27].

Another recovery technique for DNA recovery is the use of FTA paper. The effectiveness of tape lifts and FTA paper, for the recovery of DNA from car stirring wheels, was compared to the traditional double swabbing technique [28]. FTA paper, moistened with water, provided the greatest physical quantity of DNA from the sample site. There was no significant difference in the quantity of DNA recovered when either the tape lifts or double swabbing methods were used. As the authors highlighted, the major advantage for the FTA technique is the fact that it is much faster compared to carrying out the double swabbing method. FTA paper could therefore be used across a large surface area, making it especially effective when the exact contact location is unknown, or a large volume of surfaces need to be tested. Kirgiz and Calloway sampled only one non-porous surface type and more research is required to assess its effectiveness across a range of surface types [28].

Technological Advances in LT DNA Analysis

The technology and methodologies used for the analysis of LT DNA have significantly evolved and recent advances have aimed to minimize issues that may occur during the analysis stage. One such example is reducing the effects of allelic drop in/out on the DNA profiles obtained from samples. Allele drop in occurs when for a sample, alleles which do not correspond to the sample genotype are detected and allele drop out when alleles for a particular locus fail to be amplified [29]. Both of these effects are due to errors occurring during the Polymerase Chain Reaction (PCR) amplification stage, prior to analysis, with allele drop out arising from an unbalanced amplification of the LT DNA [30]. Allele drop in on the other hand, arises when background DNA (contaminant) present within the LT DNA test sample is amplified and this appears as additional allele signals which do not correspond to the sample genotype. The ability to deduce a correct genotype from the DNA sample is critical for forensic casework and allelic drop in/out could falsify the true genotype.

To reduce these effects, it is widely suggested that multiple replicates should be carried out, whereby two or more amplifications are completed using aliquots of the original LT DNA sample, analyzed, then the profiles compared [29,31]. This enables a more accurate genotype to be assigned due to a consensus being reached based on all of the resulting profiles for the same sample of DNA. Where alleles have dropped out in one replicate, they might manifest on the other replicate. Practically, conducting multiple replicates is more expensive but ultimately can aid in increasing genotype accuracy. The case scenario and the emphasis placed on the particular evidentiary material, will probably dictate the use of such procedure.

Allele drop out can also be reduced through the use of direct PCR, whereby the sample undergoes PCR without DNA extraction and can reduce the loss of DNA pre-PCR [32]. Especially in cases where LT DNA is being analyzed, it is important to maximize the level of DNA entering the analysis stages as low quantities of DNA are more susceptible to stochastic effects [30]. There are several new direct PCR kits available commercially such as AmpFlSTR® Identifiler® Direct, GlobalFiler™ Express and PowerPlex® 18D [33]. Where nylon swabs (microFLOQ®) have been used to collect LT DNA, direct PCR has shown to have variable levels of success, producing both partial and full profiles for a range of different surfaces [23] and therefore more research is needed to optimize this method further.

Massive Parallel Sequencing (MPS) is gaining importance in forensic DNA analysis. MPS enables the analysis of multiple gene markers at the same time [34]. This might be useful where LT DNA is concerned, as the technique generates a great deal of genetic information using a single extract of the original sample. Multiple markers linked to ethnicity [35,36], hair colour, eye colour [37] and stature [38], could provide vital information for a suspect's profile. The technology also enables several samples to be analyzed simultaneously, with results being obtained in a couple of days. Thus, MPS is a significant technological advancement compared to capillary electrophoresis (CE) based analysis.

Interestingly, Harbison [39] have demonstrated that MPS can also be used to sequence the resulting DNA amplified products if other analyses have been already conducted on the original DNA sample. Further research is needed to fully validate the sequencing quality of DNA from previous reactions compared to primary sampling, but this could prove a useful quality. If for example the original DNA sample has been consumed in other analyses or is no longer available, then MPS could yield further genetic information to complement that obtained in previous analyses.

LT DNA and Contamination

Contamination refers to additional DNA material within a collected sample which does not belong to the depositor in question. The introduction of background DNA can occur at three stages: at the

scene prior to recovery, during the forensic recovery or during the analysis at the laboratory.

Typically, the greatest scope for contamination is at the scene itself, prior to recovery of the forensic material. For this reason, it is vitally important that the scene is secured as soon as possible, and restrictions placed on the movement through the scene.

In order to control the contamination due to equipment, it is often advised that 'sterile' equipment should be used for DNA collection [20,40] but it is important that the equipment is also verified as DNA free [41]. For instance, sterile swabs may contain traces of DNA and this may introduce additional material to the sample. Disposable collecting equipment must therefore meet the current ISO standards of 18385:2015 [41].

It is vital that cross-contamination does not occur as this will produce inaccurate genetic information. The protocols for minimizing cross-contamination are outlined by the Forensic Science Regulator [42] in particular, changing PPE between cases/exhibits (section 8.3.5) and effectively cleaning benches (section 8.4.1). During the examination of an exhibit DNA may accumulate on the surfaces within the laboratory and the deposits can be transferred directly other evidentiary material, or to personnel/equipment then onto other exhibits [43]. Therefore, it is imperative that appropriate PPE is worn as well as vigorous cleaning regimes and environmental monitoring practiced in a forensic DNA laboratory.

Where mixed profile deposits are formed, the sample donor is likely to have the greatest profile within the mixture compared, with the rest of the profiles being contaminants. However, other factors such as shedder status may mask the donor, for instance where the donor is of a low shedder status and produces a smaller profile compared to the background DNA.

Many forensic laboratories today have specific high sensitivity areas established, dedicated to the examination of LT DNA evidence. In instances where multiple DNA exhibits have been recovered, LT DNA evidence should be examined first [44] to reduce the risk of exhibit contamination from heavily soiled items.

Conclusion

Low Template DNA testing can be extremely useful for generating a suspect profile from very small DNA quantities. However, due to the different modes of deposition of LT DNA and the generation of mixed profiles, it is important to develop robust procedures to interpret such evidence.

Due to the nature of LT DNA, it is extremely difficult to recover a low-level DNA sample without collecting background DNA and so this form of contamination is almost inevitable. Though with the use of multiple replicates to develop consensus profiling, the assigned genotype accuracy can be significantly increased.

A notable area for further research is the effect that surface properties can have on both the quantity of LT DNA deposited and subsequent collection methods used. Determining specific surfaces that are likely to yield the greatest LT DNA will enable these surface types to be targeted first, enhancing efficiency during casework. Also, the development of surface-specific guidelines for specific collecting reagents will optimize LT DNA recovery. In addition, further work on the effects of time on LT DNA recovery will also be beneficial in determining the likelihood of recovering suitable LT DNA profiles.

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