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Mechanisms to Combat DNA Degradation

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MECHANISMS TO COMBAT DNA DEGRADATION IN A FORENSIC SETTING

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Abstract:

Deoxyribonucleic acid (DNA) is a highly-susceptible molecule used in forensic analysis that can be degraded. Degraded DNA is usually unfit for analysis because information is lost as the DNA breaks, resulting in poor profiles and bad statistics. This paper looked at ways to counter or reverse the damage via mini-STRs, SNPs, and MDA. There was no methodology that was clearly better than another; all three mechanisms need to be more thoroughly researched as they have unique pros and cons to consider before they can be fully implemented. No perfect DNA damage reversal has been discovered, so perhaps our focus should shift from reversing the damage to developing smaller primers and more accurate amplification methods.

Introduction:

Throughout the relatively-recent history of forensic science, the lifespan and fragility of deoxyribonucleic acid (DNA) has been a major Achilles' heel. Vulnerable to ultraviolet light, radiation, temperature changes, and more [1], DNA may not survive for long outside of a cell if it is not being properly cared for. Despite the best lab conditions available to us today, degradation, or the breaking of the DNA into tiny, immeasurable fragments, can still occur, especially when being frozen and thawed repeatedly for analysis. Outside the laboratory in an uncontrolled environment there can be even more issues as crime scenes may not be discovered immediately after a crime has been committed, meaning any biological clues left behind by the perpetrators or victims have limited viability. Additionally, considering the nature of mass disasters for instance, the environments may be so hazardous to the DNA that it is in danger of being degraded or lost.

The problems degraded and damaged DNA causes are many, but this does not mean that there are no potential solutions. Here, methods—both forensic and otherwise—that have the potential to alleviate some of the problems caused by degraded DNA will be explored. While there are many possible techniques that could theoretically be used to treat or handle more efficiently degraded DNA, the three major methods and technologies discussed below will include: mini short tandem repeats (mini-STRs), single-nucleotide polymorphism assays (SNP assays), and multiple displacement amplification (MDA). These methods for treating DNA degradation will be compared and contrasted, with the goal of identifying if one, or a combination of more than one, are viable options for the handling and analyzing degraded DNA samples.

Obstacles Posed by Degradation:

DNA degradation is a major hindrance to forensic DNA analysis. Simply put, DNA degradation is the breaking down of DNA, where the sugar-phosphate backbone that holds the DNA together in a long strand weakens and fragments, causing the nitrogenous bases attached to the backbone to fragment and split into tiny pieces [1, 2]. It has been found that certain segments of DNA are not more or less prone to degradation than others, [3] as degradation of DNA samples can occur from a variety of outside factors, including: UV light, bacteria/mold growth, excessive moisture, changes in pH, and temperature. Ultraviolet light exposure from the sun or even lab lighting can create tiny nicks in the DNA backbone, eventually causing it to break [1, 2]. Bacteria growth can cause significant problems in a DNA setting because bacteria will have its own DNA which can contaminate the DNA sample. Additionally, bacteria, as a living organism, can break down foreign DNA which will degrade and eventually destroy the DNA sample [1]. Excessive moisture can cause similar problems; DNA exposed to high humidity will break as the ion balance is disrupted, which weakens the DNA backbone and can cause breakage [1]. Likewise, changes in pH cause the molecular interactions between the nitrogenous bases to shift and the DNA will denature, meaning the double-stranded DNA will divide into its two single halves [1]. DNA is also sensitive to temperature change; warmer temperatures cause the DNA to denature. Cold temperatures have the opposite effect, keeping the DNA moderately protected from breaking down. However, the repeated freezing and thawing of DNA in a forensic lab, DNA exposed to room temperature for an extended time, or DNA exposed to the natural elements can similarly cause denaturation and breakages in the double-stranded backbone [1].

The current method of forensic DNA analysis involves a four-step procedure: extraction, quantification, amplification, and analysis. In extraction, DNA is separated from biological fluids

and cells, then isolated from anything that is not DNA. Degradation is a problem even at this early step because more fragmented DNA means more surface area for contaminants like hemoglobin, humic acid, or indigo dyes (from blood, soil, and denim, respectively) and ions such as Mg^{2+} to bind and inhibit quantification and amplification later on [1]. While DNA can essentially be 'cleaned' of contaminants at this step, these washes are not always perfect and inhibitors can still sometimes slip through. Next, DNA is copied in quantification and amplification, though for different purposes, using a process called polymerase chain reaction (PCR). The PCR process involves the rapid heating and cooling of DNA in a solution of deoxynucleotide triphosphates (dNTPs), sequence-specific primers, and a heat-resistant DNA polymerase known as *Taq* polymerase to copy the DNA [4]. DNA degradation at this step presents a major problem because PCR relies on specific nucleotide sequences for the binding of its primers, in order for the target sequence to be accurately copied. These primers come in pairs, one forward and one reverse, that both must attach to the opposite ends of DNA sequence of interest. Then, to copy the DNA, the polymerase fills in the sequence between the primers. However, long fragments of DNA broken into many pieces will likely not have an intact region for the primer to sit down on and the polymerase to copy [2]. This will result in preferential amplification, where many of the short DNA fragments that the primers are able to bind with can be easily found and copied, but fully-intact regions of long sequences will be few in number [2]. Accordingly, that causes those larger segments of the DNA to be found and amplified less than the shorter segments. During data analysis, this may cause the samples to take on or produce a characteristic 'ski-slope' effect. In addition to preferential amplification, degraded DNA can cause allelic drop-out, where alleles that should be present are not present or visualized after capillary electrophoresis. This means entire segments of information are simply lost, which poses a severe problem for comparison or DNA

profiles and the calculation of DNA statistics, reducing the discrimination power of the profile obtained.

Overview of Possible Solutions for Degradation:

Mini Short Tandem Repeats (Mini-STRs)

Short tandem repeats, or STRs, are small units of DNA consisting of two to six nucleotides that repeat back-to-back at specific locations (or loci) on a chromosome in noncoding regions [5]. The number of times the STR sequence repeats at a specific location is the person's allele. When pattern of alleles are examined over several STR loci, they allow for genetic identification, generating a profile unique to an individual based on the number of STR repeats present at each locus. The problem with STRs, however, is their scale – because degraded DNA has been broken up, some of the STR amplicons are likely to also be fragmented, resulting in no or improper counting of repeats [5, 6]. Mini-STRs are a way to counter this problem: much like normal STRs, mini-STRs are amplified during the PCR, but the overall fragments of DNA that comprise them are significantly smaller than traditional STRs. Mini-STR primers, for use in PCR amplification, were developed by Butler, Shen, and McCord in 2003 and targeted thirteen STR loci [7]. These loci were determined to be critical to DNA analysis because of their statistical relevance and ability to discriminate between different individuals when combined. The Combined DNA Index System (CODIS), which was developed by the FBI as a database for DNA profiles to be shared across the US (so long as labs followed FBI guidelines), used these thirteen STR loci. Butler, Shen, and McCord were attempting to get the smallest amplicons possible at these locations with their mini-STRs [7]. Initially, mini-STRs were designed for use after the attack on the World Trade Center in 2001, since a majority of the DNA for identification of victims was highly damaged and

degraded [7]. Due to the small size of the amplicons, mini-STRs were an effective, alternative way to handle DNA that had been damaged in the attack and made gathering what information there was easier than the traditional STR approach [6] [8]. Today, mini-STRs still can be useful in the handling of degraded DNA samples, largely due to their small size and more reliable copying of shorter amplicons.

Single Nucleotide Polymorphism (SNP) Assays

Single-nucleotide polymorphisms (SNPs) are similar to mini-STRs, but instead of being patches of repeating nucleotides, they are points in the DNA where a single nucleotide pair is different, such as an insertion, deletion, or base substitution [9]. SNPs can vary from a single pair substitution (ex. A and T switch to C and G) which is known as binary or bi-allelic, tri-allelic (switching between three bases), or tetra-allelic (switches between all four) [10]. As expected, multi-allelic SNPs provide better discrimination power than binary SNPs [9]. Like STRs and mini-STRs, they tend to be found in noncoding regions of DNA, but not always; SNPs can be genetic markers for certain genes and can even be signifiers of underlying genetic diseases [9]. Also similar to STRs, SNPs and the surrounding DNA sequences are generally subjected to PCR as a first step in the analysis process. The SNPs, however, usually have an amplicon size of 60-80bp – significantly smaller than the approximately 250bp length of mini-STRs or the 100-450bp length of traditional STRs [11]. As a result, SNPs have excellent potential as a possible method for handling degraded DNA because the size of the DNA fragment being copied is so small [8]. However, a single SNP does not have nearly the discrimination power that one STR locus does; many more SNPs are needed to reach the same level of discrimination as a few STRs [9].

Multiple Displacement Amplification (MDA)

Multiple displacement amplification (MDA), unlike the last two methods discussed, does not rely on PCR or sequence-specific primers. MDA is an alternate form of DNA amplification that is usually done for the purpose of whole genome amplification (WGA) and involves a high-fidelity polymerase (such as Φ29, a bacterial polymerase) [12]. In a traditional PCR, sequence-specific oligonucleotide primers and the temperature-resistant bacterial enzyme *Taq* polymerase are used instead, and replication of DNA occurs through cycles of heating and cooling. MDA, however, can be done isothermally, at one temperature, and uses nonspecific hexamer primers that sit down randomly on the DNA [12]. MDA was first implemented for WGA as a method to get DNA profiles from small amounts of DNA, such as in single-cell sequencing, commonly used in analyzing DNA from museum or archeological samples. MDA can be used to obtain DNA profiles from starting template amounts of DNA below 100pg, or less than 15 cells worth of DNA, though increased allelic dropout is observable below this point [13]. To counter dropout, MDA has been paired with macromolecular crowding. Macromolecular crowding is a technique used to increase the efficiency of molecular reactions by adding in macromolecules such as proteins to a solution. This phenomenon results in molecules changing properties, which in this case increases the efficiency of the DNA amplification and results in less allelic dropout observed [13]. MDA has the potential to be used forensically, where samples are degraded and conventional PCR amplification of STRs does not produce sufficient results.

Discussion

While there may be no way to entirely reverse DNA degradation, one study in 2008 did find a partial repair mechanism. By using a combination of three different classes of enzymes (direct repair, damaged base removal, and nick translation), the researchers attempted to repair a

variety of damaged DNA samples but were met with little success [14]. The DNA could only be partially repaired and only some of that damage was even reversible in the first place. As the 2008 Nelson study discovered, the DNA damage that occurs seems to be one part repairable and another, concurrent part, is unrestorable [14]. Additionally, a major problem observed in the attempted repair of DNA is that there appears to be an even mix of allelic repair coupled with dropout. Though this study did not find massive success, it does ask the question of what other technologies or methodologies could lead to the repair of damaged and degraded DNA. Hypothetically, along the same lines as the Nelson study, if one could find a way to manipulate the DNA polymerases found within cells, this could possibly be an avenue of research for artificial DNA repair. Many methods of natural DNA repair are proofread by enzymes and polymerases; some bacterial repair mechanisms function in highly degraded environments but are prone to errors and slippage. If there was a way to manipulate or perhaps reverse engineer the proofreading capability of high-fidelity DNA polymerases and use it to essentially reconstruct the broken DNA, then scientists could use polymerases found within a victim's cells to rebuild their own DNA. If such a thing could work, there would naturally still be limitations: if the samples containing a victim's cells were old and lysed, then there would likely be no intact polymerases to reconstruct the DNA. Another study found that by using a cocktail of enzymes and ligases, including the PreCR™ mix, the Restorase™, PCRBoost™, and bovine serum albumin (BSA) within the Minifiler™ instrumentation (for mini-STR analysis), DNA showed signs of partial STR restoration [15]. Perhaps by combining these pre-amplification techniques with other DNA repair mechanisms for the goal of mini-STR or SNP analysis would be a plausible avenue for research. Additionally, such a technology might be amenable for use with MDA for purposes of better cleaning or preparing the DNA for amplification. Though more research would be needed, the prospect of enhancing

MDA with pre-amplification techniques could theoretically result in even better profiles obtained, especially if paired with the improved SNP assays. Again, there would need to be a significant amount of work put into researching and developing such technologies, including validation for use in court, but the prospect is intriguing. Because there is not currently an acceptable method to repair degraded DNA, we must focus on the techniques that are currently available to deal with degraded samples.

Mini-STRs are perhaps the most commonly known of the three technologies discussed and have been used forensically in the past, though not extensively. Mini-STRs are useful in that they can make use of smaller 'chunks' of DNA than the standard STR techniques, which makes the processing of degraded DNA more fruitful; mini-STRs would be able to glean information where standard analysis failed. Butler, Shen, and McCord's original technique of mini-STR analysis concentrated on the thirteen (at the time) core CODIS loci, which meant that any profiles developed with the mini-STR technology should have been CODIS admissible [7], but the FBI never approved their use and they were not allowed into CODIS. Additionally, of the loci targeted by the mini-STR primers, not all of them were core CODIS loci in the first place. Obviously, CODIS-admissibility would be enormously helpful in a forensic setting because even if samples obtained were severely degraded and damaged, access to CODIS would allow for a comparison to be made even where no suspect was identified. Having access to CODIS via mini-STRs would also let analysts compare family relations, such as in missing persons cases, which would allow police to narrow their investigative focus. As a result, having CODIS-admissible data from degraded DNA would be beneficial, especially in cases where standards of comparison and/or suspects are unknown. However, since the FBI updated its rules and regulations for using CODIS in 2017 by adding seven more core loci to the list of required criteria needed to upload data for a

new total of twenty core CODIS loci, mini-STRs have even further to go before they could be CODIS admissible. Though this does not make the technology any less valuable, it does restrict its effectiveness in a forensic setting. Until researchers develop primers for CODIS loci, companies develop kits using these primers, and the FBI approves the use of mini-STRs in CODIS (or sets up a separate database), mini-STRs will not be able to be used in CODIS. Disclaimer: There are some missing persons cases which *may* be allowed to use special types of DNA analysis---such as mitochondrial and Y-STRs---for comparison on CODIS, but this is highly regulated and very limited; we will not be delving into that topic in this paper for sake of length and topic clarity.

Another problem posed by mini-STRs is that they require separate kits for analysis. Although mini-STRs use the same thermocyclers and capillary electrophoresis (CE) instruments as standard STRs, they use different forensic kits that include mini-STR primers which must be validated. Not all labs have the resources to validate such kits, train the analysts to use them appropriately, nor a budget fit to accommodate their purchases. As a result, attempting to make mini-STR analysis more mainstream could face problems at labs, both private and governmental. Similarly, mini-STRs require separate training to interpret because of unique effects that appear during analysis which would put more strain on labs attempting to validate them. However, since mini-STRs are, relatively-speaking, in the same family as the standard STRs that are presently examined during forensic analysis, the technology is not too far of a step for courts or scientists to make. Mini-STRs, process-wise, are no different than the full-size STR analysis, which means that use of mini-STRs on non-degraded samples should not change the result or analysis. If all samples were routinely treated with mini-STRs, then no additional tests or analyses would have to be performed for samples that were degraded. Technically, mini-STR data *can* already be entered into CODIS when using a GlobalFiler or Fusion kit because ABI and Promega (two well-known

forensic instrumentation companies) used mini-STR primers in their kits after the FBI's expanded CODIS loci in 2017. Although, these profiles are still considered STR profiles, not specifically mini-STR profiles. The main problems with switching entirely over to mini-STRs at present is the lack of real-estate in an STR electropherogram, limiting the number of loci that can be examined in a single space, and the burden that would fall to labs across the United States in validating and using a new kit. This, also, is not addressing any potential backlash from court systems or lawyers regarding the transition, inquiring as to why mini-STRs are a better alternative and if standard-STRs are in some way, incorrect or inferior.

MDA could provide valuable insight into degraded DNA via pairing it with mini-STR analysis. Since MDA is an amplification method developed for use in single cell ecological experiments, it can handle low amounts of DNA with little error. If paired with STR analysis, the DNA would need to be chopped or cut into its desired segments after the amplification period, as MDA copies an entire sequence with nonspecific hexamer primers, which could be achieved with appropriate nucleases. PCR uses polymerases and nucleases which make copies that progressively contain just the segment of interest that the primer binds to (ex. the STR or SNP region). Despite this, if the two could be successfully combined and implemented, this would produce amplified DNA without the drawbacks of PCR. MDA forms products that are created from a branched amplification resulting from the polymerase displacing the DNA strand and as a result, have longer amplicons with less amplification bias. Yes, while MDA does still fall prey to similar problems of PCR, such as preferential amplification and primer-primer interactions, the polymerase it uses, Φ29, does not share the many problems posed by *Taq* polymerase in PCR, such as a less-than-stellar error rate, unequal amplification of smaller sequences, and the formation of dimers at high temperatures. In regard to instrumentation, theoretically, MDA would need a closed system where

a temperature and timer can be set—such as a thermocycler used in PCR, which should theoretically also be able to work with MDA—as MDA is done isothermally. Kits containing the Φ29 polymerase and other essential enzymes and nucleotides would be needed for amplification, as well as nucleases to cut the STRs zones of interest, so they can be injected and observed on the CE for analysis.

Similar to the above problems with mini-STRs, validation and implementation of new kits and, potentially, instrumentation could pose monetary and integration problems. Additionally, the possible development of an intermediate step to try to get MDA to mesh with PCR-centric technology could prove highly difficult. Along these lines, perhaps the most glaring problem is the fact that MDA has not been validated or used extensively in a forensic setting. Presently, MDA has been used primarily in WGA for ecological purposes, such as replicating bacterial DNA. Attempting to use MDA kits and instrumentation in court and prove that the science is appropriate and valid forensically would require policy changes backed by thorough analysis, which undoubtedly take a significant amount of time. Also, new training for forensic scientists would be required as analysts must be trained to use and interpret a new method or technology—this would take time as well and put a financial burden on labs that must pay to train their employees and purchase appropriate equipment. As a result, while this technology could be better in the long run, it would not be an effective solution to the analysis of degraded DNA at present due to the amount of time it would take to validate, the relative ‘newness’ of the methodology forensically, the lack of appropriate, widespread instrumentation and equipment, and the financial burden that would fall to forensic labs.

One advantage of SNPs is that they can get more information from highly degraded samples than mini-STRs; since SNPs rely on single base pair changes, so long as the 60-80 base

pair region the assay is targeting is undamaged, information can still be discovered. Therefore, even highly degraded DNA, broken into many tiny chunks, which could normally never produce good STR results, may still be useful. Several studies have even found that SNPs are more accurate and better at handling degraded DNA than mini-STRs [16] [17]. Next generation sequencing (NGS) with SNPs has proven to have several orders of magnitude higher statistical results than the standard STR CE analysis [16]. With this in mind, SNP analysis may be a way into the future of forensic DNA analysis, though it too is not without its limitations. SNPs, like mini-STRs, are also not CODIS admissible. SNP profiles are entirely different than mini-STRs and cannot be compared to them. SNPs would have to be compared to other SNP profiles in an entirely new database and because they are not currently widespread, this could make comparison---especially if no suspect is identified---significantly harder. Without a standard to compare to, no matter how good the DNA profile is, there is little value in the gathered profile. Since SNP-related data cannot be uploaded to CODIS, the information learned from degraded DNA run by SNP assays may likely have little use unless a new, separate SNP database is established. This does not take away from the value of the science or the enormous benefit highly-accurate SNPs could bring to the forensic lab, but it does limit their effectiveness. Another weakness of SNPs is, ironically, their scale; mini-STRs and regular STR-based technology have a significantly high discrimination power when combined---one of the main reasons why only thirteen core CODIS loci were reasonable to make a comparison. SNPs, because they are only single base pair polymorphisms, do not have the same discrimination power and therefore would require more of them to make an appropriate comparison. Theoretically, tri- and tetra-allelic SNPs could be used to greater effect, but these have not been thoroughly researched nor used forensically. As a result, current SNP assays simply lack the power and current research to pose a serious alternative to STR analysis, but perhaps they could be used

as an additional comparison point, such as in tandem with STR or mini-STR data. If a sample were split to be run focusing on mini-STRs and SNP assay, then perhaps it would be possible to integrate the two technologies into one's statistical calculations. If a kit could be created that utilized both SNP and mini-STR primers, scientists could benefit from both methods of analyzing degraded DNA. A hybrid profile using both mini-STR data and SNP data would likely not be admissible in a court case without sufficient study and scientific support of its efficacy and accuracy purely. Unfortunately, such a hybrid profile would also not be useful in finding familial relations because it would not be CODIS admissible. Therefore, while SNPs would be better for getting information from degraded DNA samples because of their small scale, they lack a solid background and support system both forensically and legally that would make them worthwhile as of now. With more research and perhaps with an integration into other techniques, they could one day be more useful forensically. One way to harness the potential benefits of SNPs might be to pair them with MDA. With MDA's high fidelity and ability to work with very small segments of DNA, many copies of short SNP fragments could easily be made.

Conclusion

No one technology nor methodology is a perfect answer to the problem of DNA degradation. With issues around CODIS-admissibility, limits of the technology, reagent and instrumentation costs, validation, and court admissibility, there is likely never going to be a single solution to the problems posed by degraded DNA. Despite this, a temporary solution may lie in consolidating techniques into a tiered system. If labs were to implement using mini-STR first in the face of degraded DNA and used MDA as a means of amplification in place of PCR, the results would likely have fewer random errors produced by *Taq* polymerase in PCR and more accuracy.

Additionally, if the DNA proved too degraded to handle, labs could implement SNP assays for a better profile. However, even with both of these combined strategies, the issue of court validation still arises. Practically, this could pose a problem, but if further investigation into these methodologies proves sound and suggests a better, more effective alternative to the current STR analysis and PCR method, then it would be in the best interest of forensic scientists, law enforcement, and the legal system to validate them. Overall, more research must be done into these topics and the proposed solution. Pairing technologies might create unforeseen errors but also may unlock more avenues of exploration. While DNA degradation may be a constant problem---as of now, that is---that does not mean that it always will be. By continuing to delve and experiment with polymerases and already-existing mechanisms, the answer we may be looking for may not be too far off.

Sources

[1] Butler, J.M. (2010). *Fundamentals of Forensic DNA Typing*. 2nd Ed. Academic Press/Elsevier.

[2] Alaeddini, R., Walsh, S.J., & Abbas, A. (2010). Forensic implications of genetic analyses from degraded DNA—A review. *Forensic Science International: Genetics, 4(3)*: 148-157. https://doi.org/10.1016/j.fsigen.2009.09.007.

[3] Nataas Hanssen, E., Lyle, R., Egeland, T., & Gill, P. (2017). Degradation in forensic trace DNA samples explored by massively parallel sequencing. *Forensic Science International: Genetics, 27*: 160-166. https://doi.org/10.1016/j.fsigen.2017.01.002.

[4] Saiki, R.F., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., & Arnheim, N. (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science, 230(4732)*: 1350-1354. DOI: 10.1126/science.2999980.

[5] Panneerchelvam, S. & Norazmi, M.N. (2003). Forensic DNA Profiling and Database. *Malaysian Journal of Medical Sciences, 10(2)*: 20-26. PMID: 23386793.

[6] Senge, T., Madea, B., Junge, A., Rothschild, M.A., & Schneider, P.M. (2011). STRs, mini STRs and SNPs – A comparative study for typing degraded DNA. *Legal Medicine, 12(2)*: 68-74. https://doi.org/10.1016/j.legalmed.2010.12.001.

[7] Butler, J.M., Shen, Y., & McCord, B.R. (2003). The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA. *Journal of Forensic Science, 48(5)*: 1054-64. DOI: 10.1520/JFS2003043.

[8] Westen, A.A., & Sijen, T. (2009). Degraded DNA sample analysis using DNA repair enzymes, mini-STRs and (tri-allelic) SNPs. *Forensic Science International: Genetics Supplement Series, 2(1)*: 505-507. https://doi.org/10.1016/j.fsigss.2009.08.007.

[9] Budowle, B. & van Daal, A. (2008). Forensically relevant SNP classes. *Biotechniques, 44(5)*: 603-610. https://doi.org/10.2144/000112806.

[10] Phillips, C., Amigo, J., Carracedo, Á., & Lareu, M.V. (2015). Tetra-allelic SNPs: Informative forensic markers compiled from public whole-genome sequence data. *Forensic Science International: Genetics, 19*: 100-106. https://doi.org/10.1016/j.fsigen.2015.06.011.

[11] Dixon, L.A., et. al. (2006). Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise. *Forensic Science International, 164(1)*: 33-44. https://doi.org/10.1016/j.forsciint.2005.11.011.

[12] Dean, F.B., et. al. (2002). Comprehensive human genome amplification using multiple displacement amplification. *Proceedings of the National Academy of Sciences of the United States of America, 99(8)*: 5261–5266. doi: 10.1073/pnas.082089499.

[13] Ballantyne, K.N., van Oorschot, R.A.H., & Mitchell, R.J. (2007). Increasing amplification success of forensic DNA samples using multiple displacement amplification. *Forensic Science, Medicine, and Pathology, 3*: 182–187. https://doi.org/10.1007/s12024-007-0017-2.

[14] Nelson, J. (2008). US Department of Justice. *Repair of Damaged DNA for Forensic Analysis.* https://www.ojp.gov/pdffiles1/nij/grants/227498.pdf.

[15] Robertson, J.M., et. al. (2014). Assessing PreCR™ repair enzymes for restoration of STR profiles from artificially degraded DNA for human identification. *Forensic Science International: Genetics, 12*: 168-180. https://doi.org/10.1016/j.fsigen.2014.05.011.

[16] Butler Gettings, K., Kiesler, K.M., & Vallone, P.M. (2015). Performance of a next generation sequencing SNP assay on degraded DNA. *Forensic Science International: Genetics, 19*: 1-9. https://doi.org/10.1016/j.fsigen.2015.04.010.

[17] Freire-Aradas, A., et. al. (2012). A new SNP assay for identification of highly degraded human DNA. *Forensic Science International: Genetics, 6(3)*: 341-349. doi:10.1016/j.fsigen.2011.07.010.