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Review Paper

Analyzation of degraded DNA samples-The need for advancement

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ABSTRACT

DNA fingerprinting is one of the significant methods incorporated while forensic investigation. It is considered the golden standard while investigating cases involving identical disputes. The methods used earlier for analysis involved the use of RFLP or STR. The methods stated proved to be of paramount importance but had a few cons involving the higher time consumption, lacking in specificity, being a dominant marker, and most significant being the marker dominant. The issues stated added on to the problem of analysis of degraded blood samples, which is the most common evidence found on the scene of occurrence. To solve this issue, methods involving the use of cloning via gel electrophoresis by using Sequence Characterized Amplified Region technique that enhanced the performance and make it appropriate for the co-dominant gene. The techniques such as SNP's and SCAR can be used, for analyzing the degraded blood sample, proving to be of paramount importance as a technique.

Keywords: *Degraded blood sample, DNA fingerprinting, Electrophoresis, Sequence characterized amplified region technique*

DNA fingerprinting is one of the most common methods used in identification of an individual. Analyzation of DNA remains the most crucial connection between biotechnology and forensics [1]. The science of using DNA fingerprinting as a method of identification began back in 1985 with the discovery of tandemly repeated DNA sequences unique to an individual. RFLP came into existence that enabled the certainty of identity determination to be fixed.

The very basic concept of "hypervariable region" being broken into fragments as a result of restriction endonuclease being acting so to part them from pre-known spots in the strand used for identification. The technique requires a great variety in its minisatellite composition which becomes its limitation while applying on the DNA samples being broken due the high temperature, microbial and environmental degradation. Most of the cases being masked due to the sample remaining unanalyzed due to either inability of profiling or individualization not being clear.

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Reasons for degradation

Degradation primarily is caused due to the microbial attack. The other reasons causing damage to DNA includes being exposed to harsh environmental conditions such as UV, high temperature, immersion in water and tissue decomposition [4].

The damage caused in DNA includes its hydrolysis, oxidation, cross linkages of DNA-protein as well as DNA-DNA and pyrimidine dimerization.

- **Oxidation:** The most affected site is the C=C of the pyrimidine and the imidazole ring that leads to DNA being fragmented, leading to free radical formation. Thus, modifying the base pairs.
- **Hydrolysis:** N-glycosyl bond of DNA is affected, leaving the bond without a base. Heat and humidity act as enhancers in hydrolysing the tissue. AP sites break into nick that creates a single strand break with 10-12 base pairs in between.
- **Exposure to UV:** Dimerization of C=C and T=T takes place which hangs DNA polymerase activity and the replication during PCR cannot be proceeded further.
- **Microbial digestion:** microbial flora and fauna being the most common DNA contaminant at the scene of occurrence. Increase in DNA breakage upto 25-fold [5] is seen due to microbial attack. Other factors including heat and humidity are found to enhance the microbial growth on the sample to be tested.

Problems with the degraded samples

Colonisation of PM tissue is done as a result of hydrogen peroxide and superoxide generation during anaerobic bacterial, microbial metabolism. The problem here lies the hindrance in the passage of the product which was supposed to be formed, due to the inability of Taq DNA polymerase to pass through the lesion. Hydrolysed samples could lead to either partial or complete failure of amplification. Loss of evidentiary value is seen in cases of combined UV exposure, giving a halt in PCR. Microbial degradation leads to the non -typeability of the samples to be tested, either leading to false positive or false negative test errors. The next error that exists is due to the PCR inhibitors that are extracted along with the substrate to be tested including microbial DNA, heme in blood, melanin etc leading to loss of alleles from STR loci.

ANALYSING DEGRADED DNA

Short tandem repeats

DNA has identification capacity from almost all the tissues but the integrity comes under question when a sample is highly degraded. DNA typing currently being able to use 9-21 loci of nDNA having a length of 100-500 bp. The analysis of the profile of degraded samples is done using nDNA, enabling the optimization of extraction techniques so as to enhance amplification and detection techniques [2].

The issue lies with the use of smaller pieces of DNA showing “ski-slope effect” (when analysis done on bone) with the ones having a larger loci being affected.

Mini STRs

Decreasing the target amplicon size remains the primary concern while examining highly degraded DNA [3]. The sequence was found to be disturbed by the degradation and so smaller the target, more likely to give a positive result for identification. Mini-STRs can be used for genotyping highly degraded DNA samples, having a target region of 70-280bp [5]. The basic concept behind using mini-STR is the inverse relation between sample degradation and the length of loci enabled for amplification.

Mini- STRs have proven to be a golden standard enabling testing. Studies [5] found mini-STR being successful for loci below 120 bp of degraded DNA samples. The disadvantages being lower number of loci being amplified and further separated. Comparison of STR with mini-STR shows the inability to provide more genetic information of mini-STR even after having 100% success rate.

Mini-STR are better than the previous techniques used but still it fails to meet the conditions of DNA extraction in extreme degradation conditions. The next drawback being its inability to provide complete genetic information due to restricted loci being available for analysis.

PCR inhibitors can be targeted by dilution to extract through various mixtures, or by adding Taq polymerase to increase the enzymatic activity. Quantitative real-time PCR is used for the detection of inhibitors and so enabling amplification and further fragmentation to be done.

Single Nucleotide Polymorphisms (SNPs)

Abundance of SNP is the major reason for them being used as an identification marker [6]. SNPs are generally the substitution in base or any kind of insertion, deletion that enables genomes sequencing but not variation in length. The next important reason for it to be used as a forensic marker is its(10^{-8}) low mutation rate as compared to STR($10^{-3}$to 10^{-5}).

SNPs are mostly reliably used in cases involving phenotypic character identification as well as pedigree analysis. Being divided in two types -SNP for ID and SNP intelligence. SNP for ID identifies highly degraded samples, plus point being the sample can be analysed even after having resultant DNA fragments smaller than PCR typing, loci being 45-50 bp. 49-SNP has a sensitivity of 100pg input DNA making analysis of degraded DNA easier. SNP for intelligence aims primarily on DNA phenotyping, monitoring on the key genes namely HERC2, MC1R, OCA2, SLC24A5, APIP and the other genes controlling visible characters.

SNPs having the ability to design short amplicons (45-55 bp) makes it the most suitable alternative for highly degraded DNA sample analysis.

The limitations of SNPs include them being less informative as lower numbers of loci are present [7] and the bi-allelic nature it possesses. Multiple reactions take place during SNPs thus making a poorly amplified sample. SNP bas having two alleles makes it difficult for the profile making.

Short Amplification Biomarkers (SABs)

Analysis of insertion or deletion being analysed in amplicon having less than 160bp are used for analysis of degraded samples. Studies have shown SABs have 10-fold improvement than STR and mini-STR if typically referring to analysis of degraded skeletal material.

Real time PCR

Real time PCR can detect the components on the basis of fluorescence dyes attached to the probe. The amplified copies thus formed are found to be of higher accuracy as well as sensitivity.

Quantifiler analyses the Y-chromosome target addition to the qPCR assay enables quantification for knowing the gender. Having a target of 20 pg DNA limit and amplification of 62 bp hTERT is an important forensic tool for analysis. The major reason for using commercial kit was the ability of being able to identify 1: 1000 male/female DNA [8].

Recently various alternate approaches have been used in quantification of degraded DNA including the use of Alu based assays having a detection up to 4pg. Approach used also involves quantification of single and multi -copy so as to reduce the chances of error while amplification and identification, giving better sensitive results [9].

Multiple Displacement Amplification

Multiple displacement amplification when used was able to be used as a successful circularisation of degraded sample DNA below 10ng but the very disadvantage being the loss of genetic information.

DNA REPAIR

DNA repair is of the significant approaches to be performed including whole genome amplification and the enhancement in PCR. Forensic investigation involves the major evidence composition, being degraded and in low quantity. Thus, creating a hindrance in rendering justice.

The techniques such as multiple displacement amplification have been successful in improving the quality of degraded DNA to a certain extent for STR-typing. But when carried out on highly degraded DNA the technique is not useful as the end quality product was not as desired. The need to "repair" enzymes is the future of DNA identification from degraded samples while downstream analysis.

CONCLUSION

The current techniques used for analysis have been successful in identifying degraded DNA samples (50 bp) but the end results are either not of desirable quality or unable to be processed further. STRs and mini STRs have proven to be a golden standard in identifying low amounts of DNA but still successful approaches used are not sufficient and the need of repair enzymes is of paramount importance so as to enhance the evidentiary value of the degraded blood sample recovered.

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Conflict of Interest
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