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ReviewArticle

Implications of Microarray Technology in Forensic Medicine

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ABSTRACT

Background: In Forensic medicine, microarray technology has an immense impact in the areas of investigation, to conduct in-depth Single Nucleotide Polymorphism (SNP) analysis in forensic phenotyping. That allows for the probabilistic prediction of externally visible characteristics (EVCs), such eye and hair colour, and yields useful leads for identifying suspects. The use of microarray technology to estimate the Postmortem Interval (PMI), or time since death, is another area of active research. Moreover, by observing post-mortem, gene expression, microbial DNA, and identification through biological fluids like saliva, CSF, venous blood, menstrual blood, and semen, researchers hope to increase PMI accuracy. These developments and implications of microarrays in forensic science allows for more enhanced and informative genetic analyses, ultimately strengthening forensic investigations.

Objective: This review aims to evaluate the current applications and future directions of microarray-based techniques in forensic medicine.

Methods: A systematic search was conducted in PubMed, Scopus, Web of Science, Research Gate and Google Scholar for studies published between January 2000 to March 2025. Studies focusing on forensic applications of microarrays, including gene expression profiling, identification of body fluids, time of death estimation, and toxicological assessments were included.

Results: Out of 789 initially identified articles, 34 studies met the inclusion criteria. Microarrays demonstrated significant utility in identifying specific gene expression patterns related to postmortem interval estimation, tissue-specific markers, and toxin exposure. However, variability in sample preservation, data interpretation,

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and lack of standardization limit its widespread forensic adoption.

Conclusion: Microarray technology shows promising potential in forensic medicine. Integrating it with traditional methods and next-generation sequencing could enhance accuracy and reliability in forensic investigations.

KEYWORDS

• DNA Microarray • Single Nucleotide Polymorphism (SNP) • Postmortem Interval (PMI) • Complimentary Hybridization • Toxicogenomics • RNA Profiling

Key Messages: Microarray technology enhances forensic investigations by enabling SNP-based forensic phenotyping, postmortem interval estimation, and genetic profiling. It provides high-throughput, accurate DNA analysis, surpassing traditional methods like PCR, making it indispensable in modern forensic medicine.

INTRODUCTION

Microarray technology is a vital tool in forensic investigations since it is a high-throughput analytical instrument that can analyse thousands of DNA sequences at once. The microarray is primarily founded on the basic idea of complementary hybridisation, in which samples are first amplified and labelled before being placed on a microarray chip. Special probes on the chip are used to identify genetic differences such as gene expression patterns, short tandem repeats (STRs)[1], and single nucleotide polymorphisms (SNPs). It works similarly to methods like polymerase chain reaction (PCR), Northern blot (for RNA), and Southern blot (for DNA) in this respect. Its primary benefit, though, is its capacity to produce a profile of gene expression throughout the human genome in a single hybridisation procedure.

METHODOLOGY

- Databases searched: To collect relevant literature, databases like PubMed, Scopus, Web of Science, Google Scholar, ResearchGate, and EMBASE were searched using relevant terms.
- Search terms: The study was formulated based on the following keywords/titles/concepts.
  1. Microarray and Forensic Medicine
  2. Microarray and Profiling in Forensic
  3. Microarray and Forensic Phenotyping
  4. RNA Profiling and Forensic Body Fluids
  5. Microarray and Toxicogenomics
- ***Inclusion criteria:*** Involved Free Full-text original research articles, Books and documents, Case Reports, Classical Article, Clinical Trial, Review on forensic microarray applications, published in English, maintaining time frame from January 2000 to March 2025.
- ***Exclusion criteria:*** Non-forensic applications, Newspaper Article, Observational Study, Animal only studies, Editorials, and Conference Abstracts etc were filtered and excluded in the study.
- ***Data extraction:*** Information related to study design, application domains, significant results, and envisioned future directions were systematically extracted.

Microarray Technology in Forensic Medicine: A Workflow Approach

DNA microarray technology is a powerful platform for analysing genetic diversity and gene expression, which includes a workflow from array preparation to complex data analysis. As observed in (*Figure 1*), the diagrammatic representation of DNA microarray, in this technique, the differentially expressed genes or particular SNP profiles can be identified when a sample containing target DNA is applied, Complementary sequences hybridize and fluorescence signals are recorded, and data is analysed by specialised software and databases. By examining variations in gene expression in breakdown, it helps PMI assessment. Also aids the body fluid analysis and individual identification. At the end, this technology helps researchers to understand intricate biological processes and promote progress in a number of domains.

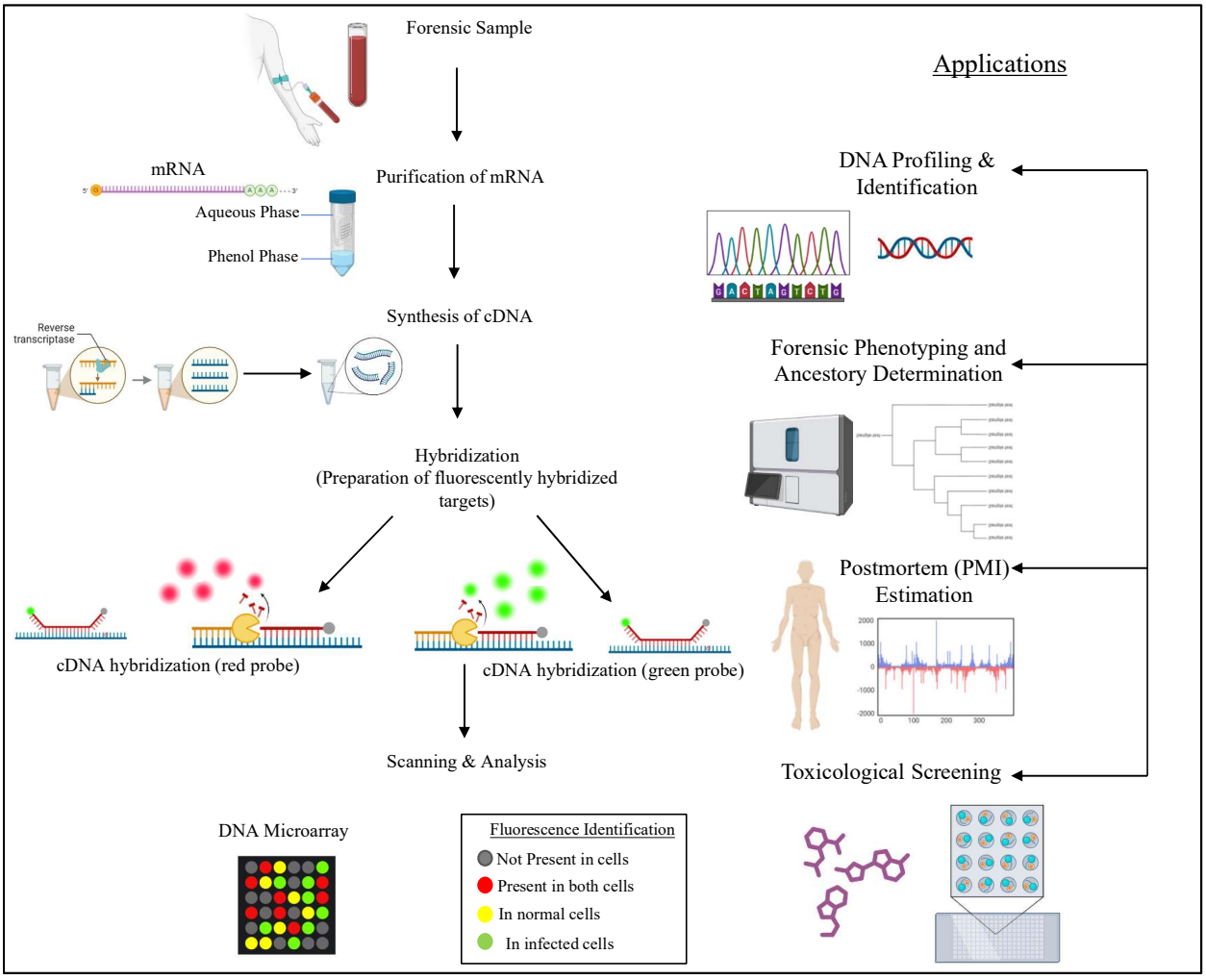

Figure 1: Pictorial Representation of Microarray Technique & its applications

APPLICATIONS OF MICROARRAY TECHNOLOGY

1. DNA Profiling and Identification

DNA testing has transformed forensic investigations by the ability to precisely identify people through the use of microsatellite amplicons (STRs), which produce a highly distinctive genetic fingerprint based on changes in the amount of short DNA sequence repeats.[1,2]

SNP microarrays are specialised technologies used to detect Single Nucleotide Polymorphisms (SNPs), which are differences in a single base pair of the genome. SNP is based on complementary hybridisation principle; it uses a single hybridisation technique in which DNA samples attach to probes on the chip for analysis. The copy number at each location is determined by comparing signal intensities from various samples SNP microarrays employ probes that match particular SNP sites in order to identify various SNP variations. The genotype of each SNP can be determined when sample DNA binds to these probes. The signal strength is then measured to determine the frequency of the different genotypes.[3]

2. Estimation of time since death or Postmortem Interval (PMI)

The post-mortem interval (PMI) refers to the estimated time elapsed between a person's death and the discovery of their cadaver. The precise estimation of this interval is the core element forensic investigations, holding critical significance in both civil and criminal proceedings. The accurate estimation of the post-mortem interval (PMI) remains a difficult and complex problem despite almost a century of intensive research on this topic. Decomposition processes are influenced by a

variety of biological and environmental factors, making it challenging to pinpoint a precise time. Despite the enhancement in estimating technique in the forensic science, forensic investigators still continued to face challenges related to consistently getting accurate results. This could be due to various factors, including microbiological decomposition, biochemical markers, physicochemical processes (rigor mortis), and physical changes (algor mortis, livor mortis), to evaluate the post-mortem interval (PMI).[4]

Furthermore, to improve PMI estimation, entomological and botanical data are also essential. Therefore in forensic research miRNAs could be important biomarker due to low molecular weight and tissue-specific expression and used for determining the post-mortem interval (PMI), quantifying wound age, and body fluids and tissues identification [5]. Scientific literature highlights that miRNA, due to their inherent stability and resistance to degradation, serve as reliable endogenous markers for post-mortem interval (PMI) estimation. Due to its property of low molecular weight and gene expression, the inference of the data obtained confirms the use of miRNAs for PMI assessment.[3] Modern technology makes it possible to detect several targets at once. High sensitivity for precise analysis is made possible by these improvements. Microarray or Next-Generation Sequencing (NGS) can be used to analyse miRNA. With these methods, hundreds of miRNAs can be found in a single experiment. Because of their excellent sensitivity, they can detect miRNAs with extremely low copy numbers. They are therefore useful instruments for biological and forensic research.[6]

3. Forensic Phenotyping and Ancestory Determination

Forensic DNA phenotyping goes beyond traditional DNA profiling, which uses STR markers to identify individuals and determine sex. This advanced method analyzes crime scene DNA to predict a person's physical traits and ancestral background, helping investigators gather more information about unknown suspects.[7] The development of forensic techniques for DNA-based predictions from biological samples found at crime scenes, as well as years of research on the genetic basis of physical traits and biogeographic ancestry, gave rise to the debate over the legalisation of forensic DNA phenotyping.[8] DNA phenotyping seeks to narrow down the pool of possible suspects by identifying the individual who are most closely match the externally visible traits and biogeographic ancestry inferred from the crime scene DNA. Even in situations when there are no eyewitnesses, it can be used. In contrast to eyewitness testimony, it offers case-specific error margins and provides probability estimations, enabling law enforcement to evaluate the accuracy of the data used in their investigation.[9]

4. Identification of Biological Fluids

Various body fluids found at crime scenes, which provides crucial evidence for forensic investigation such as plasma, tears, cerebrospinal fluid, and saliva. These body fluids provide important information for reconstructing events and identifying those involved. Each fluid type possesses unique characteristics for accurate body fluid identification at crime scene reconstruction in forensic investigations. mRNA profiling offers high sensitivity and specificity for forensic use & Tissue-specific mRNA markers remain detectable in aged biological stains stored at ambient conditions, demonstrating their potential for forensic body fluid identification[6,10] using a microarray platform, 718 human miRNAs were analyzed in total RNA from saliva, semen, vaginal secretions, and both venous and menstrual blood. The resulting distinct miRNA expression profiles enabled clear differentiation between each fluid, demonstrating the potential of miRNA analysis for robust forensic identification. Quantitative reverse transcription PCR assays currently validate microarray markers, consistently demonstrating strong overexpression of specific miRNAs within their targeted body fluids. Specifically, these assays confirm elevated miRNA expression in venous blood and semen, reinforcing the microarray's initial findings. This ongoing validation process strengthens the reliability for forensic body fluid identification. To identify distinct miRNA markers for each body fluid a multi-faceted statistical approach was employed[11]. Hierarchical clustering, SAM, and ANOVA initially delineated broad expression patterns.

Primarily, they are utilized to diagnose malignancy and pinpoint the primary tumor site, providing critical diagnostic information.[13]

The Pathwork Tissue of Origin Test, developed by Pathwork Diagnostics, provides a microarray-based approach to tumor classification. This test analyzes the expression levels of approximately 2000 genes to determine a tumor's likely origin. By comparing the gene expression patterns of the unknown tumor to those of 15 known primary tumor sites, the test generates similarity scores. These scores enable clinicians to classify the tumor, aiding in diagnosis and treatment planning[14].

5. Toxicological Assessment of Drugs

Identification of disease-associated gene expression profiles is made possible by genome-wide microarray analysis, which can measure thousands of genes simultaneously. This method is a potent screening tool in neurotoxicology that helps to clarify molecular toxicity pathways. By identifying certain genes and pathways that mediate cellular responses to hazardous chemicals, researchers can identify both susceptibility and resistance[15]. To improve healthcare, we need better drugs. Gene Chip arrays are used by pharmaceutical companies to streamline drug development.

Gene Chip microarray profiling is used in more than 40 clinical trials to predict trial outcomes and drug efficacy as well as detect disease indicators. One potent approach to pharmacogenomics is whole-genome DNA analysis, especially with microarrays that can genotype more than 100,000 SNPs. In order to identify genes associated with efficacy and toxicity the genetic profiles of drug responders and non-responders are compared. In personalised medicine, scientists are looking at large populations to determine the genetic basis of different drug sensitivities. Microarray genotyping analysis for patient classification in late-stage research ensures safer and more effective medication development. After a medicine is approved, this approach helps prove its therapeutic benefits and expand its usage to new patient groups.

Future Perspective and Emerging Trends

The need for forensic DNA analysis of evidence samples greatly surpasses existing capabilities Optimizing enzymatic processes and protocols may allow DNA microarray assays to bypass purification. Advancements in research indicate that DNA microarrays offer valuable support to criminal investigation teams. DNA microarrays can enhance case resolution from a forensic intelligence perspective. Microarrays offer immense potential for forensic investigations by screening numerous DNA sequences simultaneously. Undoubtedly, the high-throughput capability enhances forensic analysis and evidence processing. Significant progress has shown their effectiveness as powerful forensic tool paving the way of improved forensic laboratory applications. The use of Microarray technologies is growing in forensic science with continued advancements and Forensic labs are adopting it because to its increased price and efficiency. Microarray analysis is getting closer to being a common technique in forensic science. The criminal justice system is making great progress in effectively handling DNA evidence. These techniques would be used precisely for analysing even severely damaged DNA and are being improved by forensic labs. DNA analysis is becoming faster and more accurate due to innovative research. Forensic investigations are being transformed by this advancement, which guarantees quicker and more trustworthy results.

CONCLUSION

Microarray technology is transforming forensic science by aiding rapid, high-throughput genetic analysis. Despite cost challenges, advancements in software and robotics are improving its accessibility and reliability. Integration with next-generation sequencing microarrays improve toxicology, crime scene investigations, and forensic human identification, Forensic DNA phenotyping offers probability estimations supporting criminal investigations even in the absence of eyewitnesses. Furthermore, due to its stability miRNA analysis offers precise post-mortem interval estimations by advancing forensic intelligence. Beyond forensics, microarrays technology integrated with proteomics help to analyze bacterial virulence, helping vaccine development. Their potential in drug discovery and personalized medicine further underscores their significance.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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