FUTURE TRENDS IN DNA MICROARRAY TECHNOLOGY

Abstract

This article examines recent advancements in DNA microarray technology as well as applications for it. We go into great detail on the various DNA microarray or DNA chip technologies, their manufacturing procedures, and applications. This covers both low-density microarrays for various diagnostic uses and high-density microarrays for high-throughput screening applications. The described microarray fabrication methods include various inkjet and microjet deposition or spotting techniques, electronic DNA probe addressing mechanisms, and in situ or on-chip photolithographic oligonucleotide synthesis. The applications of DNA microarray hybridization that are discussed span the major domains of gene expression analysis and genotyping for point mutations, single nucleotide polymorphisms (SNPs), and short tandem repeats (STRs). In addition to the previously described molecular biology and genomic research objectives, this study involves applications of microarray devices and systems for pharmacogenomic research and pharmaceutical creation, infectious and genetic sickness and cancer diagnostics, forensic and genetic identification, and more. Additionally included is a synopsis of the advancement and application of microarray technology in the realms of contemporary cellular and proteomic research.

Keywords: DNA hybridization, DNA arrays, DNA microchips, and DNA chips.

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I. INTRODUCTION

Currently, a number of DNA microarray and DNA chip systems are offered for sale. These methods enable the highly parallelized microminiaturized analysis of hybridization between DNA and/or RNA. Research on gene expression and SNP (single nucleotide polymorphism) identification are two common uses of DNA microarray hybridization. [1]

Such microarray systems are employed for pharmacogenomic research, viral and genetic illness and cancer diagnoses, forensic and genetic identification, in addition to molecular physiologically related investigations and genomic research applications. As microarray technology continues to improve in sensitivity and selectivity, it is becoming an increasingly valuable tool for study. Genetic analysis and other significant diagnostic fields will continue to undergo revolution because of the application of DNA microarrays. The original purpose of microarray technology was DNA analysis, but it is now being applied to other areas of cellular and proteomic study.

Gene expression provides a unique insight into how cells and organisms react to changes in the external environment since it is a sensitive indication of disease state, toxicant exposure, and cellular metabolism. Gene expression levels can be measured in order to determine the mechanism of action of toxicants and to create a type of "genetic signature" based on the pattern of changes in gene expression that a chemical causes in vivo and in vitro. By contrasting unknown or suspected toxicants with recognized toxicants, these gene expression patterns would allow for quick screening of unknown or suspected toxicants. The field of toxicogenomic research has grown as a result of the ability to examine how medications and environmental stressors affect a large number of genes in a single experiment. Toxicogenomic proponents plan to use technology for mRNA and protein expression to investigate how toxins affect biological systems.

The microarray is a popular laboratory method for quickly determining gene expression or mutations. Gene chips and DNA chips are other names for these slides. Every probe is put on nylon membranes or microscope slides. Each probe has thousands of small dots with a known DNA sequence or gene. Thanks to developments in DNA sequencing technology, it is now possible to learn more about an individual's genetic composition. [2]

As technology advances, so do the techniques employed in molecular biology research. A significant fraction of genes are unsuitable for standard gene-study techniques. LAMP and GS. In a typical microarray experiment, an mRNA molecule is hybridized to the DNA template from which it was created. A range of DNA samples make up a DNA array. The amount of mRNA linked to each spot on the array represents the different genes' expression levels. They could number in the thousands. After gathering all the information, a gene expression profile for the cell is generated. [3]

A complex synthesis of many technologies and research fields, including mechanics, microfabrication, chemistry, DNA behavior, microfluidics, enzymology, optics, and bioinformatics, microarray technology has many applications, including large-scale genotyping, gene expression profiling, resequencing, and comparative genomic hybridization. [4]

The successful implementation of microarray technology required the development of many approaches and techniques for the creation of the microarrays and the identification of the probes, the performance and detection of the hybridization processes, and informatics for data processing. The signal generated by the binding of a reporter probe (fluorescent, chemiluminescent, colorimetric, radioisotope, etc.) to the target DNA sequence is frequently identified while analyzing DNA hybridization on microarrays. the microarray patterns. Among the most widely used methods for "reading" the microarrays are mass spectrometry and fluorescence scanning/imaging. Various bioinformatic techniques have been employed to convert the complex data obtained from high-density microarrays into significant insights. Automation makes using DNA microarray devices easier and simpler. It also helps to lower the number of errors caused by humans when multiplex hybridization analyses are done by hand. The development of microarray technology has involved the integration of various fields such as molecular biology, genetics, advanced microfabrication and micromachining technologies, nucleic acid chemistry, surface chemistry, analytical chemistry, software, robotics, and automation. Microarray technology is a truly successful combination of these many different scientific and technical areas. The following sections offer a summary of the major microarray technologies, along with some examples of microarray technologies that have incorporated techniques from the microelectronics industry, a collection of recent general reviews and commentary on microarray technologies and their applications, and a concluding overview of the diverse applications of DNA microarrays in research and diagnostics. [5]

II. THE EARLY HISTORY OF DNA ARRAYS

One could argue that the original DNA array was created using the colony hybridization method described by Grunstein and Hogness (1975). In this procedure, target DNA was cloned at random into E. coli plasmids, which were subsequently utilized to transform bacteria. Next, nitrocellulose filter-containing agar petri dishes were seeded with the altered bacteria. Replica plating was used to create more agar plates. The colonies on the filters were lysed, their DNA was then denatured and adhered to the filter, resulting in an unorganized and random collection of DNA spots that represented the cloned fragments. In order to swiftly identify clones with complementary DNA among hundreds of colonies, a radiolabeled probe of an intriguing DNA or RNA was hybridized. [6]

In order to create ordered arrays, Gergen et al. (1979) adjusted this method by selecting colonies into 144-well microplates. They created a jig and a mechanical 144-pin mechanism that allowed them to duplicate several microtiter plates on agar and create arrays of 1728 different colonies in a 26 by 38-cm area. A second transfer of colonies to squares of Whatman filter paper was necessary for the creation of DNA arrays on reusable filters. This was followed by the growth, lysis, denaturation, and fixing of the DNA to the filter. During the next 10 years, filter-based arrays and techniques were widely used for many various applications, such as physical mapping, cloning genes of interest, finding SNPs, and cloning genes with differential expression between two samples. [7]

In the late 1980s and early 1990s, Hans Lehrach's group automated these processes by using robotic machinery to rapidly arrange clones from microtiter plates onto filters. The simultaneous development of cDNA cloning in the late 1970s and early 1980s, along with multinational programs to completely sequence both the human genome and the human

transcriptome, led to attempts to produce reference sets of cDNAs and cDNA filter arrays for human and other genomes. Hans Lehrach's team mechanized these procedures in the late 1980s and early 1990s by quickly arranging clones from microtiter plates onto filters using robotic equipment. In the late 1970s and early 1980s, as cDNA cloning progressed concurrently with global initiatives to fully sequence the human transcriptome and genome, attempts were made to create reference sets of cDNAs and cDNA filter arrays for the human and other genomes. [8]

III. THE BIRTH OF THE MODERN DNA ARRAY

In the late 1990s and early 2000s, DNA array technology improved quickly due to the application of novel production and fluorescence detecting methods. Furthermore, improvements in our comprehension of the DNA sequences of different genomes provided us with the raw data required to build arrays that accurately represented all of a genome's genes, all of its sequence, or a significant amount of its sequence variation. It is important to note that during this time, marking relatively long DNAs on arrays was gradually superseded by the process of creating arrays using 25- to 60-bp oligos. The transition to oligo arrays was made possible by the expansion of DNA sequence data that is available to the general public. The usage of oligos, as opposed to longer sequences, boosted the specificity for the intended binding target since they could be tailored to target certain sections of the genome or genes that were most unique from other regions or genes. In-situ synthesized arrays, self-assembled arrays, and spotted arrays on glass were the three primary array types in use at this time. [9]

1. Advantages

- Information is given for thousands of genes.
- Perform just one experiment rather than several.
- Results are readily and rapidly attained.
- A significant advancement in the search for illnesses and cancer treatments.
- Different DNA fragments can be utilized to research genes.

2. Disadvantages

- The biggest disadvantage of DNA chips is their high production costs, which need a laborious and time-consuming inspection because of the massive data creation.
- The DNA chips' brief shelf life is yet another important flaw in the method.

IV. MICROARRAY DEVICES AND SYSTEMS

The development of various microarray technologies, apparatuses, and instrument systems during the last few years has made the production of DNA microarrays cheaply possible. These microarrays and systems are used in genotyping, gene expression, and other applications. Numerous methods and techniques can be used to create microarray [10] There are presently several microarray spotting technologies and methods available. The utilization of pin-based fluid transfer systems and piezo-based inkjet dispenser systems are two of the more significant spotting approaches. [11] Two more methods for producing DNA arrays are Affymetrix's in-situ photolithography-based synthesis of high-density DNA microarrays and Nanogel's electronic-based addressing of microarrays. Below, we'll discuss each of these

strategies. for additional discussions and analysis on other approaches to producing microarrays and associated technologies. [12]

1. **Spotted Arrays:** DeRisi et al. (1996) published a method that enables the synthesis of incredibly high-density DNA arrays on glass substrates. To recognize various glass slide arrays from DNA kept in crotiter plates, a robotic spotter was created. Glass microscope slides with poly-lysine coating provided efficient DNA binding. Using slotted pins, which resemble fountain pens in design, one dip of the pin in DNA solution may highlight multiple slides. Additionally, the sample may be fluorescently labeled by spotting onto glass. Using fluorescence detection provides several advantages over filter-based arrays that use radioactive or chemiluminescent markers. To begin with, fluorescence detection is quite sensitive and has a good dynamic range. Second, fluorescent labeling is frequently less expensive and difficult than radioactive or chemiluminescent marking. Third, the ability to hybridize two (or maybe more) samples to the same array while labeling them with different colors was made possible via fluorescent labeling. Since producing spotted arrays proved very difficult, comparing individually hybridized samples to presumably identical arrays may introduce illusory differences due to array-to-array variance. A two-color approach is used to measure the ratio of signals on the same array, and it is much more repeatable. [13]

2. **In-Situ Synthesized Arrays:** In 1991, Fodor and associates revealed a method for chemical synthesis that is directed by light and can be addressed spatially. To perform chemical synthesis on a solid substrate, photolithography is combined with photolabile protecting groups. The scientists demonstrated in their initial study how to independently create arrays of di-nucleotides and 10-amino-acid peptides. In 1994, Fodor and associates at the recently founded company Affymetrix demonstrated the use of this method to create DNA arrays with 256 unique octa-nucleotides. By 1995 and 1996, the reverse transcriptase and protease genes of the highly polymorphic HIV-1 genome were being mutated, and Affymetrix arrays were being utilized to measure the variety in the human mitochondrial genome. With this method, Affymetrix finally produced a wide range of DNA arrays for use in genotyping, sequencing, and expression analysis. [14]

   Blanchard and associates introduced an oligo array creation method in 1996 that fused traditional oligonucleotide synthesis chemistry with inkjet printing technology. Using customized inkjet printer heads, the four different nucleotide phosphonamidites were supplied on a glass slide that was pre-patterned with hydrophilic (with exposed hydroxyl groups) surrounded by hydrophobic (semi-transparent) patches. A surface for the phosphonamidites to attach to was given by the hydroxylated sections, while the surrounding hydrophobic portions contained the droplet(s) released by the inkjets in particular locations. This method was eventually made economically viable by Rosetta in pharmatics, and Agilent Technologies was granted a license to manufacture these arrays. The advantage of the inkjet array method is comparable to that of the Affymetrix/Nimblegen method in that an array may be made using a minimal number of chemicals. Furthermore, much like the Nimblegen method, creating a new type of array only requires sending a different set of sequence information to the printer. This has led to the inkjet array technology finding tremendous application in the production of custom, low-volume arrays. [15]

3. **Self-Assembled Arrays:** A new approach to creating arrays was created by the David Walt-led Tufts University team, which was ultimately licensed by Illumina. They used a procedure that involved synthesizing DNA on small polystyrene beads, which were then positioned on the end of a fiber-optic array that had been etched to form a well just large enough to accommodate a single bead. A range of beads would be used to manufacture different types of DNA, and when these beads were randomly put to the fiber-optic cable, an array would result. In the initial versions of these arrays, it was feasible to determine which oligo was situated at a given location on the array by optically encoding the beads with different fluorophore combinations. The greatest number of unique beads that could be recognized was limited by fluorescent labeling and optical decoding. Later and contemporary methods involve a series of steps for hybridizing and detecting many briefs, fluorescently tagged oligos in order to decode the beads. This allows a wide range of different types of beads to be used on a single array and allows the array to be functionally tested prior to use in a biological assay. Later versions of the Illumina arrays used a pitted glass surface to hold the beads instead of fiber-optic arrays. [16]

The material above is not intended to be a comprehensive history or synopsis of all DNA microarray technologies. It does, however, cover the most popular methods for creating arrays as well as the major advancements in the field. [17]

V. PRINCIPLE

Southern blotting, which entails affixing fragmented DNA to a substrate and then probing it with a known DNA sequence, is where the DNA microarray technique got its start. The idea of nucleic acid strand hybridization lends support to the DNA microarray. Complementary nucleic acid sequences can form hydrogen bonds with one another to complement nucleotide sequences. Fluorescent dyes are used to identify and hybridize at least two samples to the chip. Because there are a lot of complimentary base pairs in the nucleotide sequence, the non-covalent bonds between the two strands are stronger. [18]

Hybridization between two DNA strands is the fundamental idea behind microarrays. Complementary nucleic acid sequences can specifically pair with each other by forming hydrogen bonds between complementary trinucleotide base pairs and complementary trinucleotide base pairs and complementary trinucleotide base pairs and complementary trinucleotide base pair and complementary trinucleotide base pair and complementary trinucleotide base pair and complementary trinucleotide base pair and complementary trinucleotide base pair and complementary trinucleotide base pair and complementary tri The non-covalent bond between the two strands of a nucleotide sequence is stronger when there are a lot of complementary base pairs in it. [19]

Target sample concentration influences the signal, which is influenced by hybridization conditions (temperature, post-hybridization washing, etc.).[20]

Steps for preparing microarray experiment

- Sample collection
- mRNA isolation
- The production of labelled cDNA

- Hybridization of DNA
- Data gathering and analysis

1. **Sample collection:** Any cell or tissue that we choose to investigate can serve as a sample. Two types of tests—infected and non-infected cells—are frequently collected in order to investigate and ascertain the result.

2. **MRNA isolation:** An extractor such as phenol-chloroform or a column can be used to remove RNA from a sample. The extracted RNA is split into messenger RNA, transfer RNA, and mRNA. Poly-T tail column beads bind mRNA because it has a poly-A tail. Buffer is used to wash the column after extraction to remove the mRNA from the beads.

3. **Production of labelled DNA:** Reverse transcription of mRNA results in the creation of cDNA. The different fluorescent probes are then mixed with both samples to form fluorescent cDNA strands that allow different cDNA sampling classes to be identified.

4. **Hybridization of DNA:** Each sample's labeled cDNA is hybridized with its matching strand on a DNA microarray. After that, they are carefully cleaned to remove any mismatched sequences.

5. **Data gathering and analysis:** The information is being obtained by a microarray scanner. The scanner consists of a laser, a computer, and a camera. Signals are produced when the fluorescence of the cDNA is stimulated by the laser. The images that the laser creates while scanning the array are captured by the camera. After that, the computer saves the information and produces prompt outcomes. We are still evaluating the data. The distinct color intensity of each spot provides information about the kind of gene that is present there. [21]

6. **Photolithography:** The Greek word photolithography means "light stone" and describes a technique that uses light to find patterns in semiconductor materials. Photolithography is used to define and transfer a design to each layer. It is a process for transferring designs into a silicon wafer-based substrate that is used for photolithography. The method yields binary patterns that are limited to depth and greyscale. In a microsystem, every layer has a different pattern. This design is transferred from a mask to a photosensitive layer via photolithography. At a separate step of the process, the pattern is transferred from the photosensitive material to the non-photosensitive substance. The resist is removed from a fresh layer that is layered on top of an old one following the pattern transfer. [22]

VI. THREE STEPS OF PHOTOLITHOGRAPHY

- Coat
- Expose
- Develop

1. **Coating Step: Surface Preparation**

- In most cases, surface conditioning occurs before to the photoresist. Wafer surface preparation is followed by implant preparation. It is possible to create photoresist by offering a spotless surface. The wafer is coated with a chemical to increase adhesion.
- Following baking to eliminate the water molecules from the wafer surface, HMDS is applied (primed) to produce a hydrophobic surface.
- Give the wafer time to thaw and come to room temperature.

A vacuum chuck holds the wafer firmly in place. A hoover holds the wafer in place. Chuck accelerates to the proper thickness and spins till the film has dried.

The photography industry had an impact in the development of photoresist ants. There are resists available for both general-purpose use and specialized purposes. Often, they are tuned to a specific frequency. The parts of a photoresist are as follows:

- **Polymer:** A polymer whose structure is altered by exposure to light. Generally, the desired feature is a change in solubility in a particular solvent.
- **Solvent:** The resist is thinned using a solvent before application. A process that spins on the wafer Generally, the soft bake process removes the solvent by heating the mixture to approximately 100 degrees Celsius.
- **Sensitizer:** These substances are employed in the process exposure to regulate the chemical reaction.
- **Additives:** Are chemicals added to a process with the intention of achieving a specific result. Optical resists, or photoresists that react to UV or visible light, are another name for photoresists. There is a distinct resistance to other types of radiation, such as x-rays and e-beams.

Photoresists are classified into two categories.

- **Positive resists:** These become more soluble when exposed to UV light.

- **Negative resists:** These resists become less effective when exposed to UV light.

- **Softback:** A softback approach is used once the photoresist has been applied to the necessary thickness. "Softback" refers to the process of removing a coating of fat from the solvents that remain after the photoresist has been cleaned. The wafer is baked and let to cool to room temperature after the softback.

- **Alignment:** The design is transferred from the mask to the photoresist on the wafers during the alignment and exposure procedure. The mask is aligned using alignment markings. The process of "alignment" is a critical one in the creation of microsystems. With an imbalance of one millimeter or less, the device, along with all the other devices on the wafer, could malfunction. According to guidelines, every layer has to be properly aligned with the levels above and below it.

2. **Expose:** The light source's UV rays pass through the mask and interact chemically with the resist. Only the parts of the face not covered by the mask receive the treatment.

3. **Developing:** After the wafers are exposed and aligned, they need to be developed. A substance called developer is applied to the wafers, and it reacts with the exposed photoresist. The resist determines which developer is best. Positive photoresists have their exposed areas removed, which makes them more soluble, in contrast to negative photoresists, which have their unexposed regions removed. The most typical kind of development is a chemical reaction that is moist. Until all of the resist is gone, the wafers are submerged in the developing solution for a certain amount of time. Bake the remaining resist at 250 degrees Celsius (sometimes known as a "hard bake") to solidify it. We are still in the early phases of this practice. The resist stops degradation of the wafer beneath. After the final pattern is produced on the wafer, the leftover resist is removed using a technique called resist stripping. This can be done dry using a solvent or wet with an acid mixture. The process is called dry oxygen plasma etching. The wafers are then dried and cleaned. [23]

VII. DNA MICROARRAY TYPES

- Microarrays based on cDNA
- Microarrays based on Oligonucleotide

1. **Microarrays based on cDNA:** Target DNA microarrays are based on the mechanical or ink jet micro spotting printing of cDNA clones with sizes ranging from several hundred DNA strands to several kilobases on a glass surface. Reverse transcriptase is used to label the sample and rRNA with fluorescent Cy5 or Cy3 dyes. Next, at night, the cDNAs are hybridized to the arrays. A fluorescence laser scanner is used to scan the slides after they have been cleaned. The red/green ratio of each spotted array piece can be used to estimate the proportion of transcripts in the samples. Chips are made with oligonucleotide bases.

2. **Microarrays based on Oligonucleotide:** While in situ synthesis on glass use oligonucleotide chemistry and photolithography, some manufacturers build their arrays by locating readily accessible oligonucleotides. In this particular form of spotted probe, each gene is represented by 20–25 short chemically produced sequences. Shorter probe lengths have a number of benefits, such as the ability to study small genomic regions and polymorphisms and fewer errors in probe manufacturing. [24] Covalent linkage is employed to bind probes to glass slides since electromagnetic immobilization and inter-linking, due to their low weight, may result in significant probe loss during washing procedures. Modified 5′ to 3′ ends are used to attach probes to the microarray ground on functionally group-coated slides. [25]

VIII. APPLICATION

Tumor formation requires many gene variants and cell changes occurring simultaneously. Researchers can benefit from the DNA chip since it allows for the simultaneous study of multiple genetic samples. The identification of single nucleotide polymorphisms (SNPs) and mutations, the classification of tumors, the discovery of tumour suppressor target genes, the discovery of cancer biomarkers, and the discovery of tumour suppressor genes all benefit greatly from its use. For instance, we may compare the expression patterns of different genes in cancer patients and healthy individuals to identify

the gene associated with that specific type of cancer. Additional examples include gene linkages, medication development, and chemical resistance. [26]

Gene microarrays have been used for genome comparative hybridization. This method uses chemically synthesized genomic DNA to search for gene loss or amplification. Using the matrix-based comparative gene hybridization (aCGH) technique, genetic abnormalities in several tumor forms, such as melanoma, lymphoma, bladder and ductal carcinoma, fallopian tubes, gastric carcinoma, and breast carcinoma, have been mapped. [27] It is imperative to take into account the possibility that non-invasive malignancies could develop into invasive tumors. The RhoC gene was found to be higher expressed in metastatic cells compared to non-metastatic cells when Clark et al. looked at the genetic makeup of metastatic melanoma cells. We can uncover gene families and important cellular and molecular events that may be connected to intricate processes like metastasis because of microarray-based expression profiling. The management of patient prognosis, diagnosis, and therapy are examples of future use cases. Clinicians will be able to verify pharmacological mechanisms of action and evaluate drug sensitivity and toxicity by using DNA microchips during therapeutic trials. They can create a new molecular categorization system for cancer by using gene expression patterns, for example, to categorize malignancies into prognostic categories. Microarray technology can be used to study functional genomes, gene function in relation to gene regulatory networks, molecular phenotype profiling, and sickness classification. Two fields of research are pharmacology and developmental biology. [28]

1. **Gene Expression Analysis:** Measuring the levels of gene expression has been the primary application of DNA microarrays. This application extracts the RNA from the target cells and uses the Eberwine amplification method to turn it into cRNA. Alternatively, the RNA can be directly labelled, transformed into a labelled cDNA, or transformed into a T7 RNA promoter-tailed cDNA. Fluorescent nucleotides labeled during synthesis, biotin-labeled nucleotides stained with fluorescent streptavidin, modified reactive nucleotides to which a fluorescent tag is subsequently added, and different signal-amplification techniques are just a few of the numerous methods that have been developed for labeling cDNA or cRNA. A preliminary analysis of several labeling techniques is given. The two most popular approaches are the addition of fluorescently tagged nucleotides at the cRNA or cDNA synthesis stage and the addition of a biotin-labeled nucleotide (as performed by Affymetrix) during the cRNA synthesis step. After being washed and hybridized to the microarray, the tagged cRNA or cDNA is then measured for fluorescence at each spot to ascertain the signal. After hybridization, fluorescently-labeled streptavidin is utilized to stain the array when biotin-labeled materials are used. A scanning confocal microscope is usually used to measure laser-induced fluorescence. The signal strength at each location determines the level of gene expression at that location. [29]

2. **Transcription Factor Binding Analysis:** Microarrays and chromatin immunoprecipitation have been used to identify the binding locations of transcription factors. Summary of DNA Microarrays in Brief 22.1.6 Expansion 101 DNA is broken up and transcriptional factors (TFs) are cross-linked to it using formaldehyde, according to Current Protocols in Molecular Biology. The necessary TF(s) are affinity purified using a TF-specific antibody or by tagging the transcription factor with a peptide that is compatible with affinity chromatography, all the while maintaining the DNA to which

they were connected. The DNA is extracted from the TF, amplified, tagged, and hybridized to the array following purification. [30]

The size distribution of the fragment length and the array's architecture are connected because TFs typically bind distant from the genes they control. For example, the array needs probes that can examine the DNA area that the TF has been attached to. The ChIP-chip technique can be performed using the same arrays used for gene expression research since yeast and bacteria have very small intergenic regions. Large intergenic regions exist in the genomes of mammals, and the TF usually binds thousands of base pairs far from the target gene. [31]

3. **Genotyping:** When using single nucleotide polymorphism (SNP) genotyping techniques, microarrays have been widely utilized. The most widely used alternate techniques for identifying SNPs are the allele-specific extension and ligation to a "bar-code" oligo hybridized to a universal array, Affymetrix's allele discrimination by hybridization, and techniques that involve extending the arrayed DNA across the SNP in a single nucleotide extension reaction. Background interferes with allelic discrimination by hybridization because complex genomes exhibit nonspecific hybridization. The SNP genotyping technologies from Illumina and Affymetrix have both seen great success and are often employed. Repeatable SNP calls have call rates (the percentage of SNPs on the array that can be reliably called) greater than 99.5 percent. Finding copy-number variants can also be done using the same arrays or variants of them. [32]

4. **Data Standards and Data Exchange:** Microarrays may have been the first technological advancement that allowed researchers to access large volumes of complex digital data prior to DNA sequencing. As the technology was embraced, it quickly became clear that detailed instructions on the array, sample, protocols, and data processing methods were required so that other researchers could duplicate a specific microarray experiment. It also became clear that other people might perform research and meta-analyses (on combinations of data) that the original data developers had not thought of if they had access to the raw and processed data. Members of the Microarray Gene Expression Data Society (now the Functional Genomics Data Society; http://www.FGED.org) developed the MIAME (Minimum Information About a Microarray Experiment) standards for the description of microarray experiments and for the exchange of microarray data in order to address these issues of reproducible science and data exchange. These efforts had an impact on the development of open databases for microarray data as well as later standardization initiatives in other fields. [33]

IX. LIMITATIONS OF DNA MICROARRAYS

Simply said, microarrays are instruments for concurrently ascertaining the relative abundances of many distinct DNA or RNA sequences. They have a few drawbacks, yet they work well for a wide range of purposes. Firstly, arrays offer a proxy for relative concentration. Stated differently, the general practice is to assume that the concentration of a single, hypothetical species in solution that has the potential to hybridize to a certain place on a microarray correlates with the signal detected there. [34]The array will become saturated at high concentrations, while at low values, equilibrium favors no binding. This results in a signal that is only linear across a small range of solution concentrations. Second, creating

arrays where numerous related DNA/RNA sequences won't bind to the same probe on the array can occasionally be difficult, if not impossible, especially for big mammalian genomes. If genes B, C, and D have a high degree of sequence similarity with gene A, then a sequence on an array intended to identify gene A may also detect genes B, C, and D. This can be especially difficult for gene families and genes that have several splice variants.It should be noted that in order to accurately design arrays to identify splice variants, array probes may be built for each exon in the genome or for exon junctions. It is difficult to design arrays that can reliably detect every exon or gene in genomes with several connected genes. [35]

Finally, a DNA array can only recognize sequences for which it was designed. In other words, species of RNA or DNA that are present in the solution being hybridized to the array but for which there is no complementary sequence on the array will not be recognized. This frequently implies that genes that have not yet been found in a genome will not be represented on the array for the purposes of gene expression analysis. An array typically lacks non-coding RNAs that have not yet been confirmed as expressed. Furthermore, for very diverse genomes, like those found in bacteria, arrays are frequently made using information from a reference strain's genome. Such arrays could lack a large amount of the genes found in a particular isolate of the same species. For instance, there may be up to a 20% difference in the gene content of any two isolates of the bacterial species Aggregatibacter actinomycetemcomitans. As a result, an array made utilizing gene annotation from a reference isolate will lack many of the genes discovered in other isolates. [36]

X. THE FUTURE OF DNA ARRAYS

With arrays' aforementioned drawbacks, it would be far preferable to have an impartial method for directly and objectively quantifying each DNA or RNA species found in a given sample. With the possible exception of genotyping, all assays now have sequencing costs that are comparable to microarrays thanks to next-generation sequencing technologies. This wasn't always the case. Sequencing is superior to microarrays in many ways when costs are same. Direct measurement of the nucleic acids in solution is made possible by sequencing. To determine the abundance of a particular sort of sequence, one need merely count how many of it there are. Because counting sequences is linear in terms of concentration, the only thing that can limit the signal to noise ratio that can be created by sequencing is the number of reads employed for each sample. [37] Sequencing may provide an objective way to identify which nucleic acids are in solution. While sample preparation or other enzymes may distort the results, sequencing does not necessitate knowledge of the possible nucleic acids present, in contrast to DNA arrays. RNA editing, unique splice variants, and closely related gene sequences that would have missed discovery on DNA microarrays owing to cross hybridization can all be found through independent sequencing. Owing to these benefits as well as a drop in sequencing costs, sequencing has quickly replaced DNA arrays for almost all previously conducted microarray studies. Over the next five to ten years, sequencing techniques are expected to completely replace DNA arrays, since the cost of sequencing is currently decreasing by a factor of two every five months. [37]

XI. CONCLUSION

This review has provided clarity on the many stages of the microarray technology. There are now just a few commercial items available due to budgetary constraints; however,

the technique's potential may expand if additional commercial products become available. Understanding molecular biology is aided by the ability to gather a large number of historical samples and analyze them for distinctive genetic variants. The research of oral disorders could greatly benefit from the use of DNA microchips. Classifying oral diseases according to DNA, RNA, or protein profiles will greatly improve our ability to identify, intervene, monitor, and care for patients. Currently, the DNA chip is primarily utilized in research. Future dental practices will be significantly impacted by DNA microchips, which offer much superior, individualized, and bio-based oral care.

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