**FUTURE TRENDS IN DNA MICROARRAY TECHNOLOGY**

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**ABSTRACT**

This article examines recent advancements in DNA microarray technology as well as applications for it. The many types of DNA microarray or DNA chip systems, as well as their manufacturing processes and applications, are thoroughly discussed. This covers both low-density microarrays for various diagnostic uses and high-density microarrays for high-throughput screening applications. In situ or on-chip photolithographic oligonucleotide synthesis, various inkjet and microjet deposition or spotting techniques, and electronic DNA probe addressing strategies are all included in the disclosed microarray production methods. The key areas of gene expression analysis and genotyping for point mutations, single nucleotide polymorphisms (SNPs), and short tandem repeats (STRs) are covered by the DNA microarray hybridization applications that are addressed. This study includes applications of microarray devices and systems for pharmacogenomic research and medication development, infectious and genetic illness and cancer diagnostics, and forensic and genetic identification in addition to the aforementioned molecular biology and genomic research goals. A summary of the development and use of microarray technology in the fields of recent proteomic and cellular research is also provided.

**Key words:** DNA hybridization, DNA arrays, DNA microchips, and DNA chips.

**INTRODUCTION**

There are now several DNA microarray and DNA chip devices and systems available for purchase. These techniques allow for the highly parallelized microminiaturized examination of DNA and/or RNA hybridization. Applications for DNA microarray hybridization frequently centre on SNP (single nucleotide polymorphism) detection or gene expression research. [[[1]](#endnote-1)]

In addition to molecular physiologically connected studies and genomic research applications, such microarray systems are used for pharmacogenomic research, infectious and genetic sickness and cancer diagnostics, forensic and genetic identification. Microarray technology is becoming a more useful research tool as its sensitivity and selectivity keep rising. Thanks to the use of DNA microarrays, genetic analysis and other important diagnostic domains will continue to experience revolution. Microarray technology, which was first developed for DNA analysis, is also being used in new proteomic and cellular research fields.

Since gene expression is a sensitive indicator of toxicant exposure, disease state, and cellular metabolism, it offers a unique perspective on how cells and organisms respond to changes in the external environment. Through the measurement of gene expression levels, it is possible to establish the mechanism of action of toxicants and to produce a kind of "genetic signature" based on the pattern of gene expression changes that a chemical generates both in vitro and in vivo. These gene expression profiles would enable fast screening of unknown or suspected toxicants by comparing unknown or suspected toxicants to known toxicants. The capacity to investigate the effects of drugs and environmental stressors on a substantial number of genes in a single experiment has led to the development of the field of toxicogenomic. Supporters of toxicogenomic intend to employ mRNA and protein expression technologies to look into the effects of chemicals on biological systems.

A common laboratory technique for swiftly identifying gene expression or mutations is the microarray. These slides are also known as gene chips or DNA chips. Each probe, which has thousands of tiny spots with a known DNA sequence or gene, is placed on microscope slides or nylon membranes. Finding out more about a person's genetic makeup is now feasible because to advancements in DNA sequencing technology. [[[2]](#endnote-2)]

The tools used in molecular biology research change as technology does. A huge proportion of genes cannot be studied using conventional methods. GS and LAMP. An mRNA molecule is hybridized to the DNA template from which it was produced in a typical microarray experiment. A DNA array consists of a variety of DNA samples. The expression levels of the various genes are represented by the quantity of mRNA associated to each location on the array. There might be thousands of them. A gene expression profile for the cell is produced when all the data has been acquired. [[[3]](#endnote-3)]

Large-scale genotyping, gene expression profiling, comparative genomic hybridization, and resequencing are just a few of the many uses for microarray technology, which is a complex synthesis of many technologies and research fields, including mechanics, microfabrication, chemistry, DNA behaviour, microfluidics, enzymology, optics, and bioinformatics. [[[4]](#endnote-4)]

The development of several methodologies and techniques for creating the microarrays and identifying the probes, performing and detecting the hybridization processes, and informatics for data analysis were all necessary for the effective application of microarray technology. When analysing DNA hybridization on microarrays, the signal produced by the binding of a reporter probe (fluorescent, chemiluminescent, colorimetric, radioisotope, etc.) to the target DNA sequence is often detected. patterns in microarrays. Two of the most popular techniques for "reading" the microarrays are fluorescence scanning/imaging and mass spectrometry. Several bioinformatic algorithms have been utilised to transform the complicated data from high-density type microarrays into meaningful information. The usage of DNA microarray devices is made more simpler and easier by automation, which also helps to reduce the number of human mistakes that would be made if multiplex hybridization analyses were performed manually. Molecular biology, genetics, advanced microfabrication and micromachining technologies, nucleic acid chemistry, surface chemistry, analytical chemistry, software, robotics, and automation have all been integrated into the development of microarray technology. A genuinely effective synthesis of these numerous various scientific and technical disciplines may be seen in microarray technology. The sections that follow provide a compilation of recent general reviews and comments on microarray technologies and their applications, a summary of the key micro arraying technologies, with a few examples of microarray technologies that have made use of methods from the microelectronics sector, and a final overview of the various uses of DNA microarrays in research and diagnostics. [[[5]](#endnote-5)]

**THE EARLY HISTORY OF DNA ARRAYS**

It might be argued that Grunstein and Hogness's (1975) colony hybridization technique was used to generate the initial DNA array. This process involved randomly cloning DNA of interest into E. coli plasmids, which were then used to convert bacteria. The transformed bacteria were then seeded onto agar petri plates containing nitrocellulose filters. Additional agar plates were made using replica plating. In order to create a disorganized and random collection of DNA spots that represented the cloned fragments, the colonies on the filters were lysed, and their DNA was then denatured and glued to the filter. A radiolabeled probe of an interesting DNA or RNA was hybridized to quickly screen hundreds of colonies for clones with DNA that was complimentary to the probe. [[[6]](#endnote-6)]

By selecting colonies into 144-well microplates, Gergen et al. (1979) modified this strategy to produce ordered arrays. In order to duplicate many microtiter plates on agar and make arrays of 1728 distinct colonies in a 26 38-cm space, they developed a mechanical 144-pin device and a jig. The fabrication of DNA arrays on filters that could be reused repeatedly required a second transfer of colonies to squares of Whatman filter paper, followed by growth, lysis, denaturation, and fixing of the DNA to the filter. The following ten years saw the widespread use of filter-based arrays and methods for a number of purposes, including the cloning of genes of particular interest, the discovery of SNPs, the cloning of genes with differential expression between two samples, and physical mapping. [[[7]](#endnote-7)]

Hans Lehrach's team mechanized these procedures in the late 1980s and early 1990s by employing robotic equipment to quickly array clones from microtiter plates onto filters. Attempts to produce reference sets of cDNAs and cDNA filter arrays for human and other genomes were made as a result of the concurrent development of cDNA cloning in the late 1970s and early 1980s together with multinational programmed to completely sequence both the human genome and the human transcriptome. The production of sets of PCR products representing all of the known open reading frames (ORFs) in small genomes was made possible by the widespread availability of sets of nonredundant cDNAs in the late 1990s and early 2000s. Individual laboratories were able to create their own cDNA or ORF arrays containing the vast majority of genes in a genome using these sets in conjunction with easily accessible robots. [[[8]](#endnote-8)]

**THE BIRTH OF THE MODERN DNA ARRAY**

DNA array technology advanced quickly in the late 1990s and early 2000s thanks to the use of new manufacturing and fluorescence detection techniques. Additionally, advances in our understanding of the DNA sequences of various genomes gave us the raw data we needed to create arrays that accurately represented all of the genes in a genome, all of its sequence, or a substantial portion of its sequence variation. It should be emphasized that at this period, the practice of manufacturing arrays using 25- to 60-bp oligos gradually replaced the practice of marking relatively lengthy DNAs on arrays. The increase in the amount of publicly accessible DNA sequence data allowed for the switch to oligo arrays. Since oligos could be made to target specific portions of genes or the genome that were most distinct from other genes or regions, their use (as opposed to larger sequences) increased the specificity for the targeted binding target. During this period, there were three main types of arrays in use: spotted arrays on glass, in-situ synthesized arrays, and self-assembled arrays. [[[9]](#endnote-9)]

**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.

**DISADVANTAGES**

* The greatest drawback of DNA chips is their expensive manufacturing costs, which also need a time-consuming and difficult examination due to the excessive production of data.
* The short shelf life of the DNA chips is another significant drawback of the technique.

**MICROARRAY DEVICES AND SYSTEMS**

The ability to create DNA microarrays is now economically feasible because to the several microarray technologies, apparatuses, and instrument systems that have been created during the past few years. Gene expression, genotyping, and other uses make use of these microarrays and systems. Microarrays may be made utilizing a variety of processes, as well as a variety of different approaches. [[[10]](#endnote-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]](#endnote-11)] Affymetrix's in-situ synthesis of high-density DNA microarrays using photolithography and Nanogel's electronic-based addressing of microarrays are two further techniques for creating DNA arrays. We'll talk about both of these approaches below. for more analyses and debates on various strategies for creating microarrays and the related technology. [[[12]](#endnote-12)]

**Spotted Arrays**

A technique that makes it possible to create extremely high-density DNA arrays on glass substrates was published by DeRisi et al. in 1996. A robotic spotter was developed to identify multiple glass slide arrays from DNA stored in crotiter plates. Poly-lysine-coated glass microscope slides offered strong DNA binding. A single dip of a pin in DNA solution might highlight numerous slides when utilising slotted pins, which have a design like that of fountain pens. The sample might be fluorescently labelled by spotting onto glass as well. Compared to the radioactive or chemiluminescent labels that are typical of filter-based arrays, fluorescence detection offered a number of benefits. First off, fluorescence detection has a good dynamic range and is highly sensitive. Second, compared to radioactive or chemiluminescent marking, fluorescent labelling is often less costly and less challenging. Third, fluorescent labelling made it possible to hybridise two (or maybe more) samples to the same array while labelling them with various colours. Comparisons of individually hybridised samples to presumably similar arrays might create spurious differences owing to array-to-array variance because it was exceedingly challenging to produce spotted arrays in a reproducible manner. The ratio of the signals on the same array is assessed using a two-color method, which is far more repeatable. [[[13]](#endnote-13)]

**In-Situ Synthesized Arrays**

 A technique for light-directed, spatially addressable chemical synthesis was disclosed in 1991 by Fodor and colleagues. It combines photolabile protecting groups with photolithography to carry out chemical synthesis on a solid substrate. In their original research, the authors showed how to produce arrays of di-nucleotides and 10-amino-acid peptides individually. Fodor and colleagues at the newly established firm Affymetrix showed the application of this technique in 1994 to produce DNA arrays with 256 distinct octa-nucleotides. By 1995 to 1996, Affymetrix arrays were being used to quantify diversity in the human mitochondrial genome and to find mutations in the reverse transcriptase and protease genes of the highly polymorphic HIV-1 genome. Affymetrix eventually created a large selection of DNA arrays for use in expression analysis, genotyping, and sequencing using this technique.  [[[14]](#endnote-14)]

In 1996, Blanchard and colleagues presented a technique for creating oligo arrays that combined inkjet printing technology with conventional oligonucleotide synthesis chemistry. The four distinct nucleotide phosphonamidites were delivered on a glass slide that was pre-patterned with hydrophilic regions (with exposed hydroxyl groups) surrounded by hydrophobic regions using modified inkjet printer heads. The surrounding hydrophobic portions confined the droplet(s) released by the inkjets in specific places, while the hydroxylated regions provided a surface to which the phosphonamidites could bond. Rosetta in pharmatics finally made this technique commercially viable and licensed it to Agilent Technologies, which currently manufactures these arrays. The benefit of the inkjet array method is similar to that of the Affymetrix/Nimblegen method in that only a few chemicals are required to create an array. Additionally, the creation of a new sort of array merely needs a different set of sequence information to be sent to the printer, much like the Nimblegen method. As a result, the creation of bespoke arrays that are manufactured in low volume has found great utility for the inkjet array technology. [[[15]](#endnote-15)]

**Self-Assembled Arrays**

The Tufts University team led by David Walt developed a different method for building arrays, which they later licensed to Illumina. As part of their technique, DNA was synthesized on tiny polystyrene beads, which were then placed on the end of a fiber-optic array with fibre ends that had been etched to create a well that was just big enough to hold one bead. Different forms of DNA would be synthesized on various beads, and when a variety of beads were applied to the fiber-optic cable, an array would be created at random. Early iterations of these arrays included optically encoding the beads with various fluorophore combinations, making it possible to identify which oligo was located at which position on the array. The maximum number of distinct beads that could be identified was restricted by optical decoding by fluorescent labelling. In order to decode the beads, later and current techniques require a series of processes for hybridizing and detecting a number of briefs, fluorescently labelled oligos. This makes it possible to employ a very big variety of various kinds of beads on a single array while also functionally testing the array before using it in a biological assay. Instead, then using fiber-optic arrays to hold the beads, later iterations of the Illumina arrays employed a pitted glass surface. [[[16]](#endnote-16)]

The information provided above is not meant to represent an exhaustive history or overview of all DNA microarray technologies. It does, however, cover the key developments in the subject as well as the most common ways to make arrays. [[[17]](#endnote-17)]

**PRINCIPLE**

DNA microarray technique originated from Southern blotting, which involves attaching broken DNA to a substrate and then probing it with a known DNA sequence. The DNA microarray is supported by the notion of nucleic acid strand hybridization. In order to complement nucleotide sequences, complementary nucleic acid sequences can create hydrogen bonds with one another. At least two samples are hybridized to the chip and are labelled with fluorescent dyes. The nucleotide sequence has many complementary base pairs, which means that the non-covalent connections between the two strands are stronger. [[[18]](#endnote-18)]

The fundamental principle underlying microarrays is hybridization between two DNA strands, the ability of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between 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 A nucleotide sequence with many complementary base pairs has a stronger non-covalent connection between the two strands.[[[19]](#endnote-19)]

The amount of target sample present affects the signal, which is affected by the hybridization circumstances (such as temperature, washing after hybridization, and so on). [[[20]](#endnote-20)]

**Steps for preparing microarray experiment**

1. Sample collection
2. mRNA isolation
3. The production of labelled cDNA
4. Hybridization of DNA
5. Data gathering and analysis

**1.Sample collection**

A sample might be any cell or tissue that we want to study. In order to examine and determine the outcome, two sorts of tests, namely infected and non-infected cells, are often gathered.

 **2.mRNA isolation**

Using a column or a diluent like phenol-chloroform, RNA may be extracted from a sample. mRNA, messenger RNA, and transfer RNA are separated from the extracted RNA. As mRNA has a poly-A tail, it is bound by poly-T tail column beads. After extraction, buffer is used to wash the column in order to separate the mRNA from the beads.

**3.production of labelled DNA**

cDNA is created as a result of reverse transcription of mRNA. The various fluorescent probes are then combined with both samples to create fluorescent cDNA strands, which enable the identification of the various cDNA sampling classes.

**4.hybridization of DNA**

On a DNA microarray, a tagged cDNA from each sample is hybridized with its corresponding strand. They are then meticulously cleansed to get rid of any unpaired sequences.

**5.Data gathering and analysis**

A microarray scanner is being used to get the data. A laser, a computer, and a camera are all part of the scanner. The laser stimulates the cDNA's fluorescence, which produces signals. The camera records the pictures produced by the laser scanning the array. The computer then stores the data and generates quick results. The data is still being assessed. Each spot's unique colour intensity tells us something about the type of gene present there. [[[21]](#endnote-21)]

 **Photolithography**

Photolithography, which translates to "light stone" in Greek, is a method that uses light to detect patterns in semiconductor materials. A design is defined and transferred to each layer using photolithography. It is a method of transferring designs into a substrate that is formed on a silicon wafer that serves as a photolithography substrate. The technique produces binary patterns that are only available in greyscale and depth. Each layer in a microsystem has its unique pattern. Photolithography is used to transfer this pattern from a mask to a photosensitive layer. The pattern is transferred from the photosensitive material to the non-photosensitive substance during a different stage of the procedure. After the pattern transfer, the resist is peeled from a new layer that is placed on top of an existing one. [[[22]](#endnote-22)]

**Three Steps of Photolithography**

1. Coat
2. Expose
3. Develop

**1. Coating Step: Surface Preparation**

1. Surface conditioning usually comes before the photoresist in most cases. After wafer surface preparation, it is prepared for implantation. By providing a pristine surface, photoresist may be made. A chemical is utilized to coat the wafer and improve adherence.

2. After the wafer surface has been baked to remove the water molecules, HMDS is applied (primed) to create a hydrophobic surface.

3. Enable the wafer to defrost and reach room temperature.

A vacuum chuck secures the wafer in position. The wafer is held in place by a hoover. Chuck spins until the film has dried after accelerating to the correct thickness.

The creation of photoresist ants was influenced by the photography industry. Both resists for general use and resists for specific applications are available. They are frequently set to a certain wavelength. The components of a photoresist consist ;

1. **Polymer** - a polymer that changes structure in response to light exposure. Usually, a change in solubility in a certain solvent is the desired property.

**2. Solvent -** A solvent is used to thin the resist before applying it.

a spin-on procedure on the wafer Typically, the solvent is eliminated by the soft bake procedure involves heating to roughly 100 degrees Celsius.

3. **Sensitizers** - these are chemicals that are used to control the chemical reaction during the process exposure.

**4. Additives** are substances that are introduced to a procedure in order to produce a certain outcome. Photoresists are sometimes referred to as optical resists, which are photoresists that respond to UV or visible light. Other radiation kinds, including x-rays and e-beams, have a unique resistance.

Photoresists are classified into two categories.

1. Positive resists - these become more soluble when exposed to UV light.

2. Negative resists - these resists become less effective when exposed to UV light.

**Softback**

After the photoresist has been applied to the required thickness, a softback technique is employed. The method of eliminating a layer of fat from the solvents that are still present after cleaning the photoresist is referred to as "softback". Following the softback, the wafer is baked and allowed to cool to room temperature.

**Alignment**

During the alignment and exposure process, the design is transferred from the mask to the photoresist on the wafers. Alignment marks are used to align the mask. In the production of microsystems, "alignment" is a crucial step. The device or every other device on the wafer might malfunction with an imbalance of just one micrometer or less. Each layer must be correctly aligned with the levels above it and below it, as required by standards.

**2. Expose**

UV rays from the light source travel through the mask and chemically react with the resist. The treatment is only applied to the areas of a face that aren't covered by the mask.

**3. Developing**

The wafers must be developed once they have been exposed and aligned. The wafers are coated with a chemical (developer), which reacts with the exposed photoresist. Which developer to use depends on the resist. In contrast to negative photoresists, which have their unexposed regions removed, positive photoresists have their exposed portions removed, making them more soluble. The most common type of development is a wet chemical reaction. The wafers are immersed in the developing solution for a set duration until all of the resist has been removed. To set the leftover resist, bake it (perform a "hard bake") at 250 degrees Celsius. The practice is still in its early stages. The resist protects the wafer underneath from deterioration. The residual resist is removed using a process known as resist stripping once the final pattern has been created on the wafer. You may either do this wet with an acid combination or dry with a solvent. Dry oxygen plasma etching is performed. After that, the wafers are cleaned and dried. [[[23]](#endnote-23)]

**DNA microarray types:**

1.Microarrays based on cDNA

 2.Microarrays based on Oligonucleotide

 **1.Microarrays based on cDNA**

The mechanical or ink jet micro spotting printing of cDNA clones with sizes ranging from a few hundred DNA strands to several kilobases on a glass surface is the basis of target DNA microarrays. Labelling the sample and rRNA with fluorescent Cy5 or Cy3 dyes is done using reverse transcriptase. The cDNAs are next hybridized at night to the arrays. The slides are cleaned and scanned with a fluorescence laser scanner. The proportional number of transcripts in the samples may be estimated using the red/green ratio of each spotted array piece. Using oligonucleotide bases, chips.

**2.Microarrays based on Oligonucleotide**

Although oligonucleotide chemistry and photolithography are used in situ synthesis on glass, some producers construct their arrays by spotting commonly available oligonucleotides. Each gene is represented by 20–25 short, chemically generated sequences in this specific type of spotted probe. Shorter probe lengths provide several advantages, including the capacity to investigate polymorphisms and small genomic areas, as well as fewer probe synthesis mistakes. [[[24]](#endnote-24)] Since electromagnetic immobilization and inter-linking might result in substantial probe loss during washing operations due to its low weight, covalent linkage is used to affix probes to glass slides. Probes are linked to the microarray ground on functionally group-coated slides using modified 5′ to 3′ ends. [[[25]](#endnote-25)]

**Application**

Multiple simultaneous cell alterations and gene variations are required for tumour development. Because it enables simultaneous analysis of several genetic samples, the DNA chip can be helpful to researchers. It especially helps with the detection of single nucleotide polymorphisms (SNPs) and mutations, the categorization of tumours, the identification of tumour suppressor target genes, the detection of cancer biomarkers, and the detection of tumour suppressor genes. For example, we may analyse various patterns of gene expression in cancer patients and normal individuals to determine the gene related with that particular malignancy. Chemical resistance, drug development, and gene connections are further examples. [[[26]](#endnote-26)]

Genome comparative hybridization has been performed using gene microarrays. This technique looks for gene deletion or amplification using genomic DNA that has been chemically synthesised. The matrix-based comparative gene hybridization (aCGH) method has been used to map genetic aberrations in a number of tumour types, including breast carcinoma, bladder carcinoma and ductal carcinoma, fallopian tubes, gastric carcinoma, melanoma, and lymphoma. [[[27]](#endnote-27)]

It's critical to consider the potential transformation of non-invasive cancers into invasive tumours. When Clark et al. examined the genetic composition of metastatic melanoma cells, they discovered that the RhoC gene was more expressed in metastatic cells than in non-metastatic cells. Thanks to microarray-based expression profiling, we may identify gene families and critical cellular and molecular events that may be implicated in complex processes like metastasis. Future use cases include the management of patient prognosis, diagnosis, and treatment. Using DNA microchips during therapeutic trials, clinicians will be able to confirm pharmacological mechanisms of action and assess medication sensitivity and toxicity. They can develop a new molecular classification for cancer by, for instance, classifying cancers into prognostic groups based on gene expression patterns. The classification of illnesses, molecular phenotype profiling, gene function in connection to gene regulatory networks, and functional genomes may all be studied using microarray technology. Developmental biology and pharmacology are two areas of study. [[[28]](#endnote-28)]

**Gene Expression Analysis**

The main use of DNA microarrays has been the measurement of gene expression levels. In this application, the target cells' RNA is extracted and either directly labelled, transformed into a labelled cDNA, or transformed into a T7 RNA promoter-tailed cDNA that is then converted into cRNA using the Eberwine amplification method. Numerous methods have been developed for labelling cDNA or cRNA, such as including fluorescently-labeled nucleotides during the synthesis, biotin-labeled nucleotides that are then stained with fluorescently-labeled streptavidin, using a modified reactive nucleotide to which a fluorescent tag is added later, and various signal-amplification methods. An early review of a number of labelling methods is provided. The two methods that are most often used are the incorporation of fluorescently tagged nucleotides during the cRNA or cDNA synthesis stage and the incorporation of a biotin-labeled nucleotide during the cRNA synthesis step (as done by Affymetrix). The tagged cRNA or cDNA is then hybridised to the microarray, washed, and the signal is determined by measuring the fluorescence at each location. When biotin-labeled materials are utilised, fluorescently-labeled streptavidin is used to stain the array after hybridization. Laser-induced fluorescence is typically measured using a scanning confocal microscope. The degree of gene expression for each place is determined by the signal intensity for that spot. [[[29]](#endnote-29)]

**Transcription Factor Binding Analysis**

The binding sites of transcription factors have been identified using chromatin immunoprecipitation and microarrays. DNA Microarray Overview in essence 22.1.6 Addendum 101 According to Current Protocols in Molecular Biology, transcriptional factors (TFs) are cross-linked to DNA using formaldehyde and the DNA is broken apart. The required TF(s) are affinity purified (while still retaining the DNA to which they were linked attached) using either a TF-specific antibody or by tagging the transcription factor with a peptide compatible with affinity chromatography. After being purified, the DNA is freed from the TF, amplified, tagged, and hybridised to the array. [[[30]](#endnote-30)]

Because TFs usually bind far from the genes they regulate, the array's design and the size distribution of the fragment length are related. For instance, the array must have probes that can look at the region of DNA to which the TF has been linked. ChIP-chip can be carried out using the same arrays as gene expression studies since the intergenic areas in bacteria or yeast are incredibly small. Mammalian genomes have enormous intergenic areas, and the TF typically binds thousands of base pairs away from the desired gene. As a result, oligo arrays with oligos evenly spaced throughout the whole genome are frequently used for mammalian genomes in ChIP-chip studies. [[[31]](#endnote-31)]

**Genotyping**

Microarrays have been used often in single nucleotide polymorphism (SNP) genotyping methods. The most popular alternative methods for finding SNPs include Affymetrix's allele discrimination by hybridization, allele-specific extension and ligation to a "bar-code" oligo hybridised to a universal array, and methods in which the arrayed DNA is extended across the SNP in a single nucleotide extension reaction. Allelic discrimination by hybridization is hampered by background due to nonspecific hybridization in complex genomes. Both Affymetrix and Illumina's SNP genotyping technologies have had considerable success and are often used. Both businesses now provide SNP arrays that can recognise more than a million unique human SNPs. SNP calls have call rates (the proportion of SNPs on the array that can be reliably named) over 99.5 percent and are repeatable. The same arrays or variations of them can be used to find copy-number variants as well. [[[32]](#endnote-32)]

**Data Standards and Data Exchange**

Before DNA sequencing, microarrays were maybe the first piece of technology that gave researchers access to vast amounts of sophisticated digital data. As the technology was adopted, it became rapidly apparent that in order for others to be able to reproduce a particular microarray experiment, a full explanation of the array, the sample, the protocols, and the data processing techniques was necessary. It also became evident that given access to the raw and processed data, others would be able to conduct research and meta-analyses (on combinations of data) that the original data developers had not considered. To address these issues of reproducible science and data exchange, members of the Microarray Gene Expression Data Society (now the Functional Genomics Data Society; http://www.FGED.org) created the MIAME (Minimum Information About a Microarray Experiment) standards for the description of microarray experiments and for the exchange of microarray data. Both the creation of open databases for microarray data and subsequent standardisation projects in other domains were influenced by these activities. [[[33]](#endnote-33)]

**LIMITATIONS OF DNA MICROARRAYS**

Microarrays are merely tools for simultaneously determining the relative concentrations of a number of different DNA or RNA sequences. Despite having several flaws, they are quite useful in a variety of applications. First, arrays provide an indirect measure of relative concentration. To put it another way, it is typical to presume that the signal observed at a particular location on a microarray is correlated with the concentration of a single, speculative species in solution that may hybridise to that location. [[[34]](#endnote-34)] At large concentrations, the array will become saturated, but at low levels, equilibrium favours no binding. Because of this, the signal is only linear over a narrow range of solution concentrations. Second, especially for large mammalian genomes, it is sometimes challenging (if not impossible) to construct arrays in which many related DNA/RNA sequences will not bind to the same probe on the array. A sequence on an array designed to identify gene A may also detect genes B, C, and D if they share a significant amount of sequence similarity with gene A. This can be particularly challenging in the case of gene families and genes with 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]](#endnote-35)]

Finally, a DNA array can only recognise sequences for which it was designed. In other words, species of RNA or DNA that are present in the solution being hybridised to the array but for which there is no complementary sequence on the array will not be recognised. 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 utilising gene annotation from a reference isolate will lack many of the genes discovered in other isolates. [[[36]](#endnote-36)]

**THE FUTURE OF DNA ARRAYS**

Given the above-mentioned limitations of arrays, it would be much more desirable to have a neutral approach to objectively directly quantify every DNA or RNA species present in a particular sample. Next-generation sequencing technologies have now made sequencing cost-competitive with microarrays for all assays, most likely with the exception of genotyping. It wasn't always like this. When the price is comparable, sequencing has many advantages over microarrays. Sequencing enables direct measurement of the nucleic acids in solution. One only has to count the quantity of a certain type of sequence in order to establish its abundance. The number of reads used for each sample is the only factor that can limit the signal to noise ratio that can be created by sequencing, because counting sequences is linear in terms of concentration. [37] Which nucleic acids are present in solution may be pretty objectively determined by sequencing. Contrary to DNA arrays, sequencing does not require knowledge of the potential nucleic acids present, even though sample preparation or other enzymes may skew the findings. Sequencing can also be used independently to discover RNA editing, novel splice forms, and closely related gene sequences that may have escaped detection on DNA microarrays due to cross hybridization. Due to these advantages and a decrease in sequencing costs, DNA arrays have been swiftly supplanted by sequencing for practically all previously performed microarray investigations. Since the cost of sequencing is now falling by a factor of two every five months, it is anticipated that sequencing techniques will fully replace DNA arrays over the next five to ten years. [[[37]](#endnote-37)]

**CONCLUSION**

The many phases of the microarray technique have been clarified by this review. Due to financial limitations, there are now just a few commercial products available; however, if more commercial products become available, the technique's potential may increase. The capacity to collect a large number of historical samples and examine them for unique genetic variations aids in understanding the concept of molecular biology. DNA microchips have a lot of potential for the study of oral diseases. By categorizing oral illnesses based on DNA, RNA, or protein profiles, we will be much better able to recognize, stop, monitor, and treat patients. The DNA chip is mostly used in research at the moment. DNA microchips, which promise to give oral therapy that is much better, personalized, and bio-based, will have a huge influence on dentistry practises in the future.

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