International Journal of Genetics 9(2): 10-23, 2019
ISSN 2222-1301

DOI: 10.5829/idosi.ijg.2019.10.23

Review on Micro Array Technology and its Application in Genetics and Gene Transcript Analysis Studies

[1]Yoseph Tilahun, [1]Yosef Deneke, [1]Raji Dugasa and [2]Garoma Desa

[1]Jimma University, School of Veterinary Medicine, P.O. Box: 307, Jimma, Ethiopia
[2]National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Kaliti Tsetse fly Mass Rearing and Irradiation Center, Addis Ababa, Ethiopia

Abstract: Electronic microarrays that contain planar arrays of microelectrodes have been developed to provide unique features of speed, accuracy and multiplexing for genomic and proteomic applications through utilizing electricfield control to facilitate analyst's concentration, DNA hybridization, stringency and multiplexing. DNA Microarray is one such technology which enables the researchers to investigate and address issues which were once thought to be non-traceable. This technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body. Microarrays are significant because they possess a huge number of genes and also because of their portable size.Microarray technologies are rapidly advancing with numerous applications in gene expression, genotyping and pharmacogenomics. The technology is now also making an impact in the area of medical diagnostics, in particular, for cancer, genetic and infectious disease applications.

Key words: Gene · Hybridization · Microarray

INTRODUCTION

Functional genomics involves the analysis of large datasets of information derived from various biological experiments. One such type of large-scale experiment involves monitoring the expression levels of thousands of genes simultaneously under a particular condition, called gene expression analysis. Microarray technology makes this possible and the quantity of data generated from each experiment is enormous, dwarfing the amount of data generated by genome sequencing projects [1].

Molecular Biology research evolves through the development of the technologies used for carrying them out. It is not possible to research on a large number of genes using traditional methods. DNA Microarray is one such technology which enables the researchers to investigate and address issues which were once thought to be non-traceable. An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules. A typical microarray experiment involves the hybridization of mRNA molecule to the DNA template from which it is originated [2, 3].

One can analyze the expression of many genes in a single reaction quickly and in an efficient manner. Many DNA samples are used to construct an array. The amount of mRNA bound to each site on the array indicates the expression level of the various genes. This number may run in thousands. All the data is collected and a profile is generated for gene expression in the cell. DNA Microarray technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body [4].

As many important diseases can be traced down to the gene level, a long-standing research problem is to identify specific gene expression patterns linking to metabolic characteristics that contribute to disease development and progression. The microarray approach

Corresponding Author: Yoseph Tilahun, Jimma University, School of Veterinary Medicine, P.O. Box: 307, Jimma, Ethiopia.

offers an expedited solution to this problem. However, it has posed a challenging issue to recognize disease-related genes expression patterns embedded in the microarray data. In selecting a small set of biologically significant genes for classifier design, the nature of high data dimensionality inherent in this problem creates substantial amount of uncertainty [1].

Once the human genome sequence was completed in 2001, it paved the way for many experiments and researches; one such area was identifying the regions of DNA which control normal and disease states. Functional genomics is the study of gene function through parallel expression measurements of a genome [5].

The most common tools used to carry out these measurements include complementary DNA microarrays, oligonucleotide microarrays, or serial analysis of gene expression (SAGE). Microarray analysis can be divided into two main steps: probe production and target (cDNA) production. Specific sequences are immobilized to a surface and reacted with labeled cDNA targets. A signal resulting from hybridization of the labeled target with the specific immobilized probe identifies which RNAs are present in the unknown target sample [6].

Prevention, diagnosis and treatment in dental practice are based on an understanding of the biology of underlying oral health and disease. Few aspects of patient care will remain untouched by today's rapid advances in biological research. In the future, dentists may use inexpensive but remarkably sophisticated diagnostic tests to diagnose infection, oral lesions, and symptoms of temporomandibular dysfunction [7]. The small variations in the DNA sequence that lead to different characteristics (such as skin color, facial features, or height) are known as polymorphisms, which also can cause or contribute to the development of many syndromes and diseases [8].

These genetic variations can be easily identified by the microarray technique. Microarray provides a basis to genotype thousands of different loci at a time, which is useful for association and linkage studies to isolate chromosomal regions related to a particular disease. This array can also be used to locate chromosomal aberrations related to cancer, such as segments of allelic imbalance, which can be identified by loss of heterozygosity. By comparative genomic hybridization techniques on genomic DNA, amplified or deleted regions in the chromosomes can be identified, such as in the case of oral cancer [1].

Microarrays are significant because they possess a huge number of genes and also because of their portable size. Microarrays are therefore, helpful when one is interested in surveying a large number of genes swiftly or when the sample of interest is small. Microarrays can also be helpful to assay gene expression within each sample or to compare gene expressions of two different cell types or tissue samples, for example, healthy and diseased tissue [9].

DNA microarrays can be used to compare the level of gene transcription in clinical conditions in order to: 1) identify diagnostic or prognostic biomarkers; 2) classify diseases (e.g., tumors with different prognosis that are indistinguishable by microscopic examination) 3) monitor the response to therapy; and 4) understand the mechanisms involved in the genesis of disease processes. For these reasons, DNA microarrays are considered important tools for discovery in clinical medicine. Microarray is being used to detect the gene expressions in prostate cancer, oral cancer, breast cancer, ovarian cancer and other type of cancer [1]. The objective of this seminar paper is to review micro array technology and its application in genetics and gene transcript analysis studies.

MATERIAL AND METHODS

The Early History of DNA Arrays: One could argue that the original DNA array was created with the colony hybridization method [10]. In this procedure, DNA of interest was randomly cloned into *E. coli* plasmids that were plated onto agar petri plates covered with nitrocellulose filters. Replica plating was used to produce additional agar plates. The colonies on the filters were lysed and their DNA's were denatured and fixed to the filter to produce a random and unordered collection of DNA spots that represented the cloned fragments. Hybridization of a radio labeled probe of DNA or RNA of interest was used to rapidly screen 1000's of colonies to identify clones containing DNA that was complimentary to the probe [1].

In 1979, this approach was adapted to create ordered arrays [11] whichpicked colonies into 144 well micro plates. They created a mechanical 144 pin device and a jig that allowed them to replicate multiple microtiter plates on agar and produce arrays of 1728 different colonies in a 26 × 38 cm region. An additional transfer of colonies to squares of Whatman filter paper followed by a growth,

lysis, denaturation and fixing of the DNA to the filter, allowed the production of DNA arrays on filters that could be re-used multiple times. During the next decade, filter based arrays and protocols similar to these were used in a variety of applications including: cloning genes of specific interest, identifying SNP's, cloning genes that are differentially expressed between two samples and physical mapping [12].

In the late 1980's and early 1990's Hans Lehrach's group automated these processes by using robotic systems to rapidly array clones from microtiter plates onto filters [13]. The concomitant development of cDNA cloning in the late 1970's and early 80's [14] combined with international programs to fully sequence both the human genome and the human transcriptome. Aaronson *et al*. [15] led to efforts to create reference sets of cDNA and cDNA filter arrays for human and other genomes [16].

By the late 1990's and early 2000's, sets of non-redundant cDNA's became widely available and the complete genome sequences of some organisms allowed for sets of PRC products representing all the known open reading frames (ORFs) in small genomes [17]. These sets, combined with readily available robotics, allowed individual labs to make their own cDNA or ORF arrays that containing gene content that represented the vast majority of genes in a genome [18].

The Birth of Modern DNA Array: Invention of microarray technology in the early 1990s revolutionized the genomic research, where it was exploited for global gene expression profiling through screening the expression of thousands of genes simultaneously [19]. Northern blotting and reverse transcription polymerase chain reaction (RT-PCR) which were the traditional techniques for monitoring changes in mRNA levels were replaced with high density arrays, which proved to impart comprehensive data and to be better in the course of time [20].

Nowadays, microarrays are used for genotyping, large scale single nucleotide polymorphism analysis, comparative genomic hybridizations, identification of new genes, establishing genetic networks and as a more routine function, gene expression profiling. Providing a unique tool for the determination of gene expression at transcriptome level, it confers simultaneous measurement of large fractions of the genome which can facilitates the recognition of specific gene expression patterns associated with a certain disease or a specific pharmaceutical [21].

Detection of the inimitable genomic signature of any active compound is deemed to be another important application of microarray technology, upon which "intrinsic genomic impacts" of any pharmacologically active agent can be clarified. And presumably, such toxicity prediction may promise notable information about each individual resulting in unique patterns of gene expression that, in turn, exhibit individual specific responses to a particular toxic substance. Basically, the discovery of diagnostic biomarkers has been the most promising feature of microarrays up to now and microarray technology has shown a great potential in predicting gene function and tracking the interactions among genetic networks too [1].

Microarray methodology has also been applied for analysis of proteins and metabolites, which are the principle controllers of gene expression, to verify the results at the molecular level. This in turn can extend our understanding of gene expression patterns and molecular pathways even though other techniques such as NMR, mass spectroscopy, gas and liquid chromatography can be employed for metabolite profiling [22].

Having exploited such techniques, for example, the identity and quantity of different molecules can be determined in the CSF, urine or any other biological sample. Thus, by merging the classical techniques with new high density microarray, the "omics" technology has been devised and implemented for investigation on genomics, proteomics, cytomics and metabolomics [23].

The microarray is by far one of the best tools for pursuing such impacts. For implementation of this technology, however, one needs to be familiar with the different methods used in microarray data analyses and the ways for more efficient applications of such methods to enhance the output of a microarray screening process. In the late 90's and 2000's, DNA array technology progressed rapidly as both new methods of production and fluorescent detection were adapted to the task. In addition, increases in our knowledge of the DNA sequences of multiple genomes provided the raw information necessary to assure that arrays could be made which fully represented the genes in a genome, all the sequence in a genome or a large fraction of the sequence variation in a genome [1].

It should also be noted that during this time, there was a gradual transition from spotting relatively long DNA's on arrays to producing arrays using 25-60bp oligos. The transition to oligo arrays was made possible by the increasing amounts of publicly available DNA

sequence information. The use of oligos (as opposed to longer sequences) also provided an increase in specificity for the intended binding target as oligos could be designed to target regions of genes or the genome that were most dissimilar from other genes or regions. Three basic types of arrays came into play during this time frame, spotted arrays on glass, in-situ synthesized arrays and self-assembled arrays [20].

Glass or Spotted Arrays: It is a method which allowed very high density DNA arrays to be made on glass substrates. Poly lysine coated glass microscope slides provided good binding of DNA and a robotic spotter was designed to spot multiple glass slide arrays from DNA stored in microtiter dishes. By using slotted pins (similar to fountain pens in design) a single dip of a pin in DNA solution could spot multiple slides. Spotting onto glass allowed one to fluorescently label the sample [21]. Fluorescent detection provided several advantages relative to the radioactive or chemiluminescent labels common to filter based arrays. First, fluorescent detection is quite sensitive and has a fairly large dynamic range. Second, fluorescent labeling is generally less expensive and less complicated than radioactive or chemiluminescent labeling [22].

Third, fluorescent labeling allowed one to label two (or potentially more) samples in different colors and cohybridize the samples to the same array. As it was very difficult to reproducibly produce spotted arrays, comparisons of individually hybridized samples to ostensibly identical arrays would result in false differences due to array to array variation. However, a two-color approach in which the ratios of signals on the same array are measured is much more reproducible [18].

In-Situ Synthesized Arrays: It is method for light directed spatially addressable chemical synthesis which combined photo labile protecting groups with photolithography to perform chemical synthesis on a solid substrate. In this initial work, the authors demonstrated the production of arrays of 10-amino acid peptides and, separately, arrays of di-nucleotides. In 1994, Fodor et.al at the recently formed company of Affymetrix demonstrated the ability to use this technology to generate DNA arrays consisting of 256 different octa-nucleotides [24].

By 1995-1996, Affymetrix arrays were being used to detect mutations in the reverse transcriptase and protease genes of the highly polymorphic HIV-1 genome and to measure variation in the human mitochondrial genome. Eventually, Affymetrix used this technology to develop a wide catalogue of DNA arrays for use in expression analysis, genotyping and sequencing [25]. A major advantage of the Affymetrix technology is that because the DNA sequences are directly synthesized on the surface, only a small collection of reagents (the 4 modified nucleotides, plus a small handful of reagents necessary for the de-blocking and coupling steps) are needed to construct an arbitrarily complex array [26].

This contrasts with the spotted array technologies in which one needed to construct or obtain all the sequences that one wished to deposit on the array in advance of array construction. However, the initial Affymetrix technology was limited in flexibility as each model of array required the construction of a unique set of photolithographic masks in order to direct the light to the array at each step of the synthesis process [22].

Authors from Nimblegen Systems Inc. published a method in which the photo protection is accomplished using micro-mirrors (similar to those in video computer projectors) to direct light at the pixels on the array. This allows for custom arrays to be manufactured in small volumes at much lower cost than by photolithographic methods using masks to direct light (which are cheaper for large volume production). One constraint with this method is that the total number of addressable pixels (e.g. unit oligos that can be synthesized) is limited to the number of addressable positions in the micro mirror device [18].

Affymetrix Gene Chips are the most ubiquitous and long standing commercial array platform in use. The arrays consist of 25-mer oligonucleotides synthesized in situ on the surface of a glass chip. A photo lithography mask, similar to that used to construct semiconductor chips, is used to control light directed DNA synthesis chemistry such that oligo sequences are built up one nucleotide at a time at defined locations on a solid substrate or glass chip [24]. Current chips contain 6.5 million unique probes in an area of 1.28 cm2. The highly precise nature of the lithographic method allows the construction of compact matrices of square patches of probes [27].

Self-Assembled Arrays: An alternative approach to the construction of arrays was created by the group of David Walt at Tufts University [28] and ultimately licensed to Illumina. Their method involved synthesizing DNA on small polystyrenes beads and depositing those beads on the end of a fiber optic array in which the ends of the

fibers were etched to provide a well that is slightly larger than one bead. Different types of DNA would be synthesized on different beads and applying a mixture of beads to the fiber optic cable would result in a randomly assembled array. In early versions of these arrays, the beads were optically encoded with different fluorophore combinations in order to allow one to determine which oligo was in which position on the array (referred to as "decoding the array") [28, 29].

Optical decoding by fluorescent labeling limited the total number of unique beads that could be distinguished. Hence, the later and present day methods for decoding the beads involve hybridizing and detecting a number of short, fluorescently labeled oligos in a sequential series of steps [30]. This not only allows for an extremely large number of different types of beads to be used on a single array but also functionally tests the array prior to its use in a biological assay. Later versions of the Illumina arrays used a pitted glass surface to contain the beads instead of fiber option arrays[29].

Microarray Principle: Messenger RNA (mRNA) is an intermediary molecule which carries the genetic information from the cell nucleus to the cytoplasm for protein synthesis. Whenever some genes are expressed or are in their active state, many copies of mRNA corresponding to the particular genes are produced by a process called transcription. These mRNAs synthesize the corresponding protein by translation. So, indirectly by assessing the various mRNAs, we can assess the genetic information or the gene expression. This helps in the understanding of various processes behind every altered genetic expression. Thus, mRNA acts as a surrogate marker. Since mRNA is degraded easily, it is necessary to convert it into a more stable cDNA form. Labeling of cDNA is done by fluorochrome dyes Cy3 (green) and Cy5 (red) [31].

The principle behind microarrays is that complementary sequences will bind to each other. The unknown DNA molecules are cut into fragments by restriction endonucleases; fluorescent markers are attached to these DNA fragments. These are then allowed to react with probes of the DNA chip. Then the target DNA fragments along with complementary sequences bind to the DNA probes. The remaining DNA fragments are washed away. The target DNA pieces can be identified by their fluorescence emission by passing a laser beam. A computer is used to record the pattern of fluorescence emission and DNA identification. This technique of employing DNA chips is very rapid, besides being sensitive and specific for the identification of several DNA fragments simultaneously [32].

Types of Microarrays: Microarrays can be broadly classified according to at least three criteria: 1) length of the probes; 2) manufacturing method; and 3) number of samples that can be simultaneously profiled on one array. According to the length of the probes, arrays can be classified into "complementary DNA (cDNA) arrays," which use long probes of hundreds or thousands of base pairs (bps) and "oligonucleotide arrays," which use short probes (usually 50 bps or less) [33].

Manufacturing methods include: "deposition" of previously synthesized sequences and "in situ synthesis." Usually, cDNA arrays are manufactured using deposition, while oligonucleotide arrays are manufactured using in situ technologies. In-situ technologies include: "photolithography" (e.g., Affymetrix, Santa Clara, CA), "ink-jet printing" (e.g., Agilent, Palo Alto, CA) and "electrochemical synthesis" (e.g., Comb matrix, Mukilteo, WA) [34].

The third criterion for the classification of microarrays refers to the number of samples that can be profiled on one array. "Single channel arrays" analyze a single sample at a time, whereas "multiple channel arrays" can analyze two or more samples simultaneously. An example of an oligonucleotide, single channel array is the Affymetrix Gene Chip [34]. Depending upon the kind of immobilized sample used construct arrays and the information fetched, the Microarray experiments can be categorized in three ways [32].

Microarray Expression Analysis: The study of gene expression profiling of cells and tissue has become a major tool for discovery in medicine. Microarray experiments allow description of genome wide expression changes in health and disease. The results of such experiments are expected to change the methods employed in the diagnosis and prognosis of disease in obstetrics and gynecology. In this experimental setup, the cDNA derived from the mRNA of known genes is immobilized. The sample has genes from both the normal as well as the diseased tissues. Spots with more intensity are obtained for diseased tissue gene if the gene is over expressed in the diseased condition. This expression pattern is then compared to the expression pattern of a gene responsible for a disease [32, 34].

Microarray for Mutation Analysis: One of the many application areas of the microarray format is to genotype or detects disease causing or disease predisposing mutations in the human genome for diagnostics, carrier identification and pharmacogenetic profiling. For this analysis, the researchers use genomic DNA (gDNA). The genes might differ from each other by as less as a single nucleotide base [34]. A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection. The recently developed single nucleotide polymorphism (SNP) array can be used to measure both DNA polymorphism and dosage changes. The development of single nucleotide polymorphism (SNP) arrays enables simultaneous detection of a large number of DNA polymorphic loci in a simple way. Further technical developments make SNP arrays capable of analyzing both signal intensity variations and changes in allelic composition in parallel. SNP arrays can also detect both copy number changes and copy neutral loss of heterozygosity (LOH) events [18].

Comparative Genomic Hybridization: In recent years, however, researchers have increasingly turned to newer cytogenetic techniques. One such method is comparative genomic hybridization (CGH), which provides an alternative means of genome wide screening for copy number variations. First developed to detect copy number changes in solid tumors, CGH uses two genomes, a test and a control, which are differentially labeled and competitively hybridized to metaphase chromosomes [35].

In an attempt to overcome some of the aforementioned limitations associated with traditional CGH, investigators have developed a newer method that combines the principles of CGH with the use of microarrays. Instead of using metaphase chromosomes, this method which is known as array CGH, or simply CGH uses slides arrayed with small segments of DNA as the targets for analysis [36].

Techniques in Performing Micro Array

RNA Isolation: Numerous RNA isolation methods have been published and a variety of RNA isolation kits are available. The key criteria in choosing a method should be to achieve a high yield of intact and pure RNA. Obtaining long RNA molecules can be problematic and the choice of purification method will influence results [37].

Critical Factors in RNA Isolation: The main factors in isolating good RNA are the composition of the cell lysis buffer, the method of cell disruption and the method used for separating RNA from protein, DNA and other compounds. The nature of the biological sample is also relevant as some RNA isolation methods may be more suitable for certain tissues. Whereas soft tissues or cultured cells disrupt quickly and efficiently and methods using mild cell lysis buffers can give good results, harder tissues containing large amounts of connective tissue, such as muscle, will require the use of strong chaotropic agents such as GITC [35].

Amersham Biosciences RNA extraction kits, such as QuickPrepTotal RNA Extraction Kit, RNA Extraction Kit and QuickPrep Micro mRNA Purification Kit, all contain either guanidium hydrochloride or guanidiumisothiocyanate in the lysis buffer and are suitable for use with a wide variety of cells and tissues. In general, RNA preparations that use chaotropic agents in the lysis buffer tend to give the best results. However, the protocols are more laborious, involve the use of toxic chemicals and take longer [37].

Isolating RNA from Difficult Samples: The nature of some biological samples may necessitate the use of modified RNA extraction strategies to avoid contamination of RNA samples with other compounds. For example, plant tissues can contain polyphenols and polysaccharides. Precipitation with polyvinyl pyrrolidone can be used to remove these substances from RNA preparations. The hardness of cell walls and outer protective structures can also pose a problem [35].

Freezing the samples followed by mechanical grinding may be necessary to efficiently disrupt cell walls and to release cellular RNA. In some cases, as with yeast that has cell walls that can form capsules, disruption of cellular structures increases access to RNA. Digestion with enzymes, such as zymolase, can be used to weaken the cell walls before mechanical disruption with bead milling to lyse the cells. Similarly, isolation of bacterial RNA benefits from the use of enzymes that digest and weaken outer supportive structures. Lysozyme treatment followed by mechanical bead milling is a suitable approach for disrupting bacterial cells for RNA extraction [38].

Purification of Eukaryotic mRNA: Most eukaryotic transcripts contain a poly-A tail and this property can be exploited to separate transcripts from other RNA

molecules. Incubation of total RNA with oligonucleotides containing a poly-T sequence, otherwise called oligo, will result in the hybridization between the poly-A tail of transcripts and the oligonucleotides. By attaching the oligo to a solid support, it is possible to specifically separate transcripts away from other RNA molecules. Quick Prep Micro mRNA Purification Kit uses oligo (dT) cellulose for extraction of mRNA [38].

Although purification of mRNA lengthens the sample preparation protocols, it provides several benefits for microarray analysis [18].

- Probes prepared from mRNA usually give higher signal to noise values on arrays than probes prepared from similar amounts of total RNA.
- Total RNA preparations are more likely to contain compounds other than RNA, which can interfere with the labelling or hybridization steps.
- The yield of labelled cDNA is higher from mRNA than from total RNA, because alternative priming strategies that use oligo (dT) can be used.
- It is easier to prepare labelled probes corresponding to the 5' ends of transcripts from mRNA populations than from total RNA.

Purification of Prokaryotic mRNA: Purification of mRNA from prokaryotes is difficult as most transcripts lack poly-A tails. However, strategies have been developed to polyadenylate 3' ends of bacterial transcripts in crude extracts. Enrichment for bacterial mRNA can also be achieved by selective degradation of ribosomal RNA. By synthesizing first-strand cDNA selectively from ribosomal RNA with the use of specific primers, RNase H can be used to degrade the RNA strand in the resulting double-stranded hybrid. DNAse I digestion can then remove the DNA strand, resulting in the enrichment of transcripts. Up to 80% enrichment can be achieved with this method [39].

In conclusion, the following general recommendations for preparing RNA for microarray analysis are given [18].

- Minimize the degradation of RNA at all handling stages.
- Choose an RNA purification method that gives good yields of pure and intact RNA from your samples, even if this means using a complicated protocol.
- Measure the amount of RNA before using it for microarray labelling.
- Verify the quality of the RNA before using it for microarray labelling.
- If possible, purify mRNA for use in microarray analysis.
- Prepare all the samples for microarray analysis with the same protocol.

RNA Labeling: In differential gene expression analysis two or more RNA samples are compared to identify differences in the abundance and identity of the transcripts they contain. In order to convert the information contained in the transcript populations into a form that can be hybridized with microarrays and subsequently detected, the transcript populations need to be labeled. This can be achieved using different methods; an ideal method retains both the information carried by the identity of the transcripts as well as their relative abundance in the sample [40].

Enzymatic Labeling Methods: Several strategies based on molecular biology or chemical reactions have been developed for labeling samples for gene expression microarray analysis. The availability of fluorescent labels in different reactive forms has contributed to the diversity of labeling methods. All these strategies have in common that they start with an RNA population. Molecular biology strategies rely on the use of enzymes to convert mRNA into new populations of nucleic acids, either DNA or RNA [41].

Combining two or more enzymatic reactions into one protocol widens the choice further. Using more than one enzyme for labeling, however, has the disadvantage that the information carried by the original population is likely to change more than by using a single enzyme. This is because some information is lost in each enzymatic conversion step and as the lost information is dependent on the sequence of the transcripts and the properties of the enzyme, the representations synthesized by each enzyme will be different. Chemical methods have the advantage that no copying of nucleic acid to another form takes place; instead the labeling moiety reacts with the nucleic acids to form covalently modified, labeled probe population [40].

Fluorescent Labels: Fluorescent dyes, especially the cyanine dyes Cy3 and Cy5, are the most popular choice for dual color microarray analysis. The main benefit of using CyDyefluors in particular is that two dyes can be excited and detected from the same slide. CyDyefluors also produce bright signals and have a wide dynamic range of detection, so both weak and strong signals can

be detected in the same experiment. Fluorescent dyes can be directly incorporated into nucleic acid by either enzymatic or chemical methods [41].

RNA Amplification: The amount of RNA sample can be a limiting factor for microarray analysis and it may be necessary to amplify RNA before analysis. In the most commonly used protocol, the mRNA population is first converted into a double-stranded cDNA that contains a promoter sequence for viral RNA polymerase, such as T7, T3, or SP6 polymerase. This can be achieved by using a modified oligo (dT) primer containing a 5' extension coding for the viral promoter. Each resulting cDNA molecule will contain one RNA polymerase promoter sequence. By including the corresponding RNA polymerase and ribonucleotides in the reaction, several RNA copies can be synthesized from each template [42].

Hybridize the Labeled Target to the Microarray: This step involves placing labeled cDNA onto a DNA microarray where it will hybridize to their synthetic complementary DNA probes attached on the microarray.Hybridization is the process of incubating the labeled target DNA with the probe DNA tethered to the microarray substrate. Fluorescent target DNA hybridizes to complementary probe DNA on the slide and the amount of immobilized fluorescence or radioactivity can be determined [43].

Hybridization of the labeled probe is ideally linear (i.e. proportional to the amount of the probe) and sensitive (low abundance genes are detected) and specific (targets hybridize only to the desired genes in the complex probe mixture). The overall procedure for a PCR product based DNA microarray hybridization is basically the same as for a Southern blot except for a few modifications. Before hybridization, most glass slides need to be treated to block or inactivate the nonspecific binding sites. The procedure, called prehybridization, depends on the slide type and spotting chemistry [43].

Washing: After hybridization, the array goes through a series of washes to eliminate all unbound labeled target cDNA. Washing breaks unstable binding of target and probe cDNA, which may be the result of cross hybridization. Washing is one of the most critical steps in obtaining consistently low backgrounds. Incomplete washing causes deposition of salts or fluorescent materials that may cover all or part of the array. The wash buffers need to be of the highest quality available (filtration through glass fiber or membrane filter is usually sufficient) and free of contaminants (dust, insoluble particles, etc.). The entire array must be immersed in the washing solutions during this process. It is extremely important not to allow the arrays to dry out between washes, as this will result in high backgrounds. The number and length of the washing steps should be increased if necessary [44].

Scan the Microarray and Quantitate the Signal: The fluorescent tags on bound cDNA are excited by a laser and the fluorescently labeled target sequences that bind to a probe generate a signal. The total strength of the signal depends upon the amount of target sample binding to the probes present on that spot. Thus, the amount of target sequence bound to each probe correlates to the expression level of various genes expressed in the sample [43].

The signals are detected, quantified and used to create a digital image of the array. If we are trying to calculate relative expression between two samples, each labeled with a different dye, the resulting image is analyzed by calculating the ratio of the two dyes. If a gene is over expressed in the experimental sample, then more of that sample cDNA than control cDNA will hybridize to the spot representing that expressed gene. In turn, the spot will fluoresce red with greater intensity than it will fluoresce green. The red to green fluorescence ratio thus indicates which gene is up or down regulated in the appropriate sample [44, 45].

Data Analysis: Microarrays provide huge amounts of data. To obtain interesting and reliable hypotheses and hence results, good mathematical and statistical tools are needed for an intelligent interrogation or “mining” of microarray data. The first step of data analysis is image processing. Images generated by a microarray scanner represent the raw data of any microarray experiment. Computer algorithms convert the image into the numerical information that quantifies gene abundance. The second transformation, called normalization, removes non biological influences on biological data, including unequal quantities of starting RNA, differences in labeling or detection efficiency. The third and last part of data analysis is one of the core goals of microarray analysis: to identify which gene is differentially expressed. Statistical methods are suitable to detect those genes and to quantify their expression rate. Clustering methods then

group the genes with a similar pattern of expression and analyze their expression behavior in a complex manner [46].

Application of Microarray

Single Nucleotide Polymorphisms (SNPs)-Based Diagnostics: Given the advances in genomic studies, more and more single nucleotide polymorphisms (SNPs) are found to be contributory factors for human disease and can be used as genetic markers for molecular diagnostics. The speed, accuracy and flexibility provided by electronic microarrays have received great interest from clinical diagnostic researchers for a variety of genotyping applications [47].

One of the many application areas of the microarray format is to genotype or detects disease causing or disease predisposing mutations in the human genome for diagnostics, carrier identification and pharmacogenetic profiling. For this analysis, the researchers use genomic DNA (gDNA). The genes might differ from each other by as less as a single nucleotide base. A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection [43].

The recently developed single nucleotide polymorphism (SNP) array can be used to measure both DNA polymorphism and dosage changes. The development of single nucleotide polymorphism (SNP) arrays enables simultaneous detection of a large number of DNA polymorphic loci in a simple way. Further technical developments make SNP arrays capable of analyzing both signal intensity variations and changes in allelic composition in parallel. SNP arrays can also detect both copy number changes and copy-neutral LOH (loss-of-heterozygosity) events [18].

Forensic Detection: Short tandem repeats (STRs) represent another type of polymorphism with important applications in forensic DNA identification. In 1990, the FBI created a combined DNA index system (CODIS), which consist of 13 polymorphic STR loci, to provide a database of forensic DNA profile for nearly all forensic laboratories in the United States. The typical STR loci are selected groups of four nucleotide repeats that are represented in the human population by 4-15 alleles distinguished by a different number of repeat units. Unlike SNPs, STRs are more difficult to analyze by conversional passive hybridization technique. However, electronic hybridization techniques have been proven to overcome these problems and allow STR analysis to be performed on electronic microarray in a rapid and high fidelity fashion [18].

Gene Expression Profiling: Another array based method in genomic studies is to simultaneously monitor global gene expression profiling of cells under a condition of interest and to identify a set of gene markers for specific disease. Towards this trend, a multiplex, targeted gene expression profiling method has been developed using electronic field facilitated hybridization on Nanochip electronic microarray [48]. In this method, target mRNA generated from T7RNA polymerase mediated amplification were detected by hybridization to sequence specific capture oligonucleotides on electronic microarray. The expression of a model set of 10 target genes in the U937 cell line was analyzed during lipopolysaccharide mediated differentiation with 2 fold changes in concentration and 64-fold range of concentration. This electronic array based expression analyzing method allows simultaneous assessment of target concentrations from multiple sample sources [49].

Cell Separation: In addition to dc current, ac voltage can also be applied to the electronic microarray in certain ways to create a dielectrophoretic (DEP) force applicable to analytes such as cells. Depending on the frequency of the ac voltage and the dielectric properties of the cells, the DEP forces can be either positive (moving cells towards electrodes) or negative (moving cells towards spaces) resulting in special separation of different types of cells on electronic microarrays. Using DEP, U937 and PBMC were separated on the array [50].

Applications in Proteomics: After the completion of human genome project, the attention of the scientific community has turned toward the gene products within the cell and tissue matrix, namely proteins. The field of proteomics is an evolving area, which may shed light on the proteins associated with diseases and tumors. Protein kinases are of particular interest because they have been shown to be key regulators of many cell functions and have been one of the main targets in drug industry. Several high throughput screening (HTS) kinase assays have been developed based on either antibodies or radioactivity for detection. Recently, an electronic, fluorescent assay for kinases, phosphatases and protease has been developed for the serine/threonine kinase PKA [51].

The use of DNA Micro Array in Medicine: Perhaps the most promising technologies of DNA microarray in medical microbiology are to use it to measure simultaneously a large number of microbial genetic targets. An oligonucleotide microarray was developed to target rRNA gene for the detection of 40 most pathogenic intestinal bacteria. The PCR assay using microarray helps to shown the rapid detection and identification of bacteria in blood culture. PCR amplification in combination with Oligonucleotide microarray is used to identify *B.anthracis* [48].

In addition to bacterial and mycobacterial organisms, microarrays following broad range PCR amplification have been used to detect and identify fungal, parasitic and viral pathogens. A new method based on microarrays for simultaneous human immunodeficiency type 1, hepatitis B and c in plasma samples was described. A microarray technique for the detection and identification of enteropathogenic bacteria at the species and subspecies levels was developed covering pathogenic *E-coli, Vibrio cholera, Vibrio Parahaemolyticus, Salmonella enteric, Campylobacter jejuni, Shigella species, Yersinia enterocolitica* and*Listeria monocytogenes*[52].

Microarray system by a panel of pathogens that cause meningitis, viral and bacterial detection was used. The system detection 9 microorganisms including *Neisseria meningitides, Streptococcus Pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Streptococcus agalactiae, Herpes simplex type virus 1 and 2* and *Varicella Zoster* Virus was detected directly from the CSF.DNA microarray of comparative genomics to study the differences between the genome of Bacillus strains of *M. tuberculosis* and BOVIS M Calmette-Guerin (BCG) is used. H. pylori species and tuberculosis were used between different isolates S. Pneumoniae [6].

New Microarray Technologies: A variety of additional technologies are being developed for making microarrays and techniques for improving their performance. Considerable efforts are being carried out related to better methods for preparing oligo nucleotides and other types of DNA probes and new procedures are being created for their attachment or immobilization onto support materials [53]. There are also new advancements relating to the detection of hybridization reactions that occur on the surface of the arrays. Le Proust and coworkers have reported a new digitally light-directed synthesis method that permits microarray platforms to be designed that can carry out rapid reaction optimization on a combinatorial basis [36].

Limitation of Micro Array: Microarrays are simply devices to simultaneously measure the relative concentrations of many different DNA or RNA sequences. While they have been incredibly useful in a wide variety of applications, they have a number of limitations. First, arrays provide an indirect measure of relative concentration. That is the signal measured at a given position on a microarray is typically assumed to be proportional to the concentration of a presumed single species in solution that can hybridize to that location. However, due to the kinetics of hybridization, the signal level at a given location on the array is not linearly proportional to concentration of the species hybridizing to the array [4].

At high concentrations the array will become saturated and at low concentrations, equilibrium favors no binding. Second, especially for complex mammalian genomes, it is often difficult (if not impossible) to design arrays in which multiple related DNA/RNA sequences will not bind to the same probe on the array. A sequence on an array that was designed to detect “gene A”, may also detect “genes B, C and D” if those genes have significant sequence homology to gene A. This can particularly problematic for gene families and for genes with multiple splice variants. It should be noted that it is possible to design arrays specifically to detect splice variants either by making array probes to each exon in the genome or to exon junctions [54].

Finally, a DNA array can only detect sequences that the array was designed to detect. That is, if the solution being hybridized to the array contains RNA or DNA species for which there is no complimentary sequence on the array, those species will not be detected. For gene expression analysis, this typically means that genes that have not yet been annotated in a genome will not be represented on the array. In addition, non-coding RNA’s that are not yet recognized as expressed are typically not represented on an array [55].

Data Standard and Data Exchange: With the exception of DNA sequencing, microarrays were perhaps the earliest technology that allowed biologists to vast amounts of complex digital data. As the technology came into use, it rapidly became apparent that in order for others to be able to reproduce a given microarray experiment a detailed description of the array, the sample, the protocols and the data analysis methods needed to be available [56].

Moreover, it also became apparent that access to the raw and processed data would allow others to perform analyses and Meta analyses (on combinations of data)

that the original data producers had not conceived. To address these issues of reproducible science and data exchange, members of the Microarray Gene Expression Data Society created the MIAME (Minimum Information about a Microarray Experiment) standards for the description of microarray experiments and for the exchange of microarray data. These efforts influenced the creation public databases for microarray data and subsequent standards efforts in other areas [57].

CONCLUSIONS

Microarrays are able to simultaneously monitor the expression levels of thousands of genes. Such gene expression information can be used in medicine for comparing clinically relevant groups (e.g., healthy vs. diseased), uncovering new subclasses of diseases and predicting clinically important outcomes, such as the response to therapy and survival.The development of DNA microarray, biochip and lab-on-a-chip technologies continues at a very rapid pace. These new technologies represent a highly interdisciplinary and synergist effort, with contributions from a range of scientific and engineering fields that have previously not worked together as closely. Microarray technologies are rapidly advancing with numerous applications in gene expression, genotyping and pharmacogenomics. The technology is now also making an impact in the area of medical diagnostics, in particular, for cancer, genetic and infectious disease applications. As the technology continues to advance, it will most certainly lead to the development of a new generation of “point of care” diagnostic devices and systems. Finally, DNA microarray technology is also forming the basis for a new generation of devices for proteomic and cell biology applications.

REFERENCES

1. Jiang, W.W., H. Fujii, T. Shirai, H. Mega and M. Takagi, 2001.Accumulative increase of loss of heterozygosity from leukoplakia to foci of early cancerization in leukoplakia of the oral cavity. Cancer, 92(9): 2349-2356.
2. Aguan, K., J.A. Carvajal, L.P. Thompson and C.P. Weiner, 2000. Application of a functional genomics approach to identify differentially expressed genes in human myometrium during pregnancy and labour. Molecular Human Reproduction, 6(12): 1141-1145.
3. Berchuck, A., E.S. Iversen, J.M. Lancaster, H.K. Dressman, M. West, J.R. Nevins and J.R. Marks, 2004. Prediction of optimal versus suboptimal cytoreduction of advanced-stage serous ovarian cancer with the use of microarrays.American journal of Obstetrics and Gynecology, 190(4): 910-923.
4. Bumgarner, R.., 2013. Overview of DNA microarrays: types, applications and their future. Current protocols in Molecular Biology, 101(1): 22-1.
5. Krepischi, A.C., M.I.W. Achatz, E.M. Santos, S.S. Costa, B.C. Lisboa, H. Brentani, T.M. Santos, A. Gonçalves, A.F. Nóbrega, P.L. Pearson and A.M. Vianna-Morgante, 2012. Germline DNA copy number variation in familial and early-onset breast cancer. Breast Cancer Research, 14(1): R24.
6. Dadkhah, K., S.H.M. Anijdan, M. Karimi, M.K. Abolfazli, F. Rezaei, F. Adel and G. Ataei, 2015. DNA microarray, types and its application in medicine.Scholars Academic Journal of Biosciences, 3(7): 598-599.
7. Lindblad-Toh, K., D.M. Tanenbaum, M.J. Daly, E. Winchester, W.O. Lui, A. Villapakkam, S.E. Stanton, C. Larsson, T.J. Hudson, B.E. Johnson and E.S. Lander, 2000. Loss-of-heterozygosity analysis of small-cell lung carcinomas using single-nucleotide polymorphism arrays. Nature Biotechnology, 18(9): 1001.
8. Wang, D.G., J.B. Fan, C.J. Siao, A. Berno, P. Young, R. Sapolsky, G. Ghandour, N. Perkins, E. Winchester, J. Spencer and L. Kruglyak, 1998. Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome.Science, 280(5366): 1077-1082.
9. Roh, S.W., G.C. Abell, K.H. Kim, Y.D. Nam and J.W. Bae, 2010. Comparing microarrays and next-generation sequencing technologies for microbial ecology research.Trends in Biotechnology, 28(6): 291-299.
10. Grunstein, M. and D.S. Hogness, 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proceedings of the National Academy of Sciences, 72(10): 3961-3965.
11. Gergen, J.P., R.H. Stern and P.C. Wensink, 1979. Filter replicas and permanent collections of recombinant DNA plasmids. Nucleic acids research, 7(8): 2115-2136.
12. Budowle, B. and A. Van Daal, 2008. Forensically relevant SNP classes. Biotechniques, 44(5): 603-610.

13. Carig, A.G., D. Nizetic, J.D. Hoheisel, G. Zehetner and H. Lehrach, 1990. Ordering of cosmid clones covering the herpes simplex virus type I (HSV-I) genome: a test case for fingerprinting by hybridisation. Nucleic Acids Research, 18(9): 2653-2660.
14. Auffray, C. and F. Rougeon, 1980. Nucleotide sequence of a cloned cDNA corresponding to secreted μ chain of Mouse Immunoglobulin. Gene, 12(1-2): 77-86.
15. Aaronson, J.S., B. Eckman, R.A. Blevins, J.A. Borkowski, J. Myerson, S. Imran and K.O. Elliston, 1996. Toward the development of a gene index to the human genome: an assessment of the nature of high-throughput EST sequence data. Genome Research, 6(9): 829-845.
16. Bonaldo, M.D.F., G. Lennon and M.B. Soares, 1996. Normalization and subtraction: two approaches to facilitate gene discovery. Genome Research, 6(9): 791-806.
17. Lashkari, D.A., J.L. DeRisi, J.H. McCusker, A.F. Namath, C. Gentile, S.Y. Hwang, P.O. Brown and R.W. Davis, 1997. Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proceedings of the National Academy of Sciences, 94(24): 13057-13062.
18. Fahmideh, L., H. Kord and Y. Shiri, 2016. Importance of microarray technology and its applications. Journal of Current Research in Science, 4(3): 25.
19. Watson, J.D. and E. Jordan, 1989. The human genome program at the national institutes of health.Genomics, 5(3): 654-656.
20. Humphries, P., M. Cochet, M. Andrée Krust, P. Gerlinger, P. Kourilsky and P. Chambon, 1977. Molecular cloning of extensive sequences of the in vitro synthesized chicken ovalbumin structural gene.Nucleic Acids Research, 4(7): 2389-2406.
21. Kim, C., K.J. Kim, J. Bok, E.J. Lee, D.J. Kim, J.H. Oh, S.P. Park, J.Y. Shin, J.Y. Lee and H.G. Yu, 2012. Microarray-based mutation detection and phenotypic characterization in Korean patients with retinitis pigmentosa.Molecular Vision, 18: 2398.
22. Chee, M., R. Yang, E. Hubbell, A. Berno, X.C. Huang, D. Stern, J. Winkler, D.J. Lockhart, M.S. Morris and S.P. Fodor, 1996. Accessing genetic information with high-density DNA arrays. Science, 274(5287): 610-614.
23. Smith, G.W. and G.J.M. Rosa, 2007. Interpretation of microarray data: trudging out of the abyss towards elucidation of biological significance. Journal of Animal Science, 85(suppl-13): E20-E23.
24. Pease, A.C., D. Solas, E.J. Sullivan, M.T. Cronin, C.P. Holmes and S.P. Fodor, 1994. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proceedings of the National Academy of Sciences, 91(11): 5022-5026.
25. Ramsay, G., 1998. DNA chips: state-of-the art. Nature Biotechnology, 16(1): 40.
26. Irizarry, R.A., Z. Wu and H.A. Jaffee, 2006. Comparison of Affymetrix GeneChip expression measures. Bioinformatics, 22(7): 789-794.
27. De Matos Simoes, R., S. Tripathi and F. Emmert-Streib, 2012. Organizational structure and the periphery of the gene regulatory network in B-cell lymphoma. BMC Systems Biology, 6(1): 38.
28. Walt, D.R., 2000. Bead-based fiber-optic arrays. Science, 287(5452): 451-452.
29. Ferguson, J.A., F.J. Steemers and D.R. Walt, 2000. High-density fiber-optic DNA random microsphere array. Analytical Chemistry, 72(22): 5618-5624.
30. Gunderson, K.L., S. Kruglyak, M.S. Graige, F. Garcia, B.G. Kermani, C. Zhao, D. Che, T. Dickinson, E. Wickham, J. Bierle and D. Doucet, 2004. Decoding randomly ordered DNA arrays. Genome Research, 14(5): 870-877.
31. Brown, P.O. and D. Botstein, 1999. Exploring the new world of the genome with DNA microarrays. Nature Genetics, 21(1s): 33.
32. Tarca, A.L., R. Romero and S. Draghici, 2006. Analysis of microarray experiments of gene expression profiling. American Journal of Obstetrics and Gynecology, 195(2): 373-388.
33. Afshari, C.A., 2002. Perspective: microarray technology, seeing more than spots. Endocrinology, 143(6): 1983-1989.
34. Fu, L.M. and C.S. Fu-Liu, 2005. Evaluation of gene importance in microarray data based upon probability of selection. BMC Bioinformatics, 6(1): 67.
35. Shaffer, L.G., J. Coppinger, S. Alliman, B.A. Torchia, A. Theisen, B.C. Ballif and B.A. Bejjani, 2008. Comparison of microarray – based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens.Prenatal Diagnosis: Published in Affiliation with the International Society for Prenatal Diagnosis, 28(9): 789-795.
36. Lucito, R., J. Healy, J. Alexander, A. Reiner, D. Esposito, M. Chi, L. Rodgers, A. Brady, J. Sebat, J. Troge and J.A. West, 2003. Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation. Genome Research, 13(10): 2291-2305.

37. Gruffat, D., C. Piot, D. Durand and D. Bauchart, 1996. Comparison of four methods for isolating large mRNA: apolipoprotein B mRNA in bovine and rat livers. Analytical Biochemistry, 242(1): 77-83.
38. Penn, S.G., D.R. Rank, D.K. Hanzel and D.L. Barker, 2000. Mining the human genome using microarrays of open reading frames. Nature Genetics, 26(3): 315.
39. Rosenow, C., R.M. Saxena, M. Durst and T.R. Gingeras, 2001. Prokaryotic RNA preparation methods useful for high density array analysis: comparison of two approaches. Nucleic acids Research, 29(22): e112-e112.
40. Badiee, A., H.G. Eiken, V.M. Steen and R. Løvlie, 2003. Evaluation of five different cDNA labeling methods for microarrays using spike controls. BMC Biotechnology, 3(1): 23.
41. Smoot, L.M., J.C. Smoot, M.R. Graham, G.A. Somerville, D.E. Sturdevant, C.A.L. Migliaccio, G.L. Sylva and J.M. Musser, 2001. Global differential gene expression in response to growth temperature alteration in group A Streptococcus.Proceedings of the National Academy of Sciences, 98(18): 10416-10421.
42. Luo, L., R.C. Salunga, H. Guo, A. Bittner, K.C. Joy, J.E. Galindo, H. Xiao, K.E. Rogers, J.S. Wan, M.R. Jackson and M.G. Erlander, 1999. Gene expression profiles of laser-captured adjacent neuronal subtypes. Nature Medicine, 5(1): 117.
43. Rick, W.Y., T. Wang, L. Bedzyk and K.M. Croker, 2001. Applications of DNA microarrays in microbial systems. Journal of Microbiological Methods, 47(3): 257-272. Ying, H., H. Dalibor, S. Daniel and L. Graham, 2007: Electronic Microarray Technology and Applications in Genomics and Proteomics, 5: 211-236.
44. Majtan, T., G. Bukovska and J. Timko, 2004. DNA microarrays-techniques and applications in microbial systems. Foliamicrobiologica, 49(6): 635.
45. Yang, T.L., X.D. Chen, Y. Guo, S.F. Lei, J.T. Wang, Q. Zhou, F. Pan, Y. Chen, Z.X. Zhang, S.S. Dong and X.H. Xu, 2008. Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. The American Journal of Human Genetics, 83(6): 663-674.
46. Tseng, G.C., M.K. Oh, L. Rohlin, J.C. Liao and W.H. Wong, 2001. Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. Nucleic Acids Research, 29(12): 2549-2557.
47. Huang, Y., D. Hodko, D. Smolko and G. Lidgard, 2006. Electronic microarray technology and applications in genomics and proteomics. In BioMEMS and Biomedical Nanotechnology (pp: 3-21). Springer, Boston, MA.
48. Wong, K.K., R.J. deLeeuw, N.S. Dosanjh, L.R. Kimm, Z. Cheng, D.E. Horsman, C. MacAulay, R.T. Ng, C.J. Brown, E.E. Eichler and W.L. Lam, 2007. A comprehensive analysis of common copy-number variations in the human genome.The American Journal of Human Genetics, 80(1): 91-104.
49. Weidenhammer, E.M., B.F. Kahl, L. Wang, L. Wang, M. Duhon, J.A. Jackson, M. Slater and X. Xu, 2002. Multiplexed, targeted gene expression profiling and genetic analysis on electronic microarrays. Clinical Chemistry, 48(11): 1873-1882.
50. Cheng, J., E.L. Sheldon, L. Wu, A. Uribe, L.O. Gerrue, J. Carrino, M.J. Heller and J.P. O'Connell, 1998. Preparation and hybridization analysis of DNA/RNA from E. coli on microfabricated bioelectronic chips. Nature Biotechnology, 16(6): 541.
51. Hughes, T.R., M. Mao, A.R. Jones, J. Burchard, M.J. Marton, K.W. Shannon, S.M. Lefkowitz, M. Ziman, J.M. Schelter, M.R. Meyer and S. Kobayashi, 2001. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer.Nature Biotechnology, 19(4): 342.
52. Zvara, Á., G. Szekeres, Z. Janka, J.Z. Kelemen, C. Cimmer, M. Sántha and L.G. Puskás, 2005. Over-expression of dopamine D2 receptor and inwardly rectifying potassium channel genes in drug-naive schizophrenic peripheral blood lymphocytes as potential diagnostic markers. Disease markers, 21(2): 61-69.
53. McLoughlin, K.S., 2011. Microarrays for pathogen detection and analysis. Briefings in Functional Genomics, 10(6): 342-353.
54. Gardina, P.J., T.A. Clark, B. Shimada, M.K. Staples, Q. Yang, J. Veitch, A. Schweitzer, T. Awad, C. Sugnet, S. Dee and C. Davies, 2006. Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. BMC Genomics, 7(1): 325.
55. Kittichotirat, W., R.E. Bumgarner, S. Asikainen and C. Chen, 2011. Identification of the pangenome and its components in 14 distinct Aggregatibacteractinomycetemcomitans strains by comparative genomic analysis. PloS one, 6(7): e22420.

56. Acin, S., M.A. Navarro, J .S. Perona, J.C. Surra, N. Guillen, C. Arnal, A.J. Sarría Arbonés, J.M. Mainar, R. Carnicer, V. Ruiz-Gutiérrez and J. Osada, 2007. Microarray analysis of hepatic genes differentially expressed in the presence of the unsaponifiable fraction of olive oil in apolipoprotein E-deficient mice. British Journal of Nutrition, 97(4): 628-638.

57. Deutsch, E.W., C.A. Ball, J.J. Berman, G.S. Bova, A. Brazma, R.E. Bumgarner, D. Campbell, H.C. Causton, J.H. Christiansen, F. Daian and D. Dauga, 2008. Minimum information specification for in situ hybridization and immunohistochemistry experiments (MISFISHIE). Nature Biotechnology, 26(3): 305.