A REVIEW ON DNA MICROARRAY TECHNOLOGY

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Abstract

Microarray analysis allows scientists to make out the molecular mechanisms principal normal and dysfunctional biological processes. It has provided scientists with a tool to investigate the structure and activity of genes on a wide scale. Microarray technology could speed up the screening of thousands of DNA and protein samples simultaneously. DNA microarrays have been used for clinical diagnosis and for studying complex phenomena of gene expression patterns. Present review article focus on history, technique and future applications of DNA micro-array technology.

Key Words: Microarray, DNA, Technology

1. Introduction

Since being introduced in the mid-1990s [[1]], the DNA microarray has become a highly used tool for the measurement of transcript abundance. Currently, Array express and gene expression Omnibus, the two major online repositories of transcriptome data, each contain nearly a million DNA microarray datasets [[1], [3]]. Like the DNA microarray, RNA-seq is high throughput, suggesting that it should be possible to use data from one technique to validate the other. Previous studies have examined the level of agreement between DNA microarray data and RNA-seq data, among them [[3]-[8]]. While several previous

studies have compared RNA-seq data to DNA microarray data, to our knowledge, only one used quantitative reverse-transcription PCR (qRT-PCR) as an independent validation technique.

The transcript abundances in human foreskin fibroblasts that were in one of two states proliferating (‘PRO’) or quiescent (‘QUI’) using both DNA microarrays and RNA-seq and then used qRT-PCR to perform an independent measure of transcript abundance for 76 genes. Specifically, we characterized the level of reproducibility of the RNA-seq data, the level of reproducibility of the microarray data, the correlations between the two techniques and the level of agreement of each technique with the qRT-PCR data.

The basic principle of DNA microarrays and their main types, the role of bioinformatics in this technology, and one of its most important applications as well of these approaches, the probe refers to the DNA sequence bound to the solid-surface support in the microarray, whereas the target is the “unknown” sequence of interest. DNA microarray technologies initially were designed to measure the transcriptional levels of RNA transcripts derived from thousands of genes within a genome in a single experiment. This technology has made it possible to relate physiological cell states to gene expression patterns for studying tumors, diseases progression, cellular response to stimuli, and drug target identification. DNA microarrays are being used to detect single nucleotide polymorphisms (SNPs) of our genome (Hap Map project] [[9]], aberrations in methylation patterns [[10]], alterations in gene copynumber [[11]], alternative RNA splicing [[12]], and pathogen detection [[13],[14]]. In the last ten or 15 years, high quality arrays, standardized hybridization protocols, accurate scanning technologies, and robust computational methods have established DNA microarray for gene expression as a powerful, mature, and easy to use essential genomic tool. Although the identification of the most relevant information from microarray experiments is still under active research, very well established methods are available for a broad spectrum of experimental setups.

The first generations of commercial microarrays introduced in the mid-nineties suffered mainly from sensitivity and reproducibility due to poor production technologies. In the early days of the technology, the first microarrays had probe sets complementary to about 120 genes and about 30 transcripts were measured in routine experiments. However, it became clear that further reduction of the feature size would ultimately allow integration of entire genomes,

which is the case today. In addition, array production and sample preparation methods underwent significant improvements resulting in robust and indispensable tools for routine applications in biomedical research [[15]].

In parallel microarray technologies for the genome wide analysis of single nucleotide polymorphisms (SNPs) [[16]], copy number variation (CNV) [[17]] or DNA methylation [[18]] were successfully developed and marketed. Today a search for "DNA microarray" yields more than 50'000 PubMed entries which showcases the success of this technology and its wide range of applications. It is quite amazing that it took only 15 years of development time until multi-parallel interrogation of entire genomes became available for the research community. Recently exceptionally efficient deep sequencing technologies became available at highly competitive prices. Measuring clone frequencies in bead libraries has the potential to replace or complement chip based fluorescence based transcript imaging in the future. One advantage of this approach is the possibility to detect any genomic transcript of an available. Today many aspects of next-generation sequencing (NGS) remain to be solved. DNA microarray technology could potentiate the identification of newer compounds which can be utilized for the treatment of incurable diseases [[19]]. Microarray analysis allows scientists to understand the molecular mechanisms underlying normal and dysfunctional biological processes. It has provided scientists with a tool to investigate the structure and activity of genes on a wide scale. Microarray technology could speed up the screening of thousands of DNA and protein samples simultaneously [[20]].

1.1. The early history of DNA arrays

One could argue that the original DNA array was created with the colony hybridization method of Grunstein and Hogness [[21]]. In this procedure, DNA of interest was randomly cloned into *E. coli* plasmids that were plated onto agar petri plates covered with nitrocellulose filters. 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 a 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.

In 1979, this approach was adapted to create ordered arrays by Gergen [[22]] who picked colonies into 144 well micro plates. 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 [[23]], cloning genes that are differentially expressed between two samples [[24]] and physical mapping [[25]]. 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 [[25];[26]].

The associated development of cDNA cloning in the late 1970's and early 80's [[27]-[29]] combined with international programs to fully sequence both the human genome [[30];[31]] and the human transcriptome [[32]] led to efforts to create reference sets of cDNAs and cDNA filter arrays for human [[33]] and other genomes([34]] 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 [[35]]. These sets, combined with readily available robotics, allowed individual laboratory to make their own cDNA or ORF arrays that containing gene content that represented the vast majority of genes in a genome.

1.2. The birth of the modern DNA array

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. 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 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.

2. Experimental principles of DNA microarrays

The known DNA samples are known as ‘probes’ and are spotted and fixed on microscope glasses or silicon chips in thousands. These can be oligo nucleotides, cDNA, or even just DNA [[36]; [38]]. On the other hand, the unknown DNA samples are the ones we want analyze their gene expression level for example and are tagged using reporter molecules like fluorophores which replaced radioactive molecules due to their potential health risks [[37]]. If our target sites in the genes are found, they will form complementary bonding with the probes and emit fluorescence signal which can be read by specialized camera and computer systems. Nevertheless, we will discuss in more detail the main steps in measuring gene expression levels in a biological sample using DNA microarray technology [[37]; [39]]. This includes sample preparation and tagging, hybridization, washing, image acquisition and normalization.

2.1. Sample Preparation and Tagging

First, mRNA has to be extracted from the biological sample of interest and purified. A control must be included in the experiment as well (e.g. diseased tissue vs. healthy tissue). This step is crucial, simply because the overall success of any gene-profile microarray experiment depends on the quality of the RNA. The sample mRNA extracted from the biological sample of interest and the reference are then converted separately into cDNA using a reverse transcriptase enzyme. Next, the tagging involves performing reverse transcription reaction to synthesize complementary DNA (cDNA) strand [[40]]. Genomic DNA probes can be used for normalization, slide quality control and comparative genomic studies. Genomic DNA can be labeled by nick translation or by random priming with the Klenow fragment of DNA polymerase [[42];[43]]. Direct chemical labeling of nucleic acids is also a commonly used method. In this method, poly T primer is attached to mRNA to start the reverse transcription process from the polyadenylation signal at the 3’ un-translated site (UTR) of the mRNA. A proportion of the nucleic acids: dATP, dGTP, dCTP, and dTTP added in this reaction are incorporated with a florescent dye via covalent bonding [[44]]. Diseased and healthy samples can be also tagged with different dyes such as Cy3 (Excited by a green laser) and Cy5 (Excited by a red laser) to distinguish between them and are used in the same microarray. Thus, cDNA probes complementary to the tagged transcripts will hybridize and eventually visualized as colored spots under the camera.

2.2. Hybridization

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 non-specific binding sites. The procedure employed depends on the slide type and spotting chemistry. Processing of array images involves three steps:- spot finding, quantification, and background estimation. Genomic hybridization on a whole-genome array detects the presence or absence of DNA regions of sufficient similarity to the sequenced isolate in other microorganisms, allowing genome-wide comparison of their genetic contents. In a single hybridization experiment, the presence of all ORFs of the sequenced genome in the investigated isolates is examined. Insertions of genes in comparison to the sequenced reference strain cannot be detected in comparative genomic hybridization DNA microarray analyses. This problem can be alleviated by adding non-redundant amplified sequences from several closely related bacteria to the array, once new genetic information is available [[45]]. The recent completion of the C. albicans genome sequencing project and the availability of C. albicans microarrays has provided a rapid means of surveying the genomes of C. albicans and C. dubliniensis using comparative genomic hybridization analysis.

In this step, the DNA probe on the micro-slide glass and the tagged target cDNA will pair according to Watson Crick configuration [[46]]. This can be accomplished either manually or using robotics system. In the first approach, the array is placed in a special chamber where the researcher injects the solution containing the target cDNA onto the array under sterile conditions and incubates it at certain temperature for 12 to 24 hours [[47], [48]]. According to the second approach, everything is performed by a programmed robot which saves time and effort, performs the protocol at specialized station, and grants a better control over the temperature that is usually between 45 and 65°C [[49]]. It is also important to mention that hybridization is affected by many conditions such as salt concentration, temperature, form amide concentration, humidity, and amount of target solution [[50]]. Before hybridization, most glass slides need to be treated to block or inactivate the non-specific binding sites. The procedure employed depends on the slide type and spotting chemistry. In order to limit or prevent cross-hybridization, a repetitive DNA sequence and poly T or poly A can be added to mask the genomic repeat sequence and the polyadenylation sites on the cDNA respectively [[51]].

3. Applications of DNA microarrays

The technology of DNA microarrays is widely used to measure levels of gene expression. Other applications can include genotyping where scientists can detect single nucleotide polymorphisms (SNPs), which are type of genetic variation among people due to a difference in single nucleotide. Many human diseases such as β-thalassemia, sickle-cell anemia, and cystic fibrosis are caused by SNPs. These are also useful in characterizing allelic diversity of certain genes and mapping of genomic loci [[52]]. Usually, using multiple microarray positions for each allele gives better signal and more accurate results [[53],[54]]. Secondly, using APEX (Array primer extension), DNA is attached on the microarray through the 5' end whereas the 3' end is one nucleotide short of the SNP. When the target DNA is hybridized to the microarray, the oligo nucleotides is extended using single nucleotide "dye terminator" sequencing reaction [[55]].

A DNA microarray was also used to explore the genome-wide changes in mRNA abundance in *B. subtilis* when grown under anaerobic conditions [[56]]. The transcriptional activities of more than 100 genes were affected by the oxygen-limiting conditions. These include the genes that are involved in energy metabolism, iron uptake, antibiotic production, carbon metabolism and stress response. Instead of comparing the mRNA levels of different samples, DNA microarrays can detect the presence of an mRNA transcript and can estimate its relative abundance to other mRNA species within the same sample.

3.1. Advantages of DNA Microarray Technology

Microarray technique has a number of advantages over traditional technology. First, it reduces the labor-intensive process of manually transferring and handling samples, saving time and reducing errors. Second, it requires smaller amount of fluids, in microlitre quantity. Third, it is an automated process and gives high degree of flexibility. In contrast to several traditional approaches such as Northern blots which limit research to one-gene-at-a-time experiments, microarray assays allow a large scale experiment which involves monitoring the expression levels of thousands of genes simultaneously at a particular time and at a particular condition. One of the most popular uses of microarray is to measure gene expression levels in two different samples under two different conditions. It allows the study of patterns of gene expression across many experiments that survey diverse cellular responses and conditions. When used properly, gene expression profiling techniques promise a wealth of data that can be used to develop a

more complete understanding of gene function regulation and interactions [[56]].

3.2. DNA microarray in diagnostics and therapeutics

Early diagnosis of an infectious disease is always desirable to prevent its spread among livestock species and thus reduce the economic losses. Microarray technology has been utilized in the identification of various infectious disease pathogens such as Avian influenza (H5N1), FMD, Viral Hemorrhagic Fever (Marburg virus), SARS virus etc. Greene chip microarrays analysis has been utilized for the investigation of samples from patients with Viral Hemorrhagic Fever like syndrome for its differential diagnosis from malarial cases. A large number of microarray related studied had been carried out to characterize diseases cells in comparison to healthy cells. The pathogenicity of coxasackievirus B3 (CVB3) was examined by comparing the murine heart infected with this virus against the non-infected murine hearts.

3.3. Array Primer Extension (APEX) Assay

One of the various approaches in genotyping single nucleotide polymorphisms (SNPs) is using Array Primer Extension Assay, also known as APEX [[57]]. This is more benefcial than other laboratory assay as it contributes to lower reagent costs due to small reaction volumes, e.g. small slides. The main principle of APEX is that oligonucleotides are placed on the microarray glass slide through their 5’ end and complementary PCR amplifed fragment from DNA sample is annealed to the oligonucleotides [[58]]. Subsequently, DNA polymerase extends the 3’ ends of primers with dye tagged nucleotides (e.g. ddNTPs) via sequence specifc single nucleotide extension [[59]].

3.4. Genome sequencing and DNA microarray technology

Genomic assembly 6 was released by the Stanford Genome Center for the first preparation of DNA microarrays of C. albicans. Recently, [[60]] have described the complete sequence of chromosome 7 (Gen Bank accession number AP006852). Different research groups identified genes within assembly 19 of the C. albicans genome, and different methods were used, mostly based on automatic identification procedures. Consequently, the lack of a unified nomenclature for C. albicans genes has become an increasing problem for communication in the scientific community. In order to solve this problem, an international effort involving 18 research groups from seven different countries has recently reviewed assembly 19 to annotate and propose a unique nomenclature for C. albicans genes [[61]]. This international

collaboration has detected 246 genes containing mutations or sequencing errors. This improved dataset has allowed the identification of fungal-specific genes and permitted a detailed analysis of several large multigene families. Once the genome of a microbial isolate is sequenced, the construction of a DNA array can quickly be achieved. Sequencing of the C. albicans genome has led to the construction of whole-genome DNA microarrays for in vitro transcription profiling by several universities and companies. The procedure of DNA microarray technology is schematized. An array is nothing but a collection of DNA probes fixed on a solid support [[62]]. Accordingly, using available sequence information for a microbe of interest, one can perform genome systematic analysis to discover optimum probe and primer choices under specified conditions [[63]]. Other analysis could include sequence alignment when many strains are expected to be found in the sample and computerized analysis to detect if PCR primers self-anneal and form hairpins in order to maximize the amplification yield [[64]].

Basically, nucleic acid hybridization is the keystone of the DNA microarray technology. When denatured, single stranded nucleic acids are incubated together under certain conditions, hybridization can occur, upon which the formation of base paired duplex molecules can be prompted through G:C and/or A:T hydrogen bounds base paring. As a matter of fact the nucleic acids hybridization process may be influenced by the concentration and complexity of the sample, which also can be improved through manipulation of time, temperature and ionic strength of the hybridization buffer [[65]]. Others use the photolithographic technology pioneered by Affymetrix (Santa Clara, CA), where oligonucleotides are synthesized in situ on glass slides. Recent technical developments currently permit the simultaneous analysis of as many as 200,000 hybridizations on one array platform [[66]]. The cDNA microarray allows gene expression profiles to be determined with high reproducibility and efficiency. Construction of cDNA microarrays presents a number of challenges, largely related to costs associated with clone validation, tracking and maintenance. Another limitation of cDNA microarrays is the difficulty, because of cross-hybridization, in discriminating expression patterns of homologous genes, alternative splice variants and antisense RNAs. Several methods have been developed to improve custom array technology [[66]], and nowadays the companies offer a variety of expression arrays that allow the study of any genome.

3.5. Microbial detection and identification

For mycobacterial detection and identification, the *gyrB*, *rpoB*, and *katG* genes have been targeted by using microarrays [[67]]. Microarrays targeting the 23S rRNA and *gyrB* genes for bacterial detection and identification using clinical specimens have been described [[68]; [69]]. In addition to bacterial and myco-bacterial organisms, microarrays following broad-range PCR amplification have been used to detect and identify fungal, parasitic, and viral pathogens [[70]-[72]]. Microarrays have also been incorporated with multiplex PCR amplification for the simultaneous detection and identification of a panel of microbial pathogens in a single reaction. Khodakov described a novel microarray-based approach for the simultaneous identification and quantification of human immunodeficiency virus type 1 (HIV-1) and hepatitis B and C viruses in donor plasma specimens [[73]]. A microarray technique for the detection and identification of enteropathogenic bacteria at the species and subspecies levels was developed, covering pathogenic *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *Campylobacter jejuni*, *Shigella* spp., *Yersinia enterocolitica*, and *Listeria monocytogenes* [[74]].

Perhaps the most promising area in applying DNA microarray technology in medical microbiology is its use for simultaneous assessment of large numbers of microbial genetic targets [[74];[76]]. Specific microbial gene amplification by either a broad-range or a multiplex PCR prior to micro-array analysis enhances test sensitivity. The amplification of universal microorganism targets by broad-range PCR followed by sequencing analysis has been considered a standard procedure. The most commonly used gene targets have been the 16S bacterial and 28S fungal and intergenic transcribed spacers (ITSs) in rRNA genes, and microarray technology has been incorporated to compensate for the time-consuming sequencing identification procedure [[77]]. Assays using broad-range PCR incorporated with microarrays have been shown to allow rapid bacterial detection and identification with positive blood cultures [[78]; [79]]. A similar procedure was developed and used for the rapid diagnosis of bloodstream infections caused by common bacterial pathogens in the pediatric and general populations [[80]; [81]]. PCR amplification, in combination with an oligonucleotide microarray, was used to identify *Bacillus anthracis* based on the rRNA ITS region. Several studies reported the use of microarrays to identify pathogenic yeasts and molds by targeting the ITS regions in fungal rRNA genes.

4. Common types of DNA microarrays

Different types of microarrays have been developed based on their target material, which can be cDNA, mRNA, protein, small molecules, tissues or any other material that allows quantitative analysis which makes them different from each other. Each of these microarrays has several applications in biomedical research. Like, in an mRNA or gene expression profiling experiment the expression levels of thousands of genes are simultaneously monitored to study the effects of certain treatments, diseases and developmental stages on gene expression [[82]]. DNA microarray can be classified according to the type of technologies that are making them. Although there two main technologies:- spotted microarrays and in situ synthesized microarrays, another type was self-assembled microarray can be mentioned as well.

4.1. Spotted Microarrays

These microarrays have a probe length of 500 bp to 1000 bp (cDNA) or 25 to 100 mers (oligose) or fragments of PCR products. In this system two samples are combined on a single slide, thus give a relative expression level on each spot. They are not as specific as oligonucleotide microarray. This method is relatively low cost than oligonucleotide microarray and primary sequence information is not needed to print a DNA sequence. The use of glass slides allows the sample to be easily fluorescently labeled. 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 chemillum-inescent labeling. Third, fluorescent labeling allowed one to label two (or potentially more) samples in different colors and cohybridize the samples to the same array. These advantages include: fluorescent labeling is quite sensitive, less expensive, and requires less complicated protocol than other two labeling methods [[83]]. DNA microarrays on coated glass slides are prepared by printing DNA products with high-speed robots. The common problems associated with glass slides are spot morphology, high background, and what appears to be batch variability. The PCR products are resuspended in an appropriate solution before spotting [[84]].

4.2. In situ synthesized microarrays

This can be done by photolithography technique in which light is directed only at some areas in the microarray with help of a mask [[85]]. However, each step of synthesis requires a unique mask and they are very expensive to produce. One main of the Affymetrix technology over spotted microarrays is that synthesizing DNA sequences directly on the surface requires fewer reagents [[86]; [87]]. This was initially

proposed by Fodor in 1991 and unlike spotted microarrays, the DNA probes are built base by base on the glass microarray surface [[88]]. Each nucleotide added to the probe has a protective group on its 5' position to avoid adding more than one base during each step of synthesis. Their methodology uses chemicals to convert the protective groups and at each step of synthesis, nucleotides are fired onto the target spot using nozzles, the same ones found in inkjet printers [[86]; [89]]. A major advantage of this technology over the Affymetrix one is that a computer input system controls the synthesis of the oligonucleotides in each microarray. Although this is very flexible, it is less efficient when making great number of identical microarrays [[86]; [88]].

4.3. Self-assembled DNA microarrays

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") [[90]-[93]]. Optical decoding by fluorescent labeling limited the total number of unique beads that could be distinguished. 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 a fiber option arrays. Their method involved synthesizing DNA on small polystryrene 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. This is another technology that is based on synthesizing DNA on minute polystyrene or silicon beads of about 3μm [[94]; [95]]. These are then deposited randomly on silicon wafers or optical fibers containing arrays of micro-wells in which each catches a certain particle and hold it in place via strong adhesion forces.

5. Future perspectives of DNA microarray

DNA Microarrays are also expected to become a routine and widely used tool for disease diagnosis and classification, which anticipates the future availability of home testing kits. Eventually, DNA microarrays could be used as a routine diagnostic tool 'DNA microarray readers, with which treatment should be tailored for an individual patient [[96]]. The use of DNA microarrays for target identification and validation is currently being explored. The potential discovery of a gene, which, when knocked-down (e.g. by RNAi), destroys only cancer cells could indicate an approach for new

cancer therapies [[97]]. The possibilities of using cell DNA microarrays for large-scale RNAi studies have been proposed by several groups [[98], [99]]. Although this technique is still in its early years, cell DNA microarrays can significantly reduce the effort essential for rapid cell-based RNAi screens. In the future, in situ oligonucleotide arrays, which dramatically increase the number of probes per array, have the capability to enhance the discriminatory power of microarrays considerably, enabling the detection of smaller and smaller genetic differences on a whole-genome scale. Even so, advances in sequencing technologies currently allow the complete decoding of an entire microbial genome in a few hours [[99]], clearly out-competing arrays for the detection of SNPs, albeit at a substantially higher cost.

New tools originating from bioinformatics approaches will help in the automatic interpretation of the massive amounts of information obtained with DNA microarray technology. International consensus needs to be achieved for data evaluation (absence or presence thresholds) to maximize the enormous potential of arrays. The application of microarrays of C. albicans in early studies on pathogenesis, cell biology, antifungal susceptibility and diagnosis confirmed its usefulness, and the results showed good correlations with data obtained with other techniques. Hence, future microarrays could be constructed in a modular fashion, with oligonucleotide-based elements being added to existing PCR amplicons as more genomic sequence information is gathered, in the absence of readily available cDNA clones. Although the design and construction of DNA microarrays are nowadays still limited to a restricted number of specialized centres, companies have discovered array technology as a viable market and are speedily expanding the construction and distribution of genome-wide microarrays. It also both oligonucleotide and PCR product-based arrays are used for the study of bacterial species. Whether one format will prevail in the future will largely depend on robustness, feasibility (cost and availability of technology) and purpose of the experiments. For example, short oligonucleotide arrays may not be suitable for comparative genomic studies for organisms that are not closely related. Additionally, an individual array could be made of both oligonucleotides and PCR products. Sequencing is a direct measurement of which nucleic acids are present in solution. One need only count the number of a given type of sequences present to determine its abundance. Counting sequences is linear with concentration and the signal to noise one can obtain by sequencing is only limited by the number of reads used for each sample.

6. Limitations of DNA microarrays

At their core, 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. At high concentrations the array will become saturated and at low concentrations, equilibrium favors no binding. Hence, the signal is linear only over a limited range of concentrations in solution.

Second, especially for complex mammalian genomes, it is often difficult to design arrays in which multiple related DNA or 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 [[101]] or to exon junctions [[102]]. If researchers are dealing with several DNA sequences that are related (e.g. mammalian genomes), it is hard and sometimes even impossible to design microarray as they might end up binding to same probe on the microarray [[102]]. This is a huge problem for genes with multiple splice variants and gene families [[103]]. In gene expression profiling, the correlation between induced mRNA and induced levels of protein are not always well aligned. Translational and post-translational regulatory mechanisms that affect the activity of various cellular proteins are not examined by DNA microarrays, although the promising field of proteomics is commencing to address these issues. Differential gene expression analysis is not a stand-alone technique; results must be confirmed through direct examination of selected genes. These analyses are typically done at the level of RNA blot or quantitative RT-PCR, to examine transcripts of specific genes, and /or at the protein level, analyzing protein concentrations using immunoblotsor ELISA.

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. Moreover, for highly variable genomes such as those from bacteria, arrays are typically designed using information from the genome of a reference strain. Such arrays may be missing a large fraction of the genes present in a given isolate of the same species [[104]]. In each microarray is designed to detect only certain DNA sequences [**Error! Reference source not found.**]. In case of gene expression analysis, if a gene is not yet included to the genome it will not be detected on the microarray [**Error! Reference source not found.**]. Hence an array designed using gene annotation from a "reference isolate" will not contain many of the genes found in other isolates.

7. Gaps in Research

The use of DNA microarrays as a tool for phylogenetic studies and strain identification merits attention. For many organisms, the 16S rRNA approach often fails to truly reflect their genetic potential. This gap can be bridged by comparative genomic methods with whole genome arrays in the absence of genome sequences. It is not difficult to envision the future construction of a DNA array that will contain unique sequences of 16S rRNA, 23S rRNA, and many key functional genes for most of the representative bacterial species. This array could be useful in food, medical, environmental, and agricultural applications. Currently, both oligonucleotide and PCR product-based arrays are used for the study of bacterial species. Whether one format will prevail in the future will largely depend on robustness, feasibility and cost and availability of technology and purpose of the experiments.

8. Conclusion

DNA microarray analysis bioinformatics tool has been applied in the identification of novel targets and discovery of different diseases therapeutics. The series of biological activities that goes on in animal and humans are too numerous how they the gene mutate to give rise to certain disease has not been fully understood thereby making it a complex biological system. Microarray technology is based on the well-established and highly exploited principle of nucleic acid hybridization. The technique offers the possibility of simultaneously conducting tens or hundreds of thousands of simultaneous hybridizations. This increased experimental efficiency permits high through put and whole genome expression profiling of pathogens and hosts.

Microarray technologies are rapidly advancing with numerous applications in Medicine, genomics, proteomics, pharmaco-genomics, molecular biology, infectious diseases, food industry, chemotherapy, antibiotic resistance and so on. While the use of microarray in the investigation of infectious diseases is still in its infancy, new innovations in this emerging technology will throw more light in understanding the molecular basis of infectious agents and the diseases. Microarray-based diagnostics is being developed in many countries and still new ideas are being incorporated into the assays to increase their versatility. Most reviewed publications highlight that highly variable genetic regions are easily detected by DNA microarrays within different strains of a species. DNA microarray technology is based on the well-established and long exploited principle of nucleic acid hybridization. It offers simultaneous detection of thousands of targets in a high-throughput environment

In conclusion, data from microarray analysis can provide potential information that helps to find the causes of disease, the mechanism of drug action and the discovery of gene products that are targets for therapy in various disorders. With the use of these approaches, more accurate diagnosis and risk assessment of various diseases can be attained, leading to more precise prognosis and new the rapeutic interventions. Thus, from today's standpoint, the ultimate scope of microarray technology will be the day when it makes its way into adoctor's clinic and becomes a routine diagnostic tool.

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