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DNA Microarray Technology

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Abstract

Microarrays are a technology to which thousand nucleic acids are attached to a surface that's used to estimate the relative concentration of nucleic acid sequences in a mixture by hybridization and hybridization event detection. In this report we will discuss the background of microarrays and the prior developments that may have led to their advancement. We then discuss the methods of manufacture, restriction and the most common biological application.

Introduction:

Human beings possess dozens of thousands of genomes, and the innovation of DNA microarrays by Patrick et al in the mid-1990s enabled many of genes to be analyzed simultaneously. Initial research utilizing microarrays relied on trying to determine which genes between normal cells and cancer cells were displayed in different manner. (3) (1)

These techniques have enabled physicians much more insight over time. For instance, microarrays as of now are a major tool in genetic analysis, assisting doctors to address specific subtypes due to differences in gene expression within a general classification of disease. In fact, doctors can also use microarray data to decide which approache of therapy would be most probable effective for a patient with cancer. However, how do microarrays operate, and how are they used to manage different mutations, shine a light on both questions. (3) (1)

Researchers acknowledge that a mutation-or modification-may contribute to a disease in the DNA of a certain gene. Because most major genes have multiple regions where mutations can occur, it could be complicated to establish a method to detect such mutations. Moreover, the chip comes in the form of a slim plastic-coated glass plate. Every chip on the surface holds thousands of tiny, synthetic, single-stranded DNA sequences, all of which add up to the completely normal gene in question, and varieties (mutations) of that same gene apparently found in human population. (3)

DNA microarrays have been used for research purposes only. Nonetheless, scientists strive to implement huge-scale population studies since. Just like with computer chips, quite large quantities of' properties' can be positioned on microarray chips, portraying a rather significant portion of the human genome. It is also possible to use microarrays to test the degree to which those genes are turned on or off. (3)

The material and methods

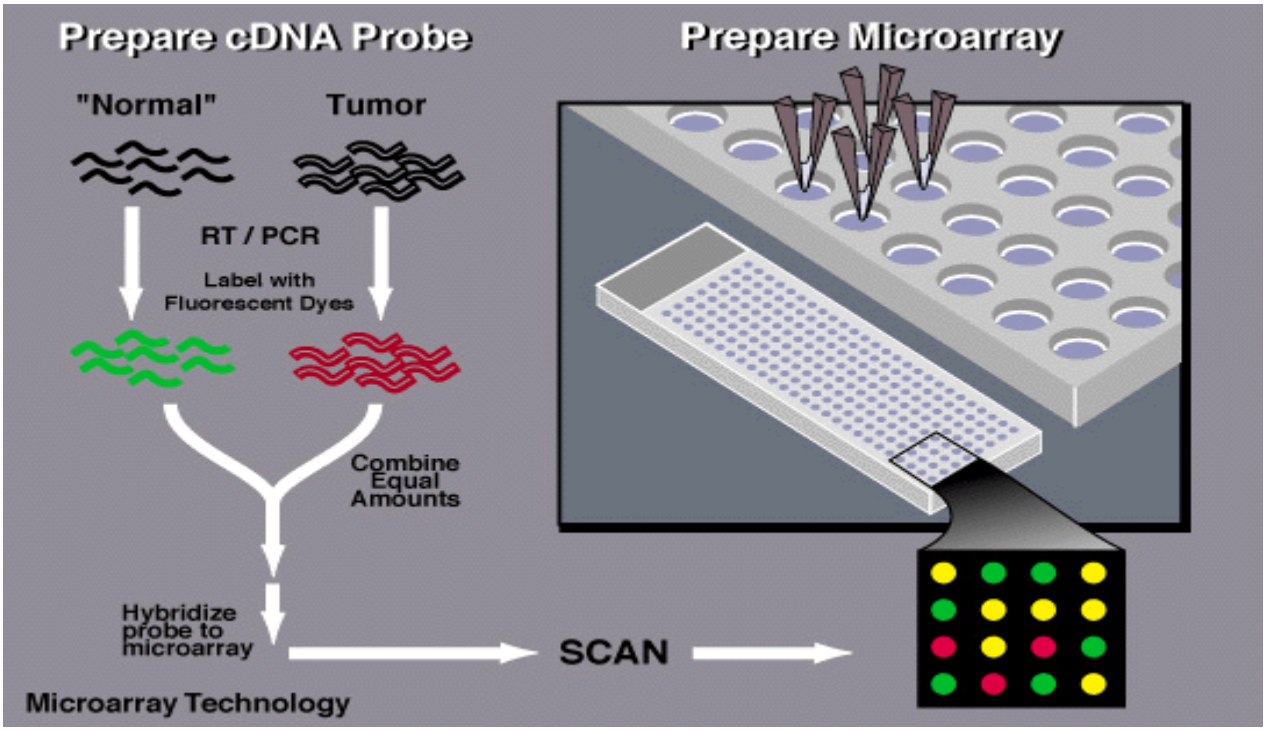
The following information that has been gathered, analyzed from respected international papers PubMed, google scholar.

Discussion/Result

Microarray scan has ninety spots organized in ten columns with nine rows. The spots appear as if O's and are red, green, and yellow against a black background. (1)

DNA microarrays exploit the flexibility of complementary strands of nucleic acids to base-pair with one another and bind. As an example, ATATGCGC can bind to its complement (TATACGCG) with a precise affinity. This method was first used by Sol Spiegelman to measure the homology (similarity) of two different nucleic acids; Spiegelman called the method "hybridization" of nucleic acids. (1)

Eventually, the DNA microarray developers dotted a set of DNA copies (cDNAs) corresponding to a wide number of known sequence mRNAs onto a glass slide. It was labeled a microarray since this array was so tiny. Although the cDNAs were double-stranded, they could be melted or denatured into single strands which could then be used to attach or hybridize to fluorescently tagged nucleic acid samples from cancerous or normal cells. After washing away the unbound molecules, bound fluorescent nucleic acid samples were identified by laser microscopy.(1)



(Figure 1) Gense chip and microarray sequences for many (all) genes of an Organism

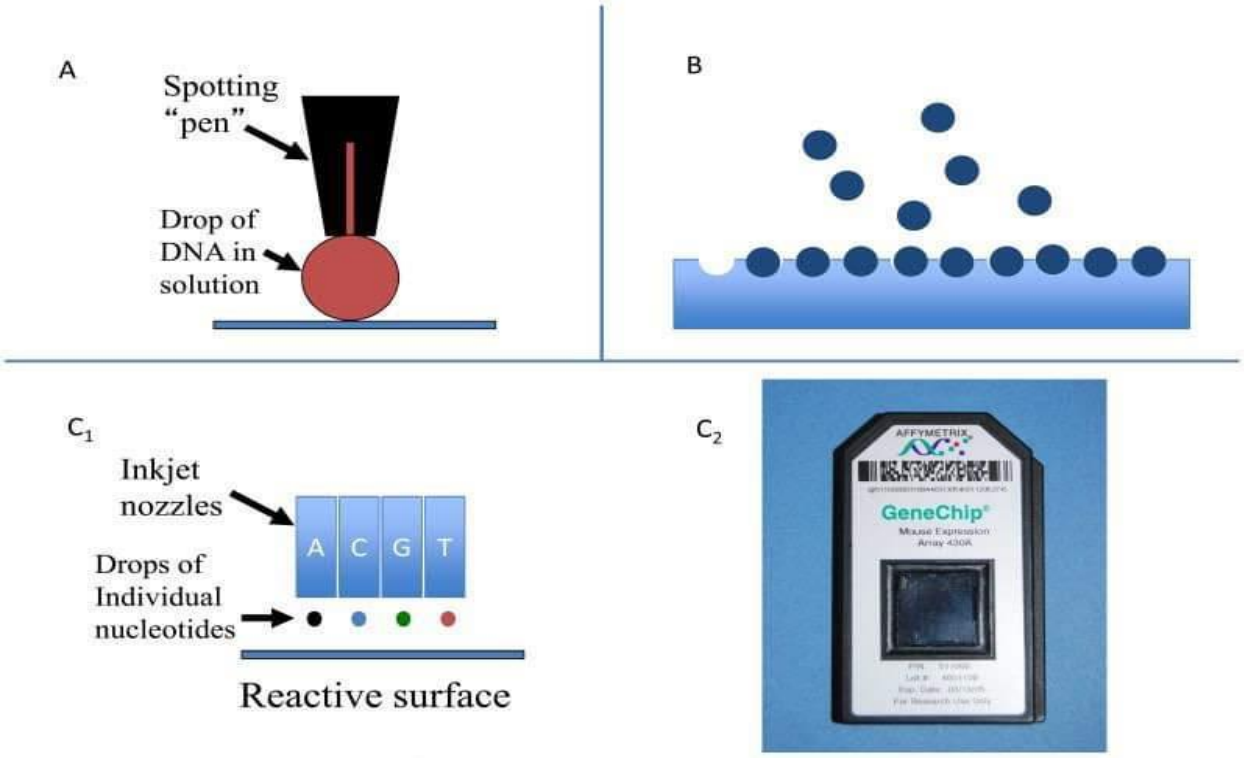
Fluorescent dots indicated expressed genes, and differences in microarray patterns between normal and cancerous cells could be quickly identified. (1) (3)

In these early microarray studies, mRNA from one type of cell was converted into a red Fluorescent dye marked cDNA and mRNA from another type of cell was converted into cDNA labeled with a green fluorescent dye. The two cDNAs were then fused and hybridized to the same microarray of DNA, resulting in red, green and yellow spots (caused by a red and green mix). [(1) (3)]

The microarray is scanned to measure the expression of each gene printed on the slide. If the expression of a gene is higher in the experimental sample than in the reference sample, then the corresponding spot on the microarray appears red. In contrast, if the expression in the experimental sample is lower than in the reference sample, then the spot appears green. Finally, if there is an equal expression in the two samples, then the spot appears yellow. [(1) (3)]
The data gathered through microarrays can be used to create gene expression profiles, which show simultaneous changes in the expression of many genes in response to a condition or treatment. [(1) (3)]

Comparative gene expression in the two samples could easily be determined by quantitating the ratio of red and green fluorescence in the spot corresponding to each gene. [(1) (3)]

There are three basic types of microarray: (A)Spotted array on the glass. (B)Self assembles the array. (C)In-situ synthesized array. [(figure 2)]



(Figure2) Types of DNA microarray (A) Spotted arrray. (B) Self assembled. (C)In situ synthesized array.

Methods of DNA microarray

Spotted Array

Derisi et.al in 1996.Presented a way to place DNA arrays on poly lysine coated glass microscope slides in a microtiter by using slotted pins (similar to fountain pen) offered a decent DNA binding site also enabled it to branded fluorescently. Fluorescent branding has various advancement then the standard labeling that are radioactive or chemiluminescent for instance fluorescent branding is not only more sensitive but also less expensive and less complicated unlike radioactive or chemiluminescent labeling. (1)

In situ synthesized array

A light-directed, flexible chemical synthesis that combines photo labile protecting groups with photolithography to conduct chemical synthesis on a solid substrate Fodor et al in 1991. In 1994, Fodor et.al. at the company of Affymetrix exhibited the ability to use this technology to generate DNA arrays consisting of 256 different octa-nucleotides. Affymetrix used this technology to develop a wide catalog of DNA arrays for use in expression analysis, genotyping and sequencing. (1)

DNA sequence are made directly on the surface which was a key benefit not only that but using only a small collection of reagents are needed to construct an arbitrarily complex array in contrasts to 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 to direct the light to the array at each step of the synthesis process. In 2002, Nimble Systems Inc. published a method that enhanced photo-deprotection step of Affymetrix by using micro-mirrors to direct light at the pixels on the array. (1)

In 1996, Blanchard et.al. Suggested a technique to produce oligo arrays by using inkjet printing technology that was modified and oligo synthesis chemistry. In brief, to deliver the four different nucleotide phosphonamidites to a glass slide that was pre-patterned to contain regions containing hydrophilic regions surrounded by hydrophobic regions, the hydrophobic region enclosed the droplet(s) deposited by the inkjet. (1)

Self-assembled arrays

A group of David Walt at Tufts University had a totally different approach to the building of array which was making numerous types of DNA on small polystyrene beads and depositing those beads on the end of a fiber-optic array in which the ends of the fibers were attached to provide a well that is slightly larger than one bead result in a randomly assembled array. In early versions of these arrays, the beads were optically encoded with different fluorophore

combinations to allow one to determine which oligo was in which position on the array (called decoding the array). [1]
The later and present methods of decoding the beads therefore include hybridizing and detecting many small, fluorescently labeled oligo in a sequence of sequential steps. This not only makes it possible to use an incredibly large number of different types of beads on a single array, but also to test the array functionally before being used in a biological assay. Later models of the arrays used by Illumina arrays used a pitted glass surface to contain the beads instead of a fiber option arrays.

The above is not supposed to be a comprehensive overview or analysis of all microarray DNA technologies. It does, but then again, cover major advances in the industry, and the prevalent array manufacturing methods.[1]

Application:

The major applications of microarrays fall into three groups:

Gene expression profiling—RNA extracted from a complex sample (such as body tissues or fluids or bacterial isolates) is applied to the microarray. The result reveals the level of expression of tens of thousands of genes, effectively all the genes in the genome, in that complex sample. This result is known as a gene expression "profile" or "signature." [1] [3]

Genotyping—Genomic DNA, extracted from an individual's blood or saliva, is amplified by the polymerase chain reaction and applied to the microarray. The genotype for hundreds or thousands of genetic markers across the genome can be determined in a single hybridization. This approach has considerable potential in risk assessment, both in research and clinical practice. [1] [3]

DNA sequencing—DNA extracted from an individual's blood is amplified and applied to specific "re-sequencing" microarrays. Thousands of base pairs of DNAs can be screened on a single microarray for mutations in specific genes whose normal sequence is already known. This greatly increases the scope for precise molecular diagnosis in a single gene and genetically complex diseases. [1] [3]

The recently developed molecular diagnostic assays based on the hybridization of probe nucleic acids with target nucleic acids from clinical samples are allowing effective detection of various diseases with high speed, sensitivity, and specificity. [1] [3]

Diagnostic DNA Microarray using disease relevant biomarkers

DNA Microarray improve diagnosis in comparison to nano specific tumor marker, DNA Microarray genes specific associated with different stages and condition. Screening by

expression profiling in leukocyte can help to diagnose and differentiate between auto immune disease (SLE and Rheumatoid arthritis) and chronic disease like osteoarthritis.
Gene expression profile utilizing DNA Microarray can help diagnosis gastrointestinal disease like Crohn's disease and ulcerative colitis and differentiating them from inflammatory bowel disease. [1] [2]

DNA Microarray for the diagnosis of genetic disorder

Genetic disorders can be chromosomal or structural (mutation in DNA sequence).
DNA Microarray have provided many advantages to the analysis of certain genetic mutation and single nucleotide pleomorphic (SNPs) in high bot manner.
May help replacing time consuming, expensive and laborious sequencing conventional method of mutation detection (PCR)Polymerase Chain Reaction, (RFLP)Restriction Fragment Length Polymorphisms, (SSCP)Single-Strand Conformation Polymorphism. [2]

Detection of chromosomal abnormalities

Kang and coworker introduced DNA Microarray for detecting chromosomal abnormalities in various genetic disorder like Down syndrome, turner syndrome Klinefeter syndrome, Edward syndrome, alpha Thalassemia, William syndrome, and hereditary neuropathy. [2] [1]

Genotyping

In comparison to several alternative approaches used to detect SNPs, Affymetrix developed a PCR based approach to reduce genomic complexity which may improve the diagnosis of certain genetic disorders. [2]

Limitation of DNA Microarray

- The biggest disadvantage of DNA chips is that they are expensive to create.
- Arrays provide an indirect measure of relative concentration.
- Complexity of designing arrays mammalian genomes.
- DNA array can only detect sequences that the array was designed to detect.
- The DNA chips don't have a very long shelf life.

Conclusion

Microarray helps in diagnosis of many genetic disorders (chromosomal and structural). It helps in the diagnosis of many auto immune and inflammatory disorders.
A total unbiased approach for direct measurement of all the DNA and RNA species found in sample would be preferable.
Gene sequencing technology maybe beneficial over microarray when cost is comparable.

References:

1. Roger Bumgarner,(2014) DNA microarrays: Types, application, and their future, Mol. Biol. 101:22.1.1–22.1.11.
2. Victor Trevino, Francesco Falciani, and Hugo A. Barrera-Saldañia, (2007) DNA microarrays: A powerful genomic tool for biomedical and clinical research, Mol Med. (9-10): 527–541.
3. RajeshwarGovindarajan, JeyapradhaDuraiyan, KarunakaranKaliyappan and MurugesanPalanisamy, (2012) Microarray and its applications, (Suppl 2): S310–S312.