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DNA Microarray Technique

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ABSTRACT

DNA Microarray is the emerging technique in Biotechnology. The many varieties of DNA microarray or DNA chip devices and systems are described along with their methods for fabrication and their use. It also includes screening and diagnostic applications. The DNA microarray hybridization applications include the important areas of gene expression analysis and genotyping for point mutations, single nucleotide polymorphisms (SNPs), and short tandem repeats (STRs). In addition to the many molecular biological and genomic research uses, this review covers applications of microarray devices and systems for pharmacogenomic research and drug discovery, infectious and genetic disease and cancer diagnostics, and forensic and genetic identification purposes.

KEYWORDS

Microrarray, Oligonucleotide array, Hybridization, Scanners, DNA Chip, Biochip

INTRODUCTION

All living organisms contain DNA, a molecule that encodes all the information required for the development and functioning of an organism. Finding and deciphering the information encoded in DNA, and understanding how such a simple molecule can give rise to the amazing biological diversity of life, is a goal shared in some way by all life scientists. Microarrays provide an unprecedented view into the biology of DNA, and thus a rich way to examine living systems. DNA is a physical molecule that is able to encode information in a linear structure. DNA encodes for genes, and regulatory elements control whether genes areon or off. For instance, all the cells of the human body contain the same DNA, yet there are hundreds of different types of cells, each expressing a unique configuration of genes from the DNA. Microarrays are a tool used to read the states of DNA.[1]

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Microarrays are, in principle and practice, extensions of hybridization-based methods which have been used for decades to identify and quantitate nucleic acids in biological samples (e.g. Southern12 and Northern blots 13, colony hybridizations, dot blots14): samples of interest are labeled and allowed to hybridize to the array; after sufficient time for hybridization and following appropriate washing steps, an image of the array is acquired and the representation of individual nucleic acid species in the sample is reflected by the amount of hybridization to complementary DNAs immobilized in known positions on the array.[2,3,4]

Conventional DNA probing and microarray analysis are two sides of the same coin. Fundamental to both processes is the binding (hybridization) of DNA, derived from a sample suspected of containing a pathogen (the 'unknown'), with highly characterized DNA derived in advance from a pathogen of interest (the 'known' DNA).[5]

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History

The Array technology was in use as early as the 1980s.It was first called ‘Macroarrays’. Until the mid 1990s when cDNA microarrays emerged as an exciting new biomolecular tool capable of probing the entire transcriptome of the cell.[6] The field of DNA microarray has evolved from Ed Southern’s key insight 25 years ago showing that labeled nucleic acid molecules could be used to interrogate nucleic acid molecules attached to a solid support. The resulting Southern blot is considered to be the first DNA array. It was only a small step to improve the technique to filter-based screening of clone libraries. The next advance was the use of gridded libraries stored in microtiter plates and stamped onto filters in fixed positions. With this system, each clone could be uniquely identified and information about it accumulated. The subsequent explosion of array technologies was sparked by two key innovations. The first was the use of nonporous solid support, such as glass, which has facilitated the development of fluorescence-hybridization detection. The second critical innovation was the development of methods for high-density spatial synthesis of oligonucleotides. Recently, a significant technical achievement was obtained by producing arrays with more than 250,000 oligonucleotides probes or 10,000 different cDNAs per square centimeter. Because this application requires the discrimination of only one mismatch, the presence of a short oligonucleotide maximizes the destabilization caused by mis-pairing. With this technology, cells or tissues are exposed to toxicants, and then gene expression is measured by collecting mRNA, converting mRNA to labeled cDNA, hybridizing it to the DNA array, staining it with an appropriate dye, and visualizing the hybridized genes using a fluorometer. The raw data are analyzed using bioinformatics software and databases. The aim is to obtain meaningful biological information such as patterns of relative induction/repression levels of gene expression, participation in biochemical pathways, and (in the most favorable cases)“genetic signatures”.[7]

Line of Recent DNA Microarray Developments

1991: Photolithographic printing (Affymetrix)

1994: First cDNA collections are developed at Stanford

1995: Quantitative monitoring of gene expression patterns with a complementary

DNA Microarray.

1996: Commercialization of arrays (Affymetrix)

1997: Genome- wide expression monitoring in S. cerevisiae (yeast)

2000: Portraits/Signatures of cancer.

2003: Introduction into clinical practices

2004: Whole human genome on one microarray[8]

Types of Microarrays

Depending upon the kind of immobilized sample used construct arrays and the information fetched, the Microarray experiments can be categorized in three ways:

1. Microarray expression analysis: In this experimental setup (Fig.1) 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.

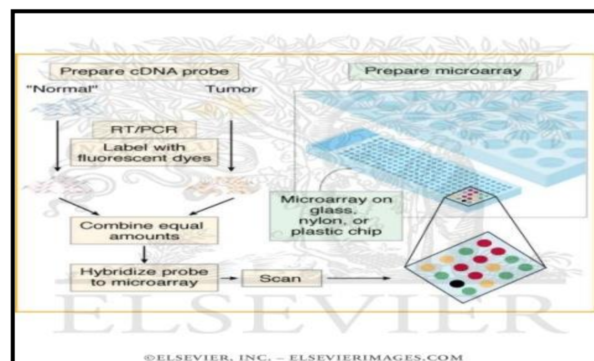

Figure 1: Microarray expression analysis

Microarray for mutation analysis: For this analysis, the researchers use 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 (Fig.2)

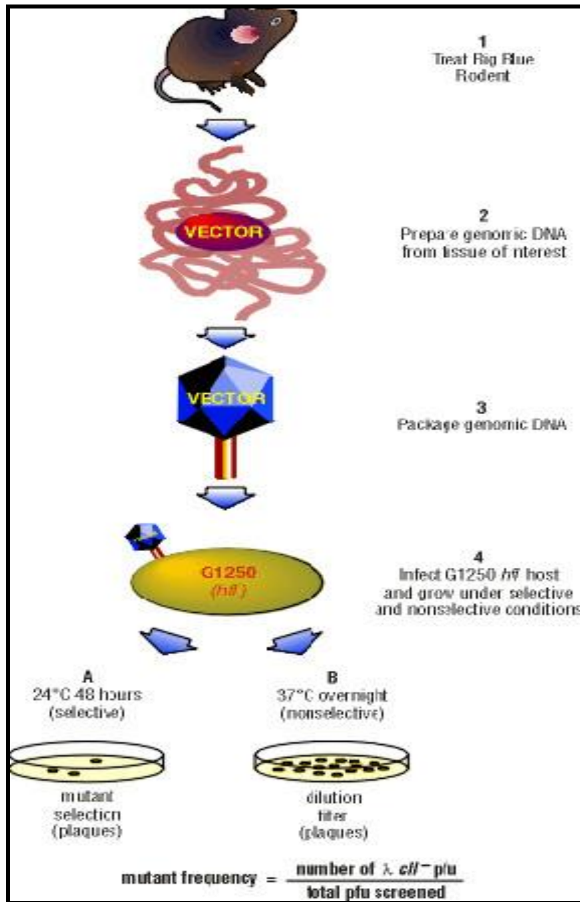

Figure 2: Microarray for mutation analysis

3. Comparative Genomic Hybridization (Fig.3): It is used for the identification in the increase or decrease of the important chromosomal fragments harboring genes involved in a disease.[5]

DNA Microarray Technique- A Technique That is Reshaping Molecular Biology

Also called

- DNA chips
- Biochips
- Gene chips
- Gene arrays
- Genome chips
- Genome arrays[9]

A DNA microarray (Fig.4.) is one of the latest multiplex technologies in the field of molecular biology and medicine. It is a technique used in combination of bioinformatics and statistical dataanalysis.[10] A microarray is so-called because it can comprise 20,000 or more different known DNAs, each DNA being spotted onto glass slides, to form the array (Fig.4). Each spot is only around 10 µm in diameter.[11] It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles (10^{-12} moles) of a specific DNA sequence, known as *probes* (or *reporters*). These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (called *target*) under high-stringency conditions.

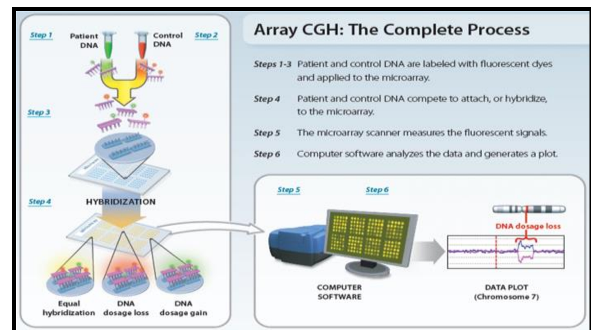

Figure 3: Comparative Genomic Hybridization

Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. The DNA microarray is an assay that can be used to measure the level of expression in a collection of cells for thousands of genes. Nearly all the cells of an organism carry the same genome. The phenotypic differences among cells of different types are

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determined by differences in the level of expression of the genes.[12]

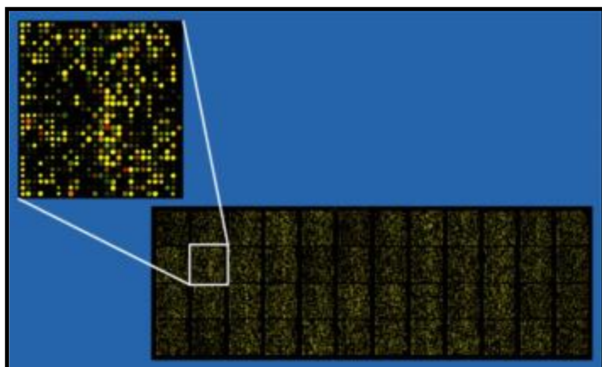

Figure 4: A DNA microarray

Principle

The core principle (Fig.5) behind microarrays is hybridization between two DNA strands, the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A high number of complementary base pairs in a nucleotide sequence means tighter non-covalent bonding between the two strands. After washing off of non-specific bonding sequences, only strongly paired strands will remain hybridized.[13] So fluorescently labeled target sequences that bind toa probe sequence generate a signal that depends on the strength of the hybridization determined by the number of paired bases, the hybridization conditions (such as temperature), and washing after hybridization. Total strength of the signal, from a spot (feature), depends upon the amount of target sample binding to the probes present on that spot.

Microarrays use relative quantization in which the intensity of a feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position. An alternative to microarrays is serial analysis of gene expression, where the transcriptome is sequenced allowing an absolute measurement[14]

Types of DNA Microarrays

Two basic types of DNA microarrays are commonly used:

1. Spotted Arrays (Stanford)

In spotted array method, a large number of cDNAs are prepared from a cDNA library and then spotted onto a glassslide by a robot. Each cDNA corresponds to one probe of length of 100-1000bpnearer to the 3'end of a gene or EST. Each spot on the slide corresponds to a particular probe. A labelled sample of mRNA is eluted onto the slide and is hybridizedovernight. The arrays are then scanned and the quantitative fluorescence image alongwith the known position of the cDNA probes is used to asses whether a gene or ESTis present and its relative abundance. Note in cDNA arrays the fluorescence image isa ratio of the abundance of mRNA of two samples. Here 2 fluorescent dyes are used (cy3, cy5).[9]

2. Oligonucleotide Arrays

Pioneered by Affymetrix (Gene Chip®)

In the oligonucletide arrays multiple probes of 20 mers are synthesized base by base built *in situ* ("on-chip") using photolithography in hundreds of thousands of different positions on

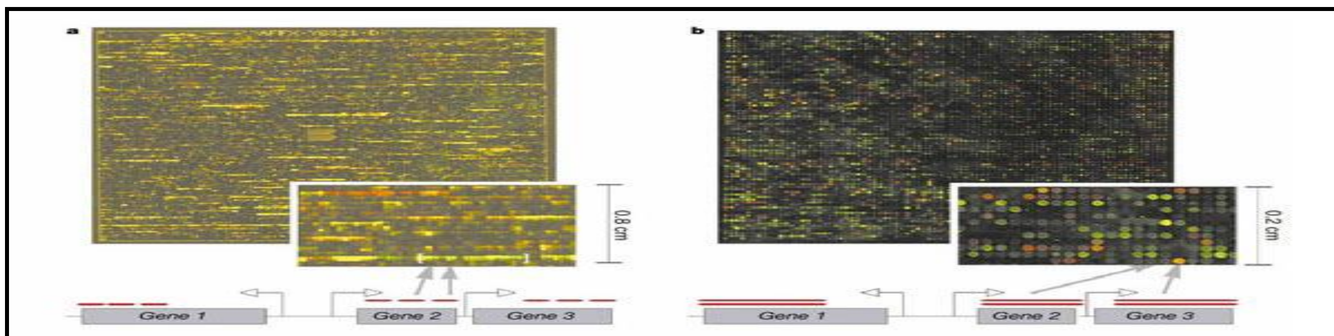

Figure 5: Oligonucleotide microarray

Figure 6: DNA microarray

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a glass plate.[12] For each gene or EST, multiple probes of length 20bp are placed in a particular position of the microarray. Again the probes are taken from the 3'end of a gene or EST. It uses 1 fluorescent dye. As in the cDNA array a labelled sample of Mrna is eluted onto the slide and is hybridized overnight. The arrays are then scanned and the quantitative fluorescence image along with the known position of the cDNA probes is used to assess whether a gene or EST is present and its abundance. In the oligonucleotide arrays the fluorescence image is an absolute measure of the abundance of mRNA of a sample.

DNA Microarray Experiment

This is an example of a DNA microarray experiment, detailing a particular case to better explain DNA microarray experiments, while enumerating possible alternatives.

Components of Microarray Fabrication

The complete process (Fig.7) involved in making DNA microarrayshas the following main steps:

- Preparation of samples
- Construction of the arrays
- Preparation of the probes
- Hybridization

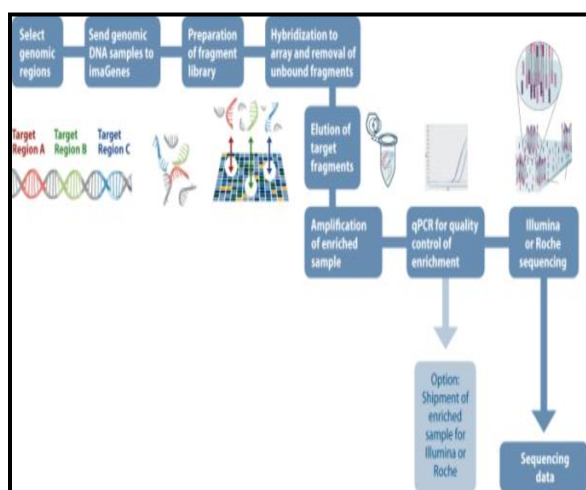

Figure 7: Flow chart of DNA Microarray Technique

Preparation of Samples

The first step in the process of microarrays is the preparation of the target DNA. This can be done from the genomic source, or Expressed Sequence Tags. The Polymerase Chain Reaction (PCR) is then used to amplify this DNA. This amplification step is vital in many applications and array formats.

- Construction of the Arrays

The DNA sequences are printed onto the microscope slides robotically, in the specific grid pattern. There are a number of methods for producing the microarray slides, and these are mainly done using robotic systems. They each have their own advantages and disadvantages. The three main methods for constructing the arrays are:

Spotting of DNA fragments directly onto the slide. Arraying of prefabricated oligonucleotides. In-situ synthesis of oligonucleotides, done on the chip [15].

- Preparation of the Probes

Fluorescent probes must be prepared, and these hybridise to the microarray.

These are prepared from messenger RNA from the cells or tissues of interest. Extraction of the mRNA is made easier by the identification of a key property by which the mRNA can be isolated. This can be done either by using a column or a Solvent system like phenol-chloroform.

Here the pH of buffer plays an important role. Generally a phosphate buffer is usedhe purified RNA is analyzed for quality (capillary electrophoresis) and quantity (by using a nanodrop spectrometer): if enough material (>1µg) is present the experiment can continue.

After the mRNA has been extracted from the cells or tissues under study, it is converted into cDNA by the use of the reverse transcriptase enzyme (Fig.8).

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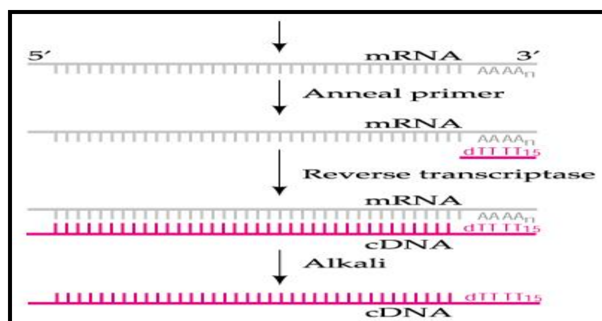

Figure 8: Preparation of the Probes

During this reaction, the DNA is labelled by the incorporation of fluorescent or radioactive nucleotides into the DNA at RT. The two samples are labelled using two different fluorescent dyes - say, red or green. The most common dyes in use are Cy3 and Cy5.The labeling can be direct (not used) or indirect which requires a coupling stage. The coupling stage can occur before hybridization (two-channel arrays) using aminoallyl-UTP and NHS amino-reactive dyes (like cyanine dyes) or after (single-channel arrays) using biotin and labeled streptavin.This labelled DNA is then hybridised to the microarray slide. Using mixtures that are differentially labelled allows the ratio of fluorescence to be measured, therefore avoiding most of the problems of hybridisation kinetics.[16]

- Hybridisation

Hybridisation (Fig.9) is the reaction that occurs between the fluorescent probes and the DNA on the microarray.

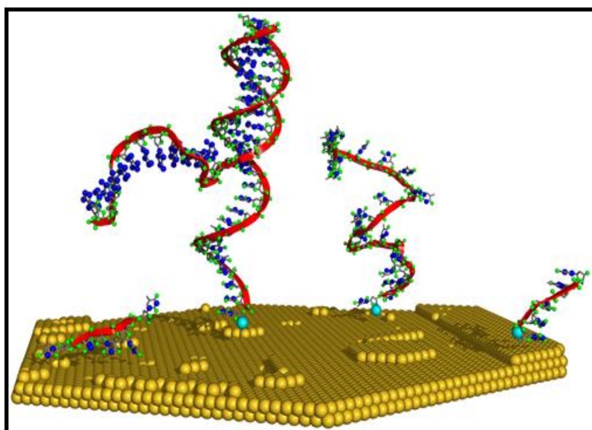

Figure 9: Hybridisation

The labeled samples are then mixed with a propriety hybridization solution which may contain SDS, SSC, dextran sulfate, a blocking agent (such as COT1 DNA, salmon sperm DNA, calf thymus DNA, PolyA or PolyT), Denhardt's solution and formamine.

The hybridisation conditions also depend on the application of the array:

Detecting mutations requires high hybridisation stringencies: lower salt concentration and higher temperature, over short time periods (hours)

Expression monitoring requires lower stringencies, to ensure low-copy number sequences anneal: overnight hybridisations with lower temperatures and higher salt concentrations.[17] After an overnight hybridization, all nonspecific binding is washed off (SDS and SSC).After hybridisation, the microarray is scanned using a laser confocal scanning microscope, which illuminates each spot of DNA and separately measures the fluorescence for each dye. This produces data to determine the ratio, and in turn the relative abundance of the sequences of each specific gene in the messenger RNA or DNA samples. The hybridisation pattern can then be used to identify the genes that are expressed differently in the tissues or cells.[18]

Microarray Equipments

- **Arrayers**

Arrayers (Fig.10) are available from a number of companies at a cost of approximately Rs.30lacs.The construction of arrayers is possible in the laboratory, but it is not easy to do. Some laboratories have relied on professional engineers.

Figure 10: Arrayers

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- **Scanners**

The scanner (Fig.11) has a laser, a computer, and a camera. The principle of scanners is to detect the different levels of fluorescence between the spots on the micro array. This produces a ratio of fluorescence, representing a ratio of hybridisation, for the different mRNA or cDNA sequences.

The laser causes the hybrid bonds to fluoresce by exciting the dye. The camera records the images produced.

The computer allows us to immediately view our results and it also stores our data.[17] After the initial capital outlay, the cost in this technique comes from the lengthy experimental procedure. The need for so many cDNAs to be printed is also a disadvantage of this technique. To decrease the cost of this technique, the number printed can be reduced.

Figure 11: Scanners

Data Analysis and Management

GREEN- the healthy sample hybridized more than the diseased sample.

RED-the diseased/cancerous sample hybridized more than the non-diseased sample.

YELLOW-both samples hybridized equally to the target DNA.

BLACK-areas where neither sample hybridized to the target DNA.

By comparing the differences in gene expression between the two samples, we can understand more about the genomics of a disease (Fig.12)[17].

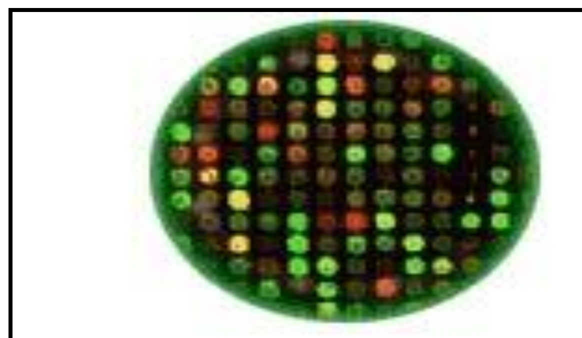

Figure 12: Analysis chart

The raw data is normalized, the simplest way is to subtract the background intensity and then divide the intensities making either the total intensity of the features on each channel equal or the intensities of a reference gene and then the t-value for all the intensities is calculated. More sophisticated methods include z-ratio, loess and lowness regression and RMA (robust multi-chip analysis) for Affymetrix chips (single-channel, silicon chip, in situ synthesized short oligonucleotides).After hybridisation and the readout of the expression levels obtained from the array, the vast quantities of data that is collected has to be stored and saved. This then enables processing and analysis - both statistical and biological - to be carried out on the data[18]

Analytical Methods

Once a dissimilarity measure has been chosen, the appropriate analytical technique can be applied. There are two commonly used techniques:

1) Unsupervised techniques

a) Hierarchical clustering

It is a commonly used unsupervised technique that builds clusters of genes with similar patterns of expression. This is done by iteratively grouping together genes that are highly correlated in terms of their expression measurements, then continuing the process on the groups themselves are used to visualize the resultant hierarchical clustering.

b) Self-organizing maps

Self-organizing maps are similar to hierarchical clustering, in that they also provide a survey of

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expression patterns within a data set, but the approach is quite different. The genes are first represented as points in multidimensional space. In other words, each biological sample is considered a separate dimension or axis of this space, and after the axes are defined, genes are plotted using expression levels as coordinates. This is easiest to visualize with three or less microarrays, but extends to a larger number of experiments/dimensions.[19]

c) Relevance Networks

Relevance networks allows networks of features to be built, whether they represent genes, phenotypic or clinical measurements[21].The technique works by first comparing all features with each other in a pairwise manner, similar to the initial steps of hierarchical clustering. Typically, two genes are compared with each other by plotting all the samples on a scatter plot, using expression levels of the two genes as coordinates. A correlation coefficient is then calculated, although any dissimilarity measure can be used. A threshold value is then chosen, and only those pairs of features with a measure greater than the threshold are kept. These are displayed in a graph.[19,21,22]

d) Principal-Components Analysis

Principal-components analysis is more useful as a visualization technique than as an analytical method. It can be applied to either genes or samples, which are represented as points in multidimensional space, similar to self-organizing maps. Principal components are a set of vectors in this space that decreasingly capture the variation seen in the points.

2) Supervised Techniques

a) Nearest Neighbours

It is commonly used in a supervised fashion to find genes directly with patterns that best match a designated query pattern. For example, acute lymphocytic leukemia was distinguished from acute myelogenous leukemia using this method. This method is also used in toxicogenomics.[20]

b) Support Vector Machines

Support vector machines address the problem of finding combinations of genes that better split sets of biological samples.[23] Although it is easy to find individual genes that split two sets with reasonable accuracy owing to the large number of genes (also known as features) measured on microarrays, occasionally it is impossible to split sets perfectly using individual genes.[15]

APPLICATIONS

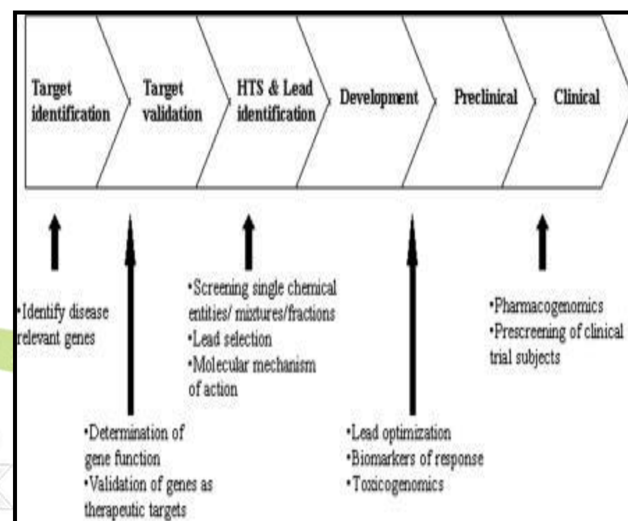

Figure 13: Applications of DNA microarray technique

- DNA microarrays have helped to define molecular features of cancer progression as well as to distinguish between the nonmetastatic and the metastatic phenotype, such as in melanoma and medulloblastoma.
- DNA microarrays to study the gene expression characteristics of the 2 inflammatory diseases rheumatoid arthritis and inflammatory bowel disease[4]
- cDNA microarrays and a clustering algorithm were used to study the patterns of gene expression in human mammalian epithelial cells. These cells were studied both in primary human breast tumors, and in culture.
- In new born babies, DNA microarrays could be used to screen for a number of treatable disorders, such as sickle cell disease. This could be done using small blood samples taken from babies[23]

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- DNA microarrays are being utilized more as a genotyping tool to identify indicators that were inherited in both maternal and paternal testing.
- In Pharmacodynamics for discovery of new diagnostic and prognostic indicators and biomarkers of therapeutic response; elucidation of molecular mechanism of action of a herb, its formulations or its phytochemical components and identification and validation of new molecular targets for herbal drug development.
- In Pharmacogenomics for prediction of potential side-effects of the herbal drug during preclinical activity and safety studies; identification of genes involved in conferring drug sensitivity or resistance and prediction of patients most likely to benefit from the drug[12]
- In Pharmacognosy for correct botanical identification and authentication of crude plant materials as part of standardization and quality control.
- DNA microarray technology has been applied extensively to the analyses of natural and anthropogenic factors in yeast for which whole-genome chips have been available for a few years.[18]
- The Family Finder test uses "automated Gene Titan Instrument and Axiom Array Plates" technology from Affymetrix (Fig.14). The test is capable of assessing nearly 570,000 genetic markers, including many that "are relevant to genealogy" and the equipment can process more than 760 samples per week. The test price is $289 US.[24]
- DNA microarrays have been used to develop a much deeper insight into the mechanism of chemical toxicity at the molecular level, used cDNA microarrays to compare the effects of arsenic, nickel, chromium, and cadmium on the expression of 1,200 human genes in human bronchial BEAS-2B cells.
- Because of the length of the probes, cDNA microarrays can be used in heterologous hybridizations across strains and closely related species as long as sequence divergence is limited for a given gene[25][26]
- **Gene Discovery:** DNA microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions. The benefits of this include being able to determine the genetic factors of disease, but the drawbacks include more opportunities for genetic discrimination.[18]
- **Disease Diagnosis:** DNA Microarray technology helps researchers learn more about different diseases such as heart diseases, mental illness, infectious disease and especially the study of cancer. Until recently, different types of cancer have been classified on the basis of the organs in which the tumors develop. Now, with the evolution of microarray technology, it will be possible for the researchers to further classify the types of cancer on the basis of the patterns of gene activity in the tumor cells. This will tremendously help the pharmaceutical community to develop more effective drugs as the treatment strategies will be targeted directly to the specific type of cancer.
- **Toxicological Research:** Microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny. Toxicogenomics establishes correlation between responses to toxicants and the changes in the genetic profiles of the cells exposed to such toxicants.[27]

Figure 14: Automated Gene Titan Instrument

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Advantages (Fig.15)

- Hundreds of pathogens can be looked for simultaneously when probing a single microarray slide[22]
- Being able to study the behaviour of many genes simultaneously is a great advantage.
- The technique is very fast: there can be as many as 150 copies of an array of 12,000 genes printed in only 1 day.
- DNA microarray technology is relatively cheap to use:
- The initial cost of constructing an arrayer is approximately Rs.27, 000,000.
- After this the cost per copy of a microarray is small, usually less than Rs.5000.
- The technique of DNA microarrays is very user-friendly:
- The technique is neither radioactive nor toxic.
- The microscope slide is a convenient base for the technique.
- Arrays are cheap and easily replaced.
- A major advantage of DNA microarrays is that information about the sequence of the DNA is not required to construct and use the DNA microarrays.
- In fact, most of the human genes that have used microarray technology in expression studies are only defined by partial EST sequences at the moment.[25,18]

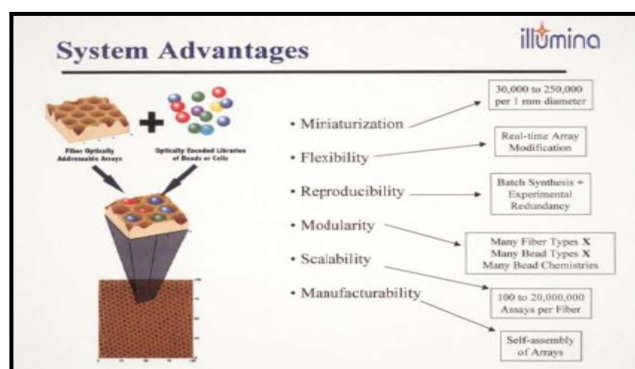

Figure 15: Advantages

MARKET SURVEYS

Since their development in the mid 1990's, DNA microarrays have become an essential tool for life science researchers interested in performing gene expression studies. While this technology was initially beset with technical challenges and was prohibitively expensive for most researchers, in recent years microarrays have become easier to use and more affordable. This has allowed the market for microarrays in the life sciences to grow significantly. In fact, this growth has even begun to expand into areas outside of basic research.

One interesting area of expansion for DNA microarrays is in the clinical/diagnostic laboratory. Some of the uses for microarrays in this setting include the identification of viral or bacterial strains in patient samples and profiling gene expression patterns of various cancers to determine the optimum therapeutic treatment. Recent advances in probe hybridization techniques, signal amplification strategies as well as signal detection and interpretation technologies have made this use of microarrays possible.

The total market for DNA microarrays and materials totaled 544.4 million USD in 2002. Rising at an AAGR (average annual growth rate) of 13.4%, this market is expected to just exceed 1 billion USD in 2007.Microarrays themselves make up the bulk of the market and sales have rised at an AAGR of 11.7% through 2007 to reach 744 million USD. Materials sales will rise faster due to increasing complexity, reaching 275 million USD in 2007 at an AAGR of 18.8%.Although DNA microarrays are entering a period of more modest growth (growth was at 30%/year just a few years ago), there are some dramatic changes taking place in the types of microarrays being produced.[28] Affymetrix is leading the DNA microarray market(50%), based on its high-intensity platform with 20% annual growth in sales.[29]

FUTURE POSSIBILITIES

Fingerprints: DNA microarrays will enable the identification of 'fingerprints' of gene

expression. These fingerprints will categorise particular diseases, or other biological states.

Genome-wide Studies: DNA microarrays are commonly applied to studies involving the transcription of genes.

However, as discussed, they can also be used to scan the whole genome of an organism.

Their application here involves finding a functional characteristic of a DNA sequence that will allow the separation of the DNA or RNA being used.[18]

CONCLUSION

DNA Microarray technology enables scientists to investigate and address tissues which were once thought to be non traceable. DNA microarray promises to be the next generation sequencer which could explain how organisms evolve and adapt looking at the whole genome. DNA microarray technology could be foreseen as an automated process for screening of compound targets, diagnostic and drug development with improved efficiency, quality and reliability of data, helping to reduce overall cost of research and development.[30]

REFERENCES

1. Emmert-Streib Frank, Dehmer Matthias (Hrsg.), Analysis of Micro array Data, http://www.wiley-vch.de/books/sample/3527318224_c01.pdf (Accessed on 03/10/2012).
2. Eisen Michael B and Brown Patrick O, DNA Arrays for Analysis of Gene Expression, http://eminentsofunn.com/biochemistry articles/geneexpressionanddnaarrays.pdf (Accessed on 03/10/2012).
3. Southern EM, J. Mol. Biol., 98, 1975, 503-17.
4. Alwine JC, Kemp DJ, Stark GR, Proc National Academy of Science, USA, 74, 1977, 5350-4.
5. Microarray Technology, Premier Biosoft, Technote, http://www.premierbiosoft.com/tech_notes/microarray.html (Accessed on 03/10/2012).
6. Sasik R, Woelk CH, Corbeil J, Microarray truths and consequences http://jme.endocrinology-journals.org/cgi/reprint/33/1/1.pdf(Accessed on 04/10/2012).
7. Lenoir Timand Giannella Eric, The emergence and diffusion of DNA microarray technology http://j-biomed-discovery.com/content/1/1/11.
8. DNA Microarray History Timeline, Microarray Station, http://www.microarraystation.com/dna-microarray-timeline (Accessed on 04/10/2012).
9. http://www.microarray.org (Accessed on 04/10/2012).
10. Kostrzynska M, Bachand A, Application of DNA microarray technology for detection, identification and characterization of food-borne pathogens, Can. J. Microbiol., 52,(2006), 1-8.
11. Boonman N, Walsh K, Smith P, Madagan K, Graham I & Parker I, Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis, J. Virol. Methods, 108, 2003, 181–187.
12. http://www.utulsa.edu/microarray/Articles/Design%20of%20Studies%20Using%20DNA%20Microarrays.pdf (Accessed on 05/10/2012).
13. Kafatos FC, Jones CW, Efstratiatis A, Nucl. Acids Res., 7, 1979, 1541-52.
14. Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, 2001.
15. Park PJ, Pagano M, Bonetti M, A nonparametric scoring algorithm for identifying informative genes from microarray data. Pac. Symp. Biocomput., 2001, 52–63.
16. DNA Microarray Technology, National Genome Research Institute, http://www.genome.gov/10000533 (Accessed on 04/10/2012).

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17. Microarrays: Chipping Away at the Institute of Science and Medicine. National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html (Accessed on 05/10/2012)

18. Introduction to DNA Microarrays available on: http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html (Accessed on 05/10/2012).

19. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns, Proc. Natl Acad. Sci. USA 95, 1998, 14863–14868.

20. Butte AJ, Tamayo P, Slonim D, Golub TR, Kohane IS, Discovering functional relationships between NA expression and chemotherapeutic susceptibility using relevance networks, Proc. Natl Acad. Sci. USA, 97, 2000, 12182–121

21. Tamayo P et al., Interpreting patterns of gene expression with self-organizing maps: Methods and application to hematopoietic differentiation, Proc. Natl. Acad. Sci. USA 96, 1999, 2907–2912.

22. Hilsenbeck SG et al., Statistical analysis of array expression data as applied to the problem of tamoxifen resistance, J. Natl Cancer Inst, 91, 1999, 453–459.

23. Toronen P, Kolehmainen M, Wong G, Castren E, Analysis of gene expression data using self-organizing maps, FEBS Lett., 451, 1999, 142–146.

24. Family Tree DNA launches Family Finder DNA testing

25. http://anglo-celtic-connections.blogspot.com/2010_04_04_archive.html (Accessed on 05/10/2012).

26. http://www.cysonline.org/article.asp?issn=44444443;year=2010;volume=1;issue=1;spage=1;epage= 5; aulast=Khan(Accessed on 05/10/2012).

27. Lettieri Teresa, Recent Applications of DNA Microarray Technology to Toxicology, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1332648/ (Accessed on 05/10/2012) http://www.aacc.org/SiteCollectionDocuments/Events/Expert%20Access/2005/microarray/historyMicro0905.pdf (Accessed on 05/10/2012)

28. Dr. Brauer Samuel, DNA Microarrays and Their Materials, BCC Library Research Report, January 2004, http://www.bccresearch.com/report/BIO049A.html (Accessed on 05/10/2012)

29. http://www.math.utah.edu/~palais/pcr/COE2.ppt#288, 62 (Accessed on October 5, 2012).

30. Leming Shi, DNA Microarray (Genome Chip) - Monitoring the Genome on a Chiphttp://www.gene-chips.com (Accessed on 05/10/2012).

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