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A Survey on Introduction of Dna Chip

Priya Mahilange; Dauli Sahu ; Purushottam Patel ; Prateek Kumar Singh

[1-2]BE Student CSE Department, CSVTU University, Chhattisgarh Kirodimal Institute of Technology, Raigarh, Chhatisgarh, India

[3]Head of Department & Assistant Professor CSE Department, CSVTU University, Chhattisgarh Kirodimal Institute of Technology, Raigarh, Chhatisgarh, India

[4]Lecturer CSE Department, CSVTU University, Chhattisgarh Kirodimal Institute of Technology, Raigarh, Chhatisgarh, India

ABSTRACT

In this report, we first give a basic introduction to the "DNA chip", also known as the "Gene Chip" or "Micro-DNA array". Micro -Array containing all the genes (roughly 40,000) in the entire Human Genome (complete Genetic Code). The rest of the report is divided into four major sections – Construction, Detection, Uses and Challenges. , we can study gene expression of thousands of genes simultaneously. DNA chip technology was developed to efficiently identify the differential expression pattern of independent biological samples. DNA chip provides a new tool for genome expression analysis that may revolutionize many aspects of human life including new drug discovery and human disease diagnostics.

I. INTRODUCTION

Scientists know that a mutation - or alteration - in a particular gene's DNA may contribute to a certain disease. However, it can be very difficult to develop a test to detect these mutations, because most large genes have many regions where mutations can occur. For example, researchers believe that mutations in the genes *BRCA1* and *BRCA2* cause as many as 60 percent of all cases of hereditary breast and ovarian cancers. But there is not one specific mutation responsible for all of these cases. Researchers have already discovered over 800 different mutations in *BRCA1* alone.

The DNA CHIP (microarray) is a tool used to determine whether the DNA from a particular individual contains a mutation in genes like *BRCA1* and *BRCA2*. The chip consists of a small glass plate encased in plastic. Some companies manufacture microarrays using methods similar to those used to make computer microchips. On the surface, each chip contains thousands of short, synthetic, single-stranded DNA sequences, which together add up to the normal gene in question, and to variants (mutations) of that gene that have been found in the human population.

History:

Deoxyribonucleic Acid (DNA) chips.

In order to understand DNA chips and various related concepts we must first be comfortable with the biological concepts that drive genetic functions. **Watson and Crick's** discovery of DNA 50 years ago has drastically changed the way we look at ourselves. DNA stands for Deoxyribonucleic Acid, which, as described earlier, is composed of four primary building blocks that are generally classified as nucleotides. Each of these nucleotides contains one of the four-nitrogen bases (Adenine, Guanine, Cytosine, and Thymine) that make up the genetic code. Also included is a phosphate sugar backbone. Due to its code-like nature, DNA makes us all the same, and all different - the same because we are all built on the same 4 letter language or code, and different because there are endless coding possibilities. DNA is the building block - the blue print for life and this is why it prompted the scientific society to find out the meaning of this code. Different techniques were developed to decode DNA but all were time consuming and expensive. In fact, the ―Human Genome Project,‖ sponsored by the US government, took 13 years to complete and $451 million dollars. The emergence of new technologies, however, has altered this trend as the DNA Chip was created. See Figure 1. These nucleotides are arranged in exceedingly long double helix strands that reside within the nuclei of all cells. Essential to DNA's functioning is the concept of genes and mRNA. A gene is a segment of DNA that defines a specific hereditary characteristic, usually corresponding to a single mRNA carrying the information for constructing a protein. Scientists have determined that only 3% of DNA contains genes.

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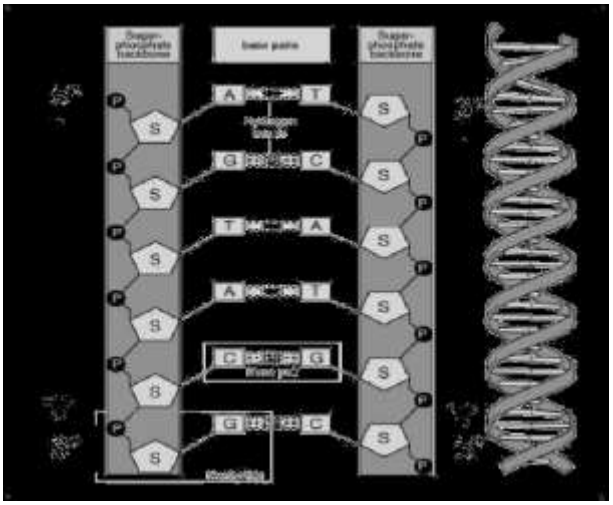

Figure 1 : Structure of DNA

These regions of active code are known as ―exons‖, and are mixed in with segments containing inactive code, also known as ―introns‖. In order for genes to synthesize proteins, the information carried on introns must be consolidated and transported outside the nucleus by mRNA. This **"Messenger" Ribonucleic Acid (mRNA)** has copied and spliced the genetic material off of a DNA strand and as stated above, corresponds to one specific gene. Protein is then synthesized in the cell's cytoplasm. See Figure 2.

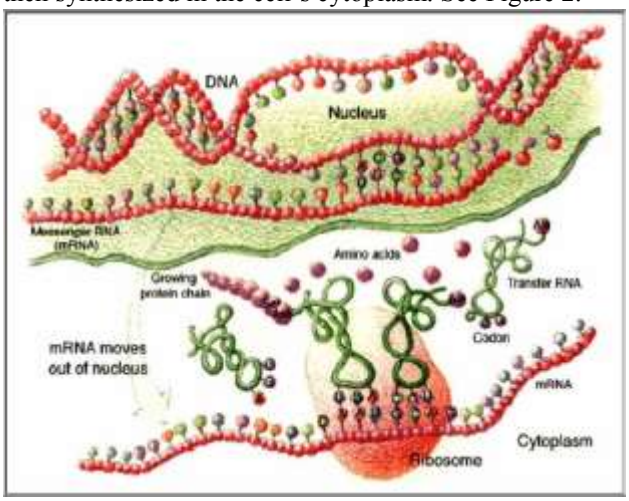

Figure 2 : DNA and mRNA at work

Another key mechanism that is utilized by gene chips is that of Hybridization. This term refers to the recombination of complimentary strands of DNA. Gene chips utilize this selective nature by planting single strands of a gene onto one location of the chip. The driving rationale being that a genetic single-strand sample or ―target‖ introduced onto the chip will chemically attach itself to the chip's resident ―probe‖ if it contains the opposite bases in the proper sequence. Thus the re-combination or Hybridization of the target and probe indicates that the two strands are the same gene and are genetic matches. ―Watson‖ and ―Crick‖ examine their preliminary model of DNA in the figure below.

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Figure 3 : Watson and Crick examine their DNA model

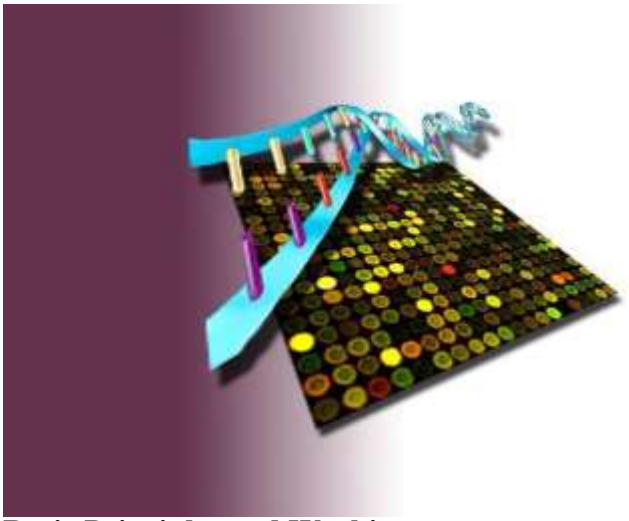
Thousands of genes and their products in a given living organism function in a complicated and orchestrated way that creates the mystery of life. The whole picture of a gene function is, therefore, hard to obtain in varying one gene per experiment, as evidenced in the human genome project. Simultaneously analyzing expression levels of a large number of genes provides the opportunity to study the activity of an entire genome much more efficiently. Successful manufacturing of the DNA chip permits these kinds of analyses, making the DNA chip an obvious choice to accelerate the characterization of a wide variety of genome experiments.

INTRODUCTION OF DNA CHIP

Figure 4: Affymetrix's Gene Chip

DNA Microarrays

Micro-Array containing all the genes (roughly 40,000) in the entire Human Genome (complete Genetic Code). Micro-Arrays quickly show the relationships between specific genes and specific traits, diseases and the like. Thus, we efficiently gain valuable insight into how our genetics specifically affect us.



Basic Principles and Working:

DNA microarrays (or **DNA Chips** as they are referred to informally) work on a pretty simple basic principle.

A basic chip consists of a substrate (usually a glass slide) on which is embedded hundreds of copies of a certain known molecule (the probes). This chip is flooded with molecules of an unknown structure. Only some of the molecules put in would 'stick' to or bond with one or more of the probes on the chip. Because we know the exact sequences of the molecular probes on the chip, we can figure out the structural details of the 'marked' fragments bound to them.

Molecules that don't find a match on the chip are simply washed away.

In the case of DNA chips, the molecules on the chip are actually oligonucleotide strands whose sequencing is known. The bonding that we refer to is actually the well-known phenomenon of hybridization of DNA strands. Since hybridizations is strongly specific to the sequencing of the two DNA molecules involved, what we can find out by these techniques is the exact sequencing of a random DNA strand. The molecules with unknown sequencing are either tagged by using fluorescence based methods or are detected by using electronic properties of Nano materials.

DNA chips promise to carry the techniques of DNA analysis to a whole new level, and to bring tools for getting DNA-sequence information out of research labs into doctors' offices.

―The brain has about a trillion neurons, and about a quadrillion interconnections‖, says Hood. What we call "consciousness" somehow "emerges" from how all these neurons interact. Wouldn't tell us one iota more about the brain's emergent properties, because they arise from the network, not a single cell," says Hood. "If we were to study each gene in isolation, we'd never know how the genome functions as a whole. DNA chips are the prototype global technology for genetics, because they let us look at the behavior of thousands of genes at once.

Layout of the report:

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In this report, we first give a basic introduction to the DNA chip. The rest of the report is divided into four major sections – Construction, Detection, Uses and Challenges.

We describe the methods of construction most commonly used and discuss their pros and cons. Following that, we talk about the different techniques used for detecting the hybridizations of DNA on the chip. We discuss both conventional and emerging methods. Finally, we elaborate on the uses with specific emphasis on the detection of cancerous tissue.

Construction of DNA Chip:

The first DNA microarray was made by Patrick Brown and his colleagues at Stanford University. They first used this to study the precise details of the process by which yeast cells make spores. From then on, they have performed a number of experiments using this technology. Of late, they are trying to find which genes are related to or cause cancer. Their goal is to characterize gene expression patterns for melanoma and other malignancies that show the presence and stage of a tumor.

The methods for synthesizing DNA chips are broadly classified into

1. **Off-Chip Synthesis**
2. **On-Chip Synthesis**

There are two major types of DNA chips available: **DNA** chips containing long DNA sequences (from hundreds to several thousand residues per strand) and **DNA** chips containing oligonucleotides (synthetic sequences of less than 100 residues).

In one method of making DNA chips, the strands of DNA are made in the laboratory and then transferred to the glass substrate. Since the preparation of the DNA probes takes place 'offsite' this method is termed as Off-Chip Synthesis.

In contrast to this, the On-Chip synthesis mechanism involves making 'hollows' on the glass surface using photolithography techniques [quote paper here] followed by a two-step in-situ reaction to obtain DNA strands.

1. off-Chip Synthesis

In 1985 Kary Mullis described the polymerase chain reaction (PCR) - a technique that allows researchers to make millions of copies of any piece of DNA they wish to study. The method is used for generating the DNA fragments for chips and has proven indispensable for almost all the genetic studies done today, including large-scale DNA sequencing of organisms from yeast to humans.

This is also hailed widely for its simplicity of execution. As Mullis himself wrote "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat." PCR, as currently practiced, requires several basic components. These components are:

DNA template, which contains the region of the DNA fragment to be amplified

Two primers, which determine the beginning and end of the region to be amplified.

Taq polymerase (or another durable polymerase), a DNA polymerase, which copies the region to be amplified.

Deoxynucleotides-triphosphate, from which the DNA Polymerase builds the new DNA .

Buffer, which provides a suitable chemical environment for the DNA polymerase.

This technique is a very vital component of Off-Chip synthesis. The substrate used for these chips is usually a specially treated, silence coated glass slide. Microarrays on glass slides are made by dispensing a small amount of oligonucleotide or PCR product using a solid dispensing mechanism. Dispensing mechanisms are classified into **contact** and **non-contact** methods. Common dispensing mechanisms are tweezers, rings, split pins, inkjets, or micro spotting pins. We deal with one example of each type here – micro spotting pins and inkjets respectively. The grids on the chip have a specific pattern to them. There is a certain basic pattern called a sub grid, which repeats throughout. This repeated arrangement of subgrades is called a met grid. Each sub grid consists of tens of thousands of *different* probes (DNA sequences). This can be used to find out the sequencing of a molecule. Since there are a number of these sub grids, this sequencing analysis can be carried out for many molecules simultaneously. This inherent parallelism of DNA microarrays contributes significantly to making them a powerful analysis tool.

Micro spotting pins – contact method:

This method uses a number of fine sized pins to transfer the solution in the PCR well onto the surface of the chip. The pins are mounted in a gridded head at the end of a robotic arm, which in turn is controlled by a software program directing the arm to create a specified grid of spots.

The process (a specific example): The basic process of printing an array depends on the arrayed. There is a slide platform which holds 50 slides in five rows and ten columns- it moves in the X direction .The robot arm moves in the Y and Z direction. The arm and platform together allow a pin to be positioned anywhere on and over the slide platform. A spotting operation consists of washing the pins in Nano pure water, dipping them in the spotting plate wells for a second or so (to allow take-up into the pin chamber), positioning the pins over each slide at the proper position, and slowly touching the pins to the slide's surface,

depositing the DNA in a coin-shaped spot. The pins are miniaturized nibs with a gap that draws up the oligonucleotide solution with a capillary action. These can load upto 1 µL of solution. Upon contact of the pin with the glass surface 1 nL of the solution is delivered. Thus one loading is sufficient to fill in about a thousand spots. One problem during this process is that some carryover occurs between spotting an oligonucleotide and going back to the source plate to pick up another one. Washing the pins in-between collecting two samples minimizes this problem. Also, spreading of the spot is observed soon after the procedure, which is rectified by administering a coating of a hydrophobic material like silence on to the glass surface. This maintains the spot size after delivery by surface tension. There are also constraints on the humidity levels that must be maintained after deposition to facilitate oligonucleotide attachment.

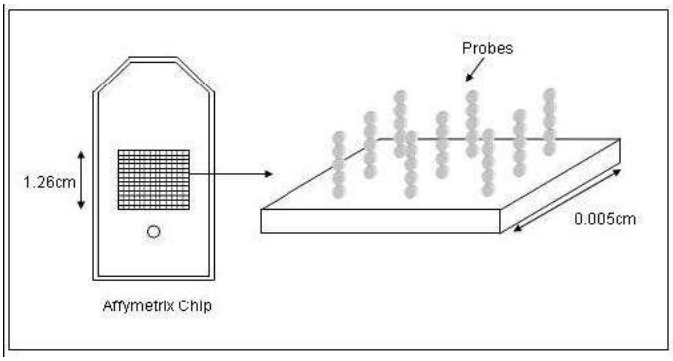

Fig. 7 : Robot used for spotting

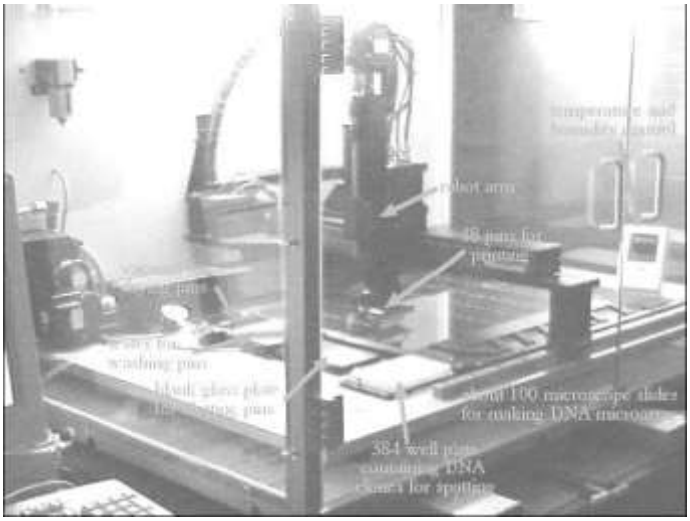

Fig. 7 : Robot used for spotting

Jets – non-contact method:

As the name suggests, in this method of delivery of reagents no contact is made with the surface of the chip. This method takes its origin in the technology that was developed for inkjet printers. This essentially has bubble jets, which are simple semiconductor devices, which consist of a nozzle from which fluid is ejected by rapid heating. Early contributions to this area were made by researchers at Combine using coaxial piezoelectric/glass capillary jets. Later this was applied to DNA by researchers at Canon who used a solvent that includes glycerin, urea and thiodiglycol as wetting agents. Unto 12 different oligonucleotide solutions could be dispersed in volumes of 24 pL and 70 µm spots were created. Recently a better technique was developed at Microfiber with ten independent fluid channels with jet openings of the order of 40 µm. These deliver Oligonucleotides in 20% aq. ethylene glycol.

2. on-Chip Synthesis

In the seemingly unrelated world of computer electronics, Robert Nonce and Jack Kilby first used a process called photolithography to build integrated circuits-tiny transistors, capacitors, resistors, and diodes-onto silicon chips. These miniature circuits allowed computers to shrink in size and this technique was a pretty revolutionary one. Not many would have thought, however, that this would be used in biology only a few decades later.Today, photolithography is one of the cornerstone techniques in On-Chip Synthesis of DNA microarrays.

Photolithography or **Optical lithography** is a process used to transfer a pattern from a photo mask to the surface of a substrate. To put it simply, it is etching by light. The broad idea in this process is to apply a coating of a special light sensitive material, called photoresist onto the surface of the chip and then exposes this to a certain pattern of light. This pattern is obtained by focusing light through a mask, which has transparent and opaque areas made on it according to the required pattern. Light goes in though the transparent regions of the mask, strikes the photoresist on the chip in only specific areas and causes it to undergo a chemical transformation. Depending on it's nature, the photoresist becomes either less or more soluble. Subsequently, we wash the surface of the chip with a solvent thus leaving a pattern identified by either the presence or the absence of photoresist. The figure below illustrates this process.

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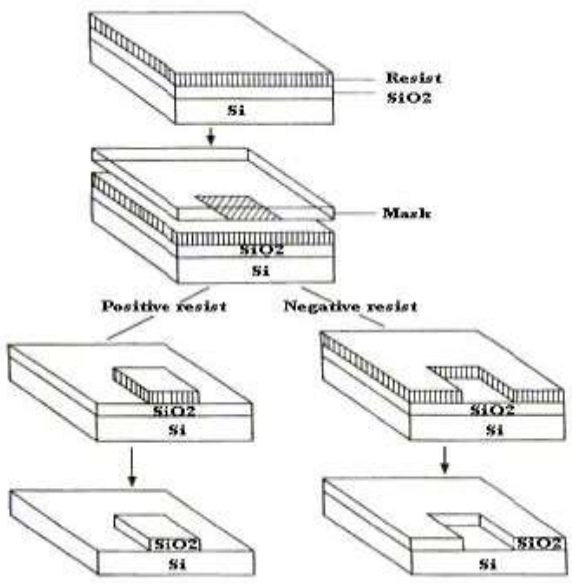

Photolithography

The two types of photoresist are called positive and negative.

1. For positive resists, exposure to the UV light changes the chemical structure of the resist so that it becomes more soluble in the developer. The exposed resist is then washed away by the developer solution, leaving windows of the bare underlying material. Negative resists behave in just the opposite manner. Exposure to the UV light causes the negative resist to become polymerized, and more difficult to dissolve.

2. Therefore, the negative resist remains on the surface wherever it is exposed, and the developer solution removes only the unexposed portions. The mask is thus made depending on the photoresist with which it will be used.

Photolithography in DNA microarrays – On chip synthesis:

Photolithography is the key step in the On-Chip synthesis of DNA microarrays. It is not used in the exact form that it is employed in electronic chip design but the essential idea remains. The general approach is to build the DNA probes on the chip by adding one monomer at a time (one of Adenine, Thymine, Cytosine, Guanine). To start with the chip is coated with linker molecules, which are just short DNA strands. The linker molecules' reactive 5` site is covered with a protecting group like dimethoxytrityl (DMT). These protecting groups have the property that they are removed on exposure to UV light. Next, a mask is made so that light is allowed through to only those sites wherein the particular strands needs to increase in size. When this mask is placed over the chip and UV light is incident on it, light passes through only to these sites, removes the protecting groups and exposes the reactive 5` site. The chip is then flooded with the solution containing a particular monomer say adenine. This attaches on to only those sites, which were DE protected in the previous step. Once the adenine molecules bond, the rest of the solution is washed away leaving the DNA strands at a specific set of sites with an A attached to the end. This process is then repeated with this chip each time adding a monomer to a certain set of sites.

This enables us to construct arbitrary DNA probes at each site by choosing an appropriate sequence of masks. To be precise, if we go through 4 x l cycles of the kind above (The four is because we alternately choose A, C, T and G at each stage) we can get 4 possible DNA sequences. This makes this an extremely powerful technique because of the large number of probes that can be produced in a few cycles.

Alternate Method :

An alternative method was developed by workers at Affymetrix in collaboration with IBM using traditional semiconductor photoresists as a two layer physical barrier to conventional chemical reagents for DNA synthesis. Initially, photolithographic techniques are used to create a grid of exposed regions on the glass wafer. Following this, the exposed glass regions are etched using HF (a standard glass etching reagent) leaving hollows or wells about 30 μm deep on the wafer. These wells are then connected to DNA synthesizers in a flush of beryl indium PGA-P (3% in CH_2Cl_2) each of which introduces an oligonucleotide. Then the deportation step is carried out by focusing light at specific points on the chip using a set of mirrors and lenses. Then the coupling reaction takes place as above, extending the length of the DNA fragments at the DE protected points.

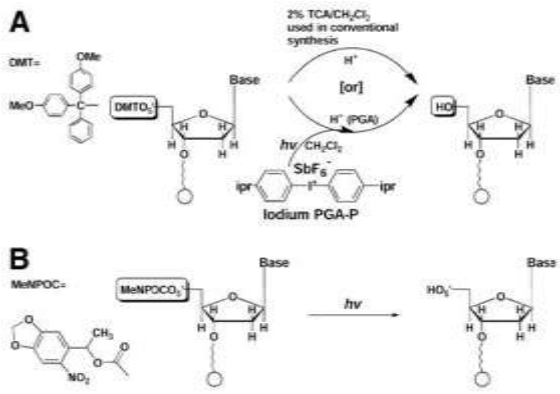

Fig : DE protection step

The gene chip thus made is ready for identifying the sequencing in the target molecule. The target molecule, placed on a sub grid undergoes what is known as hybridization to one of the probes on the grid.

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Conventional Hybridization:

In the introduction, it is the hybridization of DNA strands, the binding of complementary strands into a double helix, which allows scientists to characterize DNA using the DNA chip.

Because each strand of DNA has a thickness of only approximately 2 nm, millions of identical strands are found in each site. Each site has an associated microarray dot in a microarray image describing fluorescent sensing. Illustrated below are the hybridization processes both using conventional techniques and the DNA chip. In the following illustration, short fragments of DNA are identified. The DNA is denatured (separated) and placed in a solution, forming a reference segment for the DNA fragment of interest.

The DNA chip section represents complementary strands in the area of a single microarray dot:

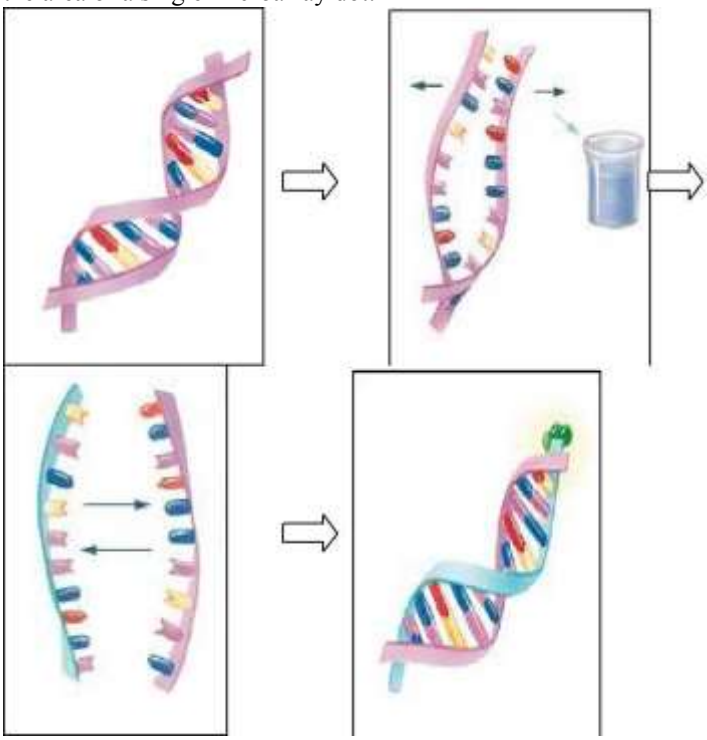

Fig : Convential hybridization characterization process

Hybridization is the process of combining complementary, single-stranded nucleic acids into a single molecule forming a double helical structure. Nucleotides will bind to their complement under normal conditions so two perfectly complementary strands will bind to each other readily. Conversely, due to the different geometries of the nucleotides, a single inconsistency between the two strands will prevent them from binding.

Once the target gets hybridized to the probe, all it remains is to find out the probe to which it sticks. In other words we need to detect hybridization at corresponding probes. This is accomplished through some well-established methods like Fluorescence Resonance Energy Transfer (FRET), as well as some recently investigated methods involving the electronic properties of Carbon Nano Tube Network Field Effect Transistors (CNTFNET) and Enhanced Absorption of Light by Oligonucleotide Modified Silver Nanoparticles.

FRET (Fluorescence Resonance Emission Transfer):

When two fluorophores (parts of molecules that are responsible for fluorescence in them) whose excitation and emission spectra overlap are in close proximity, excited energy of the donor molecule is transferred by a resonance dipole-induced dipole interaction to the acceptor molecule which results in

a) Quenching of the donor fluorescence

b) Enhancement of the acceptor fluorescence

c) Decrease in the lifetime of the donor.

DNA Chip Applications:

Three great improvements in our society. ,,

First, scientists will be able to discover new genes. These genes will not only explain the size and shapes of proteins but will also better an organism when introduced in their genome. For example, it is possible to find on the market tomatoes that take longer to rot. This allows people to keep tomatoes longer and allows farmers to let their tomatoes grow for longer times instead of picking them early. This idea can be applied to many things, limited only by ethics and personal beliefs.

The second main application is disease diagnosis. People will be able to get their karyotype checked to see if they have any genetic diseases. For example, they can see if they are prone to getting cancer in the future. This will allow a person to act in consequence such as following a certain style of life and having regular tests performed.

The third final major application is drug discovery. Some people are more resistant than others to diseases. This fact leads pharmacists to geneticists to try to understand this phenomenon. A common example would be diabetes. People suffering from diabetes lack a protein called insulin, which helps regulate the sugar level in the blood stream. Like any other protein insulin has its corresponding genetic code which scientist found by reverse engineering. It was therefore easy to implant this gene in bacteria to have it produce this insulin that now all people suffering from diabetes use. This concept can

be applied to many diseases. Finding the protein that would help cure a disease can only be done by the study of many different individuals and therefore the DNA Chip is the best way to do this.

―Today's science fiction is often tomorrow's science fact.‖
―Stephen Hawking

This statement is very true in the case of DNA chips. As no one must have thought that a biological computer chip can actually diagnosis the diseases, enhance the process of drug designing.

There are varied uses for DNA chip. The completion of the sequencing of the human genome and many other organisms makes the determination of gene function an important next step in understanding the role of the DNA in the processes of life.

DNA microarrays are excellent tool to address this question because their numerous probe sites enable the analysis of many genes simultaneously.

The stratagem of the DNA chip is mainly to collect and analyze large amounts of genomic information in quick and easy way. By just knowing the sequence of the DNA we can
find out a vast data that is required in genetic screening, disease diagnosis and new drug development.

1. DISEASE DIAGNOSIS:

The current DNA chip market runs as a device for detection of cancer. One of the biggest challenges in cancer treatment is choosing the right regimen for a given patient. Treatment strategies work differently for different tumors. Previously, oncologists rely heavily on biopsy reports that diagnose the tumor type involved but researchers report the first systematic and objective approach for identifying and classifying tumor types. This approach exploits the hot new technology of DNA microarrays. DNA chip can analyze the activity of thousands of genes at a time and could be used in the future to accurately diagnose cancer subtypes and also to predict clinical outcomes.

Scientists used a DNA chip to examine gene activity in bone marrow samples from patients with two different types of acute leukemia-acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Using a computer algorithm, they identified signature patterns that could distinguish the two types. When they cross-checked the diagnoses made by the chip against known differences in the two types of leukemia, they found that the chip method could automatically discover the distinction between AML and ALL without previous knowledge of these classes. Distinguishing the two types of leukemia is critical for successful treatment of the disease because chemotherapy regimens for the two vary considerably.

The results demonstrate the feasibility of cancer diagnosis based solely on gene expression and suggest a general strategy for discovering new subtypes of cancer, independent of previous biological knowledge.

Scientists used a sophisticated computer algorithm called a "self-organizing map" (SOM) that takes advantage of the fact that many genes in a cell behave similarly and organizes the data into clusters with similar patterns. SOM s have been used widely in data mining, particularly for large or messy data sets like stock market data, but scientists were the first to apply it to gene analysis.

Initially, DNA chip was used in expression profiling of healthy and diseased cells, this is the first time that the technology has been applied to classify cancer types. There are recent studies on the screening for the breast cancer gene (BRAC1) using a biochip system.

Now, scientists are trying to use this ―diagnosis chip‖ for other diseases like leprosy etc.

2. DRUG DESIGNING:

DNA chip is used to find out the traits that alter the drug response or leads to a disease state. This technique has helped in studying Pharmacogenomics and pharm cogenesis.

1. Pharmacogenomics:

Genetic basis of drug response from the perspective of inherited traits and ethnic differences

1) Differences in a drug receptor (pharmacodynamics)

2) Differences in drug metabolism (pharmacokinetics, includes drug transporters)

2. Pharm cogenesis :

Non-inherited genetic traits that alter drug response or leads to a disease state (i.e. point mutations or collection of mutations). e.g.SNP s: single-nucleotide polymorphisms.

SNP s involves variation at a single base that is found in at least 1% of the population.

As the human genome project is making advances, every polymorphism is noted and are trying to find the variation this single nucleotide affect the biological processes like gene expression levels etc. Usually the change in a single nucleotide can't effectively make a change on protein coding but may increase the probability of getting the disease. The drugs are also different in function as the variability caused by ―exclusively‖ different nucleotide. So we need to know the sequence and address the problem by designing the ―right‖ drug.

Previously, the genetic disorders are confirmed through family background, racial and ethical background. Even we are not sure of how genuine is this information.

But with DNA chip at hand, we can study gene expression of thousands of genes simultaneously. Using the chip, one can look at drugs that bind to DNA or RNA. This drug screening process can help tell us why a drug is more

effective than another. In this case, one can know a drug molecule binds to a region in the DNA or RNA probe, but we don't know why it happens. A lot of interesting observations can be made and later we can use the data to study the mechanisms of binding where we need Pharmacogenomics.

3. TOXICOLOGY AND DETECTION OF PATHOGEMS:

Detecting pathogens, whether from natural diseases or biological weapons, is about to get faster and more convenient, as this can be done through the new DNA chips that specially design to sense harmful DNA and immediately alert a doctor or scientist. The research, published in the April 9 issue of the Journal of the American Chemical Society,2003 uses custom-designed loops of DNA that emit coloured light in the presence of a specific creature's DNA. The loop-laden chip could be used to detect anything from a bacterium or virus, to the specific DNA of a plant or person.

The new chip is remarkable in that it eliminates many of the time-consuming steps normally taken in identifying an organism by its DNA.

Traditionally, workers in a laboratory have to make thousands of copies of a piece of DNA they want to test. Then a complex series of steps must be performed to attach a special molecule to the DNA, which will act as a fluorescent beacon, making the DNA strand easy to detect. These beacon-outfitted pieces are then mixed with control DNA sequences to see if any match. Matching sequences would adhere to one another, betraying their presence via the beacon. But now, a scientist might only have to place a drop of the solution in question onto a small chip or card and watch for a change of colour to indicate whether specific DNA is present. The chips are sensitive enough that copying may be unnecessary, as are complex beacon attachments, and the chips could be easily manufactured so doctors could instantly detect dozens or hundreds of pathogens right in their office. Future soldiers would also be able to identify unknown biological substances quickly and surely on the battlefield.

4. QUALITY CONTROL IN FOOD INDUSTRY:

A single test can now reveal the presence of meat from any of 32 different species in food samples, enabling a wide range of important questions to be answered.

These include whether chicken has been bulked up with beef or pork extracts; whether expensive albacore tuna is really cheap skipjack tuna; whether rats, mice or even bits of people fell into the mincer when your burger was being made; and whether unscrupulous companies are risking spreading mad cow disease by adding beef to cattle feed?

The test, based on a DNA chip, is being evaluated by food regulatory authorities in Europe, and could also be used by supermarkets and food companies to check on their suppliers.

"The beauty of this is that you can scan for so many things at once," says Thomas Schlumberger, director of clinical genetics at Affymetrix in California, which developed the "Food Expert-ID" chip together with bioMérieux of France.

Each chip is coated with segments of DNA unique to each species, and arranged in zones. DNA strands from the food sample are transcribed into RNA and tagged with fluorescent chemicals.

These are then brushed over the chip, and any matching sequences will stick together. The pattern of fluorescent zones read by a laser scanner can therefore tell scientists which species are present, and whether this corresponds to what is on the product label.

5. FORENSICS & PERSONNEL IDENTIFICATION:

The past decade has seen great advances in a powerful criminal justice tool: deoxyribonucleic acid, or DNA. DNA can be used to identify criminals with incredible accuracy when biological evidence exists. By the same token, DNA can be used to clear suspects and exonerate persons mistakenly accused or convicted of crimes. In all, DNA technology is increasingly vital to ensuring accuracy and fairness in the criminal justice system.

DNA is generally used to solve crimes in one of two ways. In cases where a suspect is identified, a sample of that person's DNA can be compared to evidence from the crime scene. The results of this comparison may help establish whether the suspect committed the crime. In cases where a suspect has not yet been identified, biological evidence from the crime scene can be analyzed and compared to offender profiles in DNA databases to help identify the perpetrator. Crime scene evidence can also be linked to other crime scenes through the use of DNA databases.

One of the biggest problems facing the criminal justice system today is the substantial backlog of unanalyzed DNA samples and biological evidence from crime scenes, especially in sexual assault and murder cases. The development of ―DNA chip technology‖ uses nanotechnology to improve both speed and resolution of DNA evidence analysis. This technology will reduce analysis time from several hours to several minutes and provide cost-effective miniaturized components.
DNA chip analysis can be also included in many other fields

CHALLENGES:

DNA Chip has made tremendous progress in a short span of about two decades. However, considerable work remains to

be done. The commercialization of DNA-chip technologies may depend as much on overcoming regulatory and marketplace hurdles as on advancing technology. Despite the myriad applications for the DNA chips now being introduced to research markets, demand for this technology is still relatively small. Nevertheless, most observers agree that this situation will soon change, as DNA chips begin to show up in products designed for more-traditional clinical applications. To reach this point, however, several important regulatory and technical hurdles must be overcome.

DNA chips sound simple in concept, but generating probes on a solid array surface requires considerable expertise and "technical wizardry". At present, DNA chips are much too expensive and limited in application, because their use requires prior knowledge of gene sequences and any interactions with other genes, in order to be available for use in medical practices. DNA chip making is a complex process and most of the labor is usually done by high tech and fairly expensive robotics.

A look at the challenges ahead:

1. TECHNOLOGICAL CHALLENGES:

1. Improving sensitivity and reliability:

To increase the sensitivity we need to increase the signal to noise ratio. In reality noise is introduced at every stage of the procedure like mRNA preparation (tissues, kits and procedures vary), transcription (inherent variation in the reaction, enzymes), labeling (type and age of label), amplification, pin type (quill, ring, ink jet), surface chemistry, humidity, target volume, slide in homogeneities, target fixation, hybridization parameters (time, temperature, buffering etc), unspecified hybridization (labeled DNA hybridized on areas which do not contain perfectly complementary sequences, segmentation, quantification etc. The product of yields of individual steps determines the efficiency of a multi-step sequence. So, the efficiency of the whole procedure is drastically reduced.

Because of all these noise it becomes difficult to determine whether the variation of a particular gene is due to the noise or is it a genuine difference between the different conditions tested. Furthermore, while looking at a particular gene how much of the measured variance is due to noise and how much to noise?

The various ways to improve the signal to noise ratio are:

I. **Amplification of the signal**

A variety of strategies are being employed to boost signal, ranging from better bio-electronic sensors to molecular strategies that bind probes and samples during the transcription and translation stages of DNA replication.

1. **Getting the background noise down :**

To achieve this we need to use better labeling techniques. Currently, "fluorophore labels dominate all micro formats in DNA testing. But manufacturers are experimenting with the use of time-resolved fluorescence, dendrites, new uses of iodine radioisotopes, and chemical locks derived from improved washing techniques. Semiconductor Nano crystals as well as bioluminescent proteins are being developed to improve signal-to-noise ratios.

However, virtually all of these techniques will require the laboratories that use them to perform additional, more-complicated sample preparation and detection procedures.

2. Increasing density of the arrays:

Current technologies enable manufacturers to fabricate single-chip arrays containing roughly 20,000 wells, with each well holding about 100 nl. One approach to attaining high densities is being studied by researchers at the National Institute of Standards and Technology, who have developed a chip made up of a single-molecule layer of DNA bound to a thin film of gold. The surface-tethered DNA then binds to a target strand and piezoelectric differences signal the level of hybridization. Tiny electrical charges, infrared spectroscopy, and scanning tunneling microscopy help to organize the ultrathin layers. Other researchers have displayed remarkable results with elastomer films. In fact, the progress in this area is so encouraging that Harvard University scientist George Whitesides has declared, "The days of silicon-based chips are numbered."

What is the theoretical limit of such arrays? That is a question routinely asked in this new field, as the scale of operations becomes ever smaller. One guess puts the theoretical boundary at 1 million elements on a 1-cm^2 chip. But problems with hybridization worsen at higher densities. And there also is a point where the optics of detection reach their limits of resolution.

3. Developing "lab-on-a-chip" technology:

This deals with integrating sample preparation with the detection and analysis procedures. Manufacturers are pursuing a variety of strategies to improve the microfluidics capabilities of chips. "Conventional capillary electrophoresis (CE) has been used to perform a diversity of analyses involving proteins, nucleic acid fragments, drugs, and so on. Such flexibility is a compelling reason for considering this technique for on-chip detection. CE and laser-induced fluorescence have been particularly popular for separation-detection combination. But other techniques have also been explored, including on-chip chemiluminescence, and electrochemiluminescence assays.

4. Normalization:

The aim of normalization is to account for systematic differences across different data sets (e.g. Overall intensity) and eliminate artifacts. This IS crucial if results of different experimental techniques are to be combined. While everybody agrees on the goal of normalization, the consensus seem to disappear regarding how exactly the normalization should be done.

5. Biological factors- limitation of DNA microarray:

In spite of many advantages, microarrays are not necessarily able to substitute completely other tools in the arsenal of the molecular biologist. Moat microarrays measure the amount of mRNA specific to particular to genes and the expression level of the gene is directly associated with the amount of mRNA. However the real expression of the gene is the amount of the protein produced not the amount of mRNA. Although in most cases, the amount of mRNA reflects accurately the amount of protein, there are situations in which this may not be true. Even if the amount of protein were always directly proportional to the amount of mRNA , the proteins may require a number of post-translational modifications in order to become fully active and fulfill their role in the cell. Any technology that works exclusively at the mRNA level, such as microarrays, will be blind with respect to these changes.

6. Large number of genes :

The classical metaphor of the needle in the haystack can easily become an accurate description of the task at hand when tens of thousands of genes are investigated.

2. CHALLENGES IN COMMERCIALIZATION:

1. Reducing the cost

Currently, DNA chips cost between $100(Rs.4577) and $450 (Rs.20, 250) each. In a tight managed-care marketplace that places a premium on technologies that can either show quick savings or more-efficient results, some analysts say that such high prices will limit the growth of the DNA-chip market.

2. Reimbursement

Another challenge is how to pay for the adoption of new technologies. Many clinical labs just cannot do traditional [viral load] assays because reimbursement cutbacks have resulted in a lack of training and a lack of trained technologists. The need for automated systems is increasing—but not without regard for costs. To become accepted, new instruments and related technologies must make for less-expensive tests. Making individual tests cheaper could be as much an information management challenge as it is a hardware issue.

3. Regulatory requirements

As DNA-chip products are developed for the clinical laboratory and even home-care settings, quality assurance in both design and manufacturing will become increasingly important issues. "At some point the whole industry will have to sit down at the table together to develop some basic standards and help FDA (Food and Drug Administration) establish basic quality assurance requirements," agrees Deepak Thakur, product manager for microarrays at Geometrics Inc. (Woodlands, TX). "Currently, each company in the field has its own corporate standards, which may differ from one another in significant details."

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Priya Mahilange Mahilange (Roll No. 3102212026) Persuing BE, in Computer Science & Engineering at Kirodimal Institute of Technology, Raigarh,Chhattisgarh, India.

Dauli Sahu (Roll No. 3102212302) Persuing BE, in Computer Science & Engineering at Kirodimal Institute of Technology, Raigarh,Chhattisgarh, India.

Prateek Kumar Singh Working as an Lecturer, Department of Computer Science & Engineering at Kirodimal Institute of Technology, Raigarh,Chhattisgarh, India.