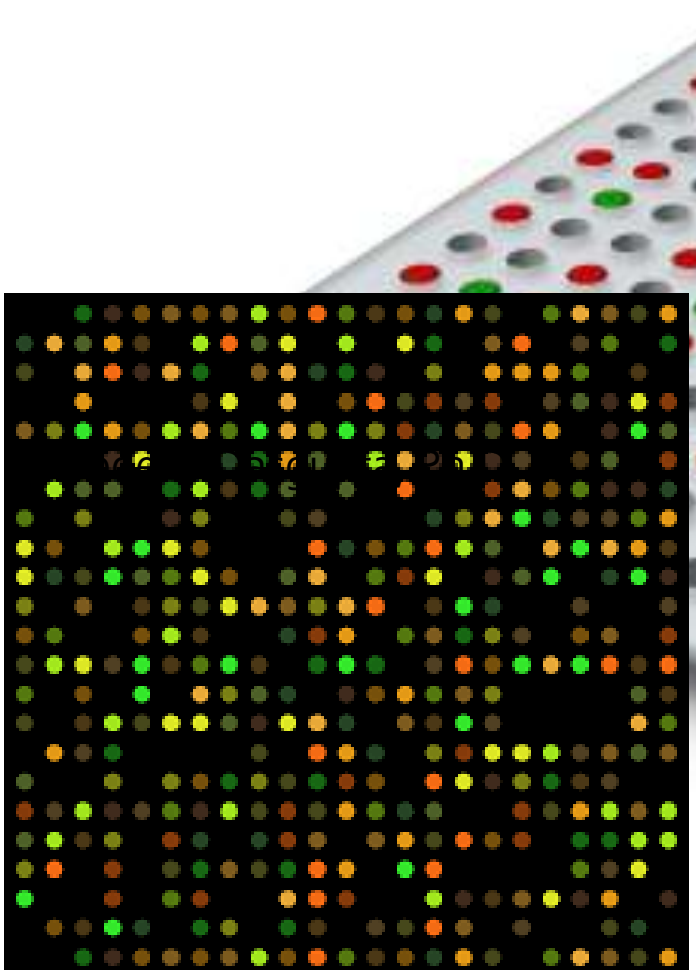
DNA MICROARRA



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- HISTORY
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- TYPES
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- ANIMATED CLIP

What is a Microarray?

- **Microarray**" has become a general term, there are many types now
  - DNA microarrays
  - Protein microarrays
  - Transfection microarrays
  - Antibody microarray
  - Tissue microarray
  - Chemical compound microarray
  - …
- We'll be discussing **DNA** microarrays

INTRODUCTION

- A **DNA microarray** (also commonly known as DNA Chip or biochip) is a collection of microscopic DNA spots attached to a solid surface.
- Each DNA spot contains picomoles (10^{-12} moles) of a specific DNA sequence, known as *probes* (or *oligos*).
- Each known gene or "probe" occupies a particular "spot" on the chip, and varying levels of fluorescent activity show varying levels of gene activity in introduced genetic material.
- Fluorescently labeled target sequences that bind to a probe sequence generate a signal.

Historical background :

Sir Edwin Southern

• Southern blotting was developed in the year 1975.

Sir Steve Fodor

• The concept of DNA microarrays began in the mid 1980s.

• **Pin based robotic system** was developed by Lehrach's group in 1990.

• **Steve Fodor** developed scanner for reading the output.

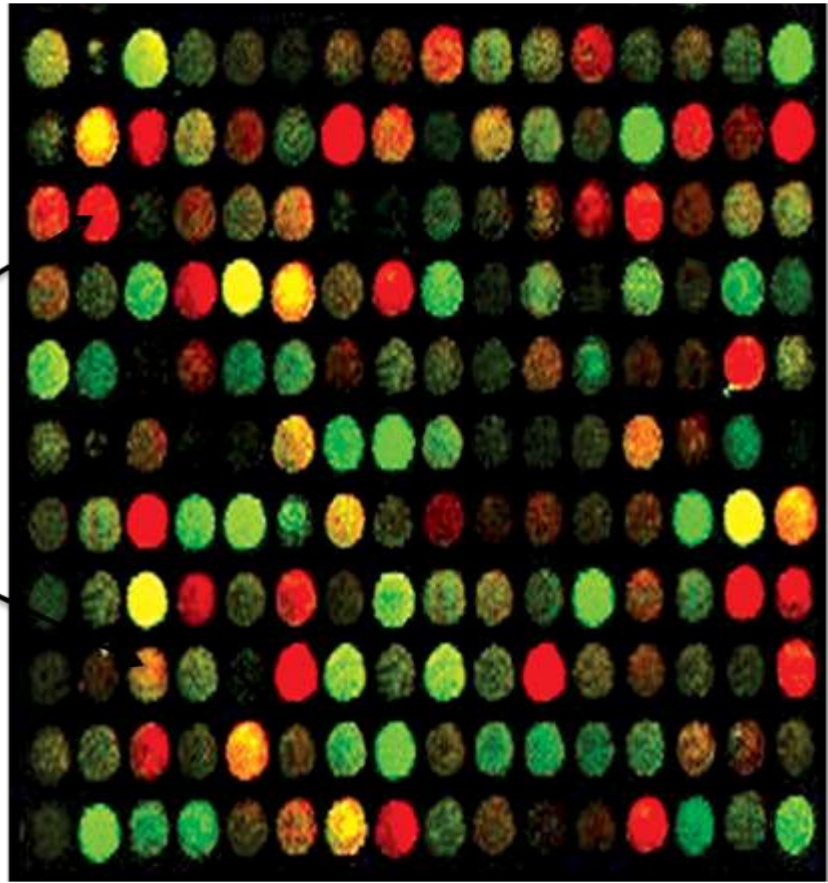
Sir Patrick Brown

•"**Quantitative Monitoring of Gene Expression Patterns with a complementary DNA microarray**" reported by **Patrick Brown, Mark Schena** and colleagues in *Science (1995).*

Mark Schena

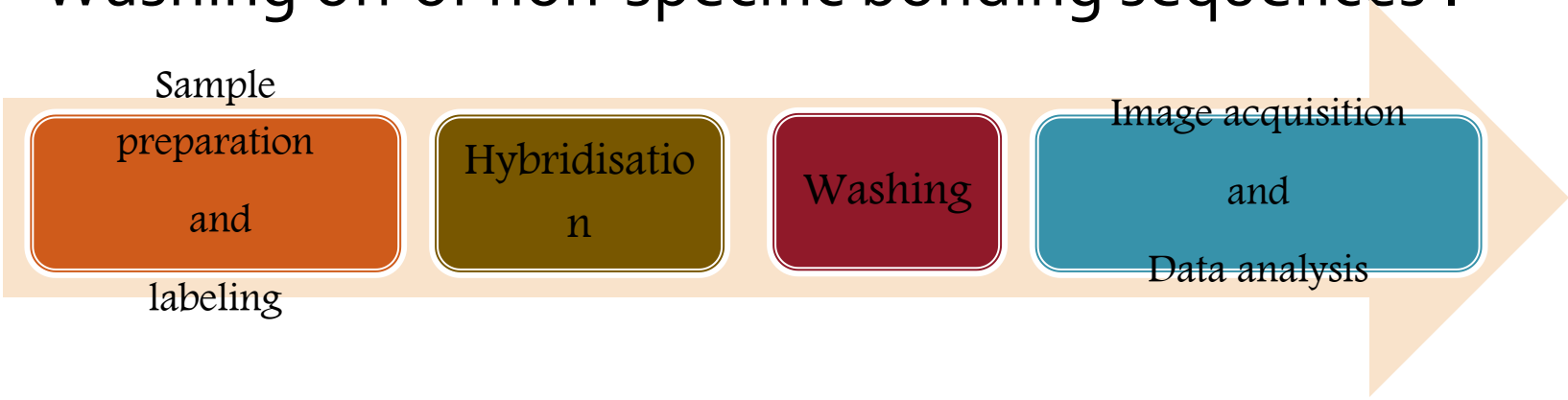
• **Mark schena** was proclaimed as the "Father of Microarray Technology".

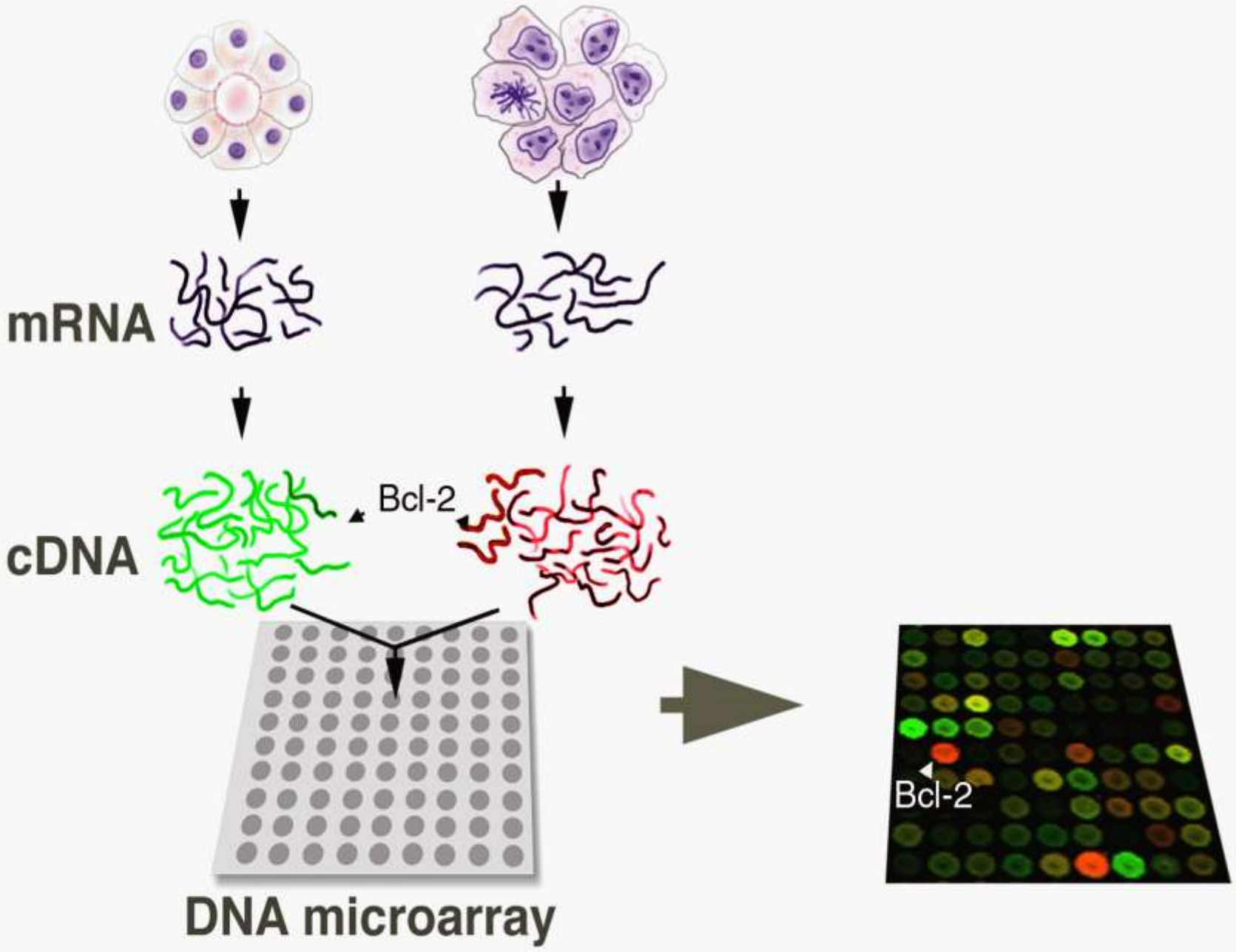
- The use of a collection of distinct DNAs in arrays for expression profiling was first described in 1987.
- The use of miniaturized microarrays for gene expression profiling was first reported in 1995, and a complete eukaryotic genome (*Saccharomyces cerevisiae*) on a microarray was published in 1997.



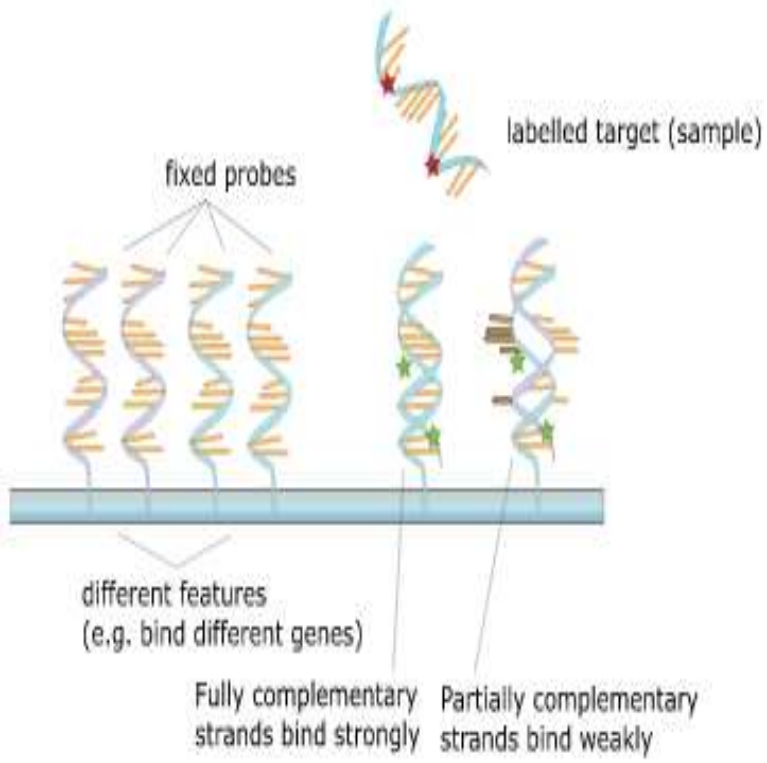
Principle

- The core principle behind microarrays is **hybridization.**
- Samples are labeled using fluorescent dyes.
- At least two samples are hybridized to chip.
- **Complementary** nucleic acid sequences get pair via hydrogen bonds.
- Washing off of non-specific bonding sequences .



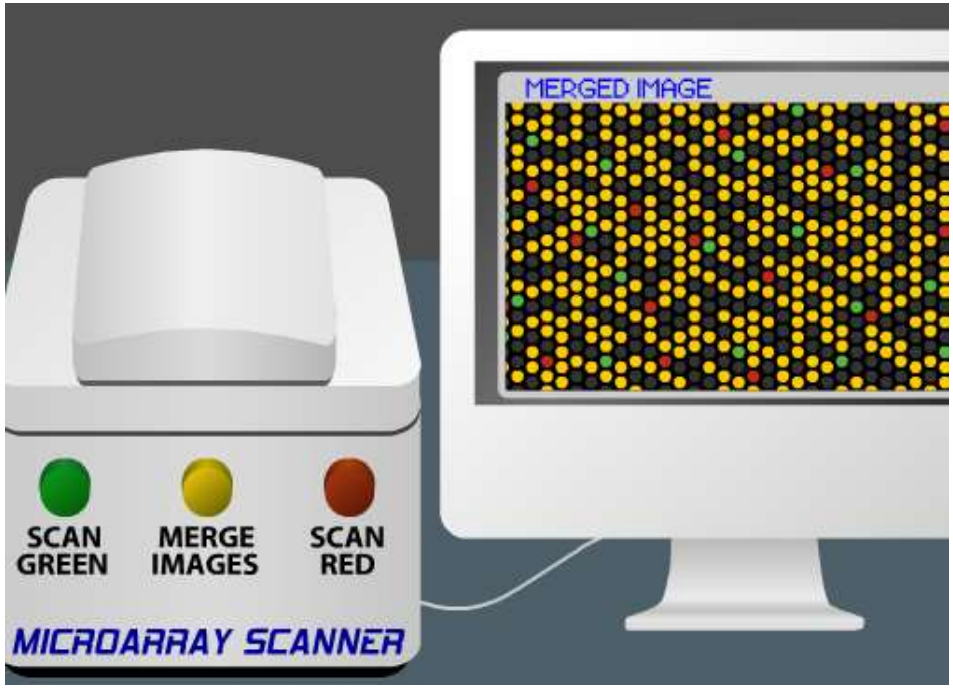

mRNA
cDNA
Bcl-2
DNA microarray
Bcl-2

- Fluorescently labeled target sequences that bind to a probe sequence generate a signal.
- The signal depends on.
  - The hybridization conditions, ex: temperature
  - washing after hybridization
- Total strength of the signal, depends upon the; amount of target sample.



Scanning the arrays

- Laser scanners
  - Excellent spatial resolution
  - Good sensitivity, but can bleach fluorochromes
  - Still rather slow
- CCD scanners
  - Low resolution
  - Sensitivity, easily adjustable (exposure time)
  - Faster and cheaper than lasers
- In all cases, raw data are images showing fluorescence on surface of chip



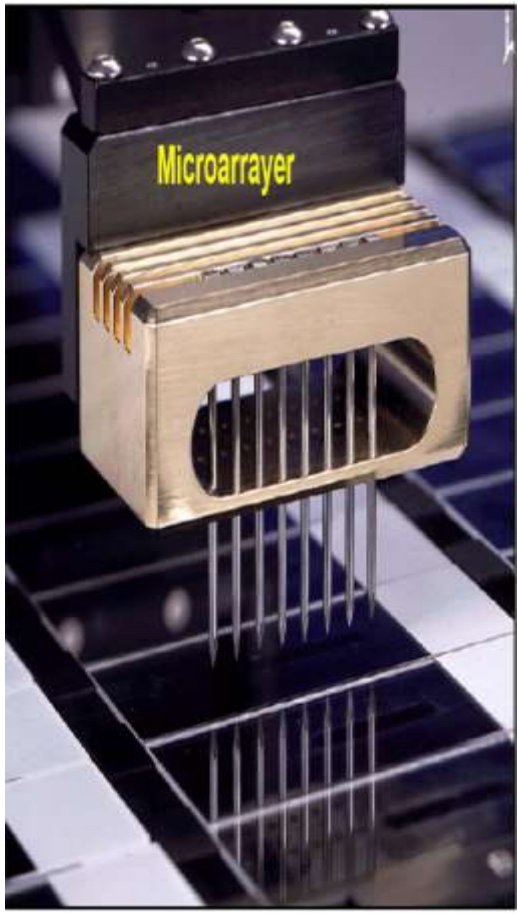
Types of Microarrays

- **Spotted DNA arrays ("cDNA arrays")**
  - Developed by Pat Brown (Stanford)
  - PCR products (or long oligos) from known genes (~100 nt) spotted on glass, plastic, or nylon support.
  - Customizable and off the shelf.

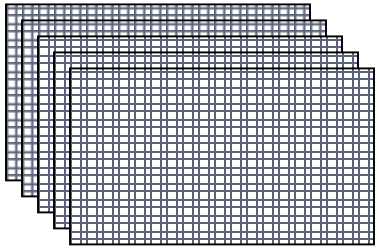
- **Gene Chips**
  - **Oligonucleotide arrays (Affymetrix)**
    - Small number of 20-25mers/gene
    - Enabled by photolithography from the computer industry
    - Off the shelf
  - **Ink-jet microarrays (Agilent)**
    - Large number of 25-60mers "printed" directly on glass
    - Four cartridges: A, C, G, and T
    - Flexible, rapid, but expensive

Spotted DNA arrays

- In *spotted microarrays*, the probes are oligonucleotides, cDNA or small fragments of PCR products that correspond to mRNAs. There probes are synthesized prior to deposition on the array surface and are then "spotted" onto glass.
- A common approach utilizes an array of fine pins or needles controlled by a robotic arm that is dipped into wells containing DNA probes and then depositing each probe at designated locations on the array surface.
- The resulting "grid" of probes represents the nucleic acid profiles of the prepared probes and is ready to receive cDNA derived from experimental or clinical samples.



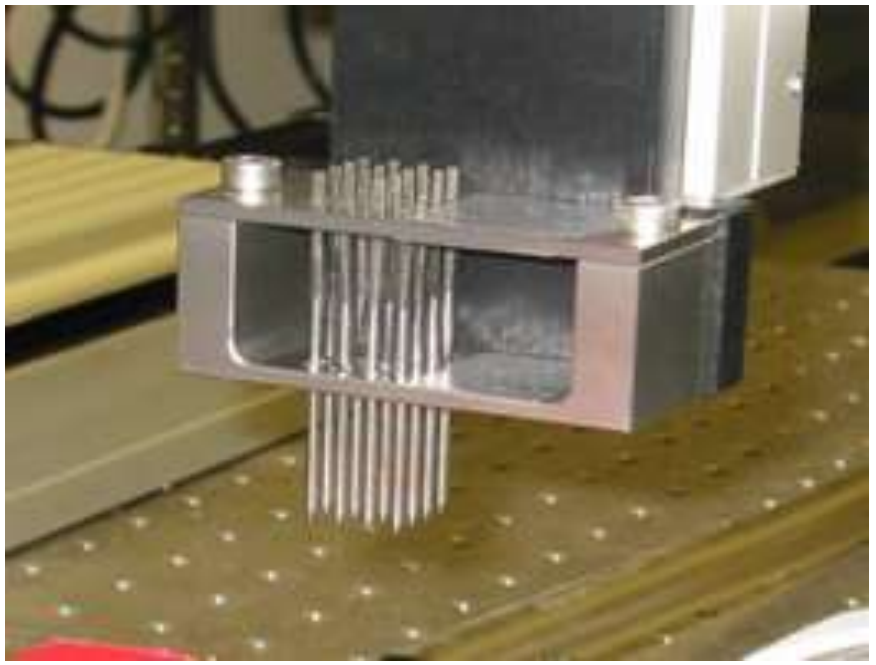
Building a cDNA chip



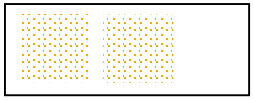
Arrayed Library
(96 or 384-well plates of bacterial glycerol stocks)

PCR amplification of target DNA
(cDNA or portion of genomic DNA)

Consolidate into plates



Spot as microarray on glass slides



These cDNAs are usually more than 500 bases long

Oligonucleotide arrays

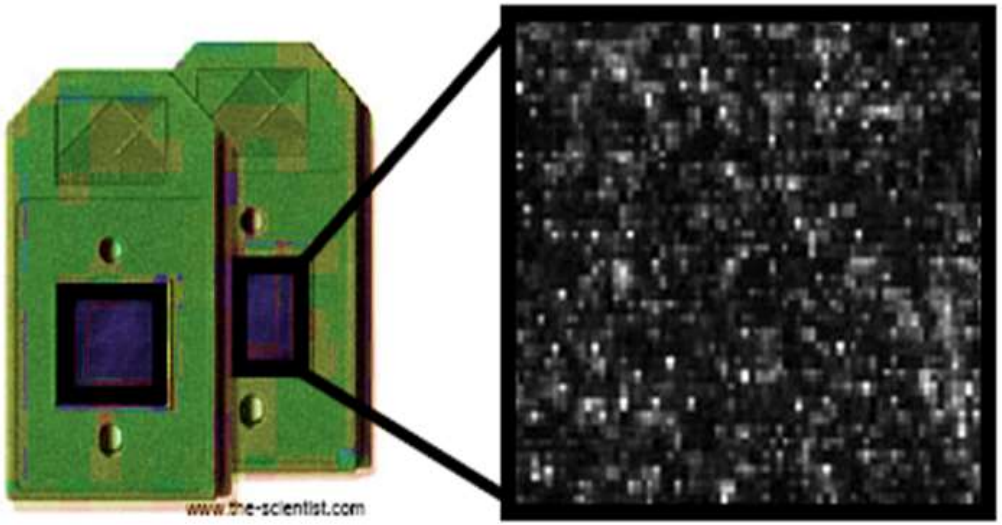
In *oligonucleotide microarrays*, the probes are short sequences designed to match parts of the sequence of known or predicted open reading frames.

- Although oligonucleotide probes are often used in "spotted" microarrays, the term "oligonucleotide array" most often refers to a specific technique of manufacturing.
- Oligonucleotide arrays are produced by printing short oligonucleotide sequences designed to represent a single gene by synthesizing this sequence directly onto the array surface instead of depositing intact sequences.

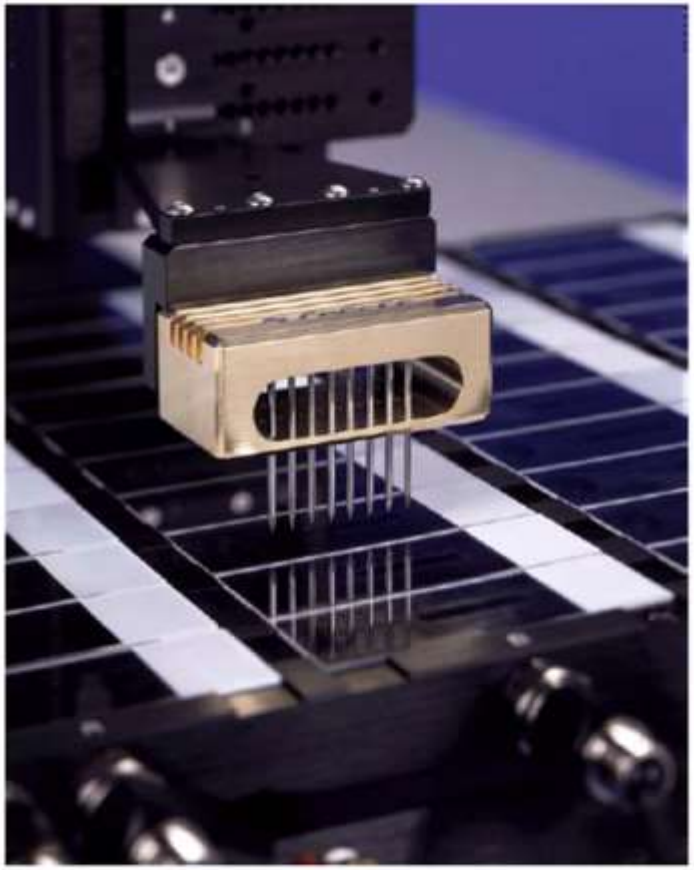
- Sequences may be longer (60-mer probes such as the Agilent design) or shorter (25-mer probes produced by Affymetrix) depending on the desired purpose; longer probes are more specific to individual target genes, shorter probes may be spotted in higher density across the array and are cheaper to manufacture.

- One technique used to produce oligonucleotide arrays include photolithographic synthesis (Agilent and Affymetrix) on a silica substrate where light and light-sensitive masking agents are used to "build" a sequence one nucleotide at a time across the entire array.

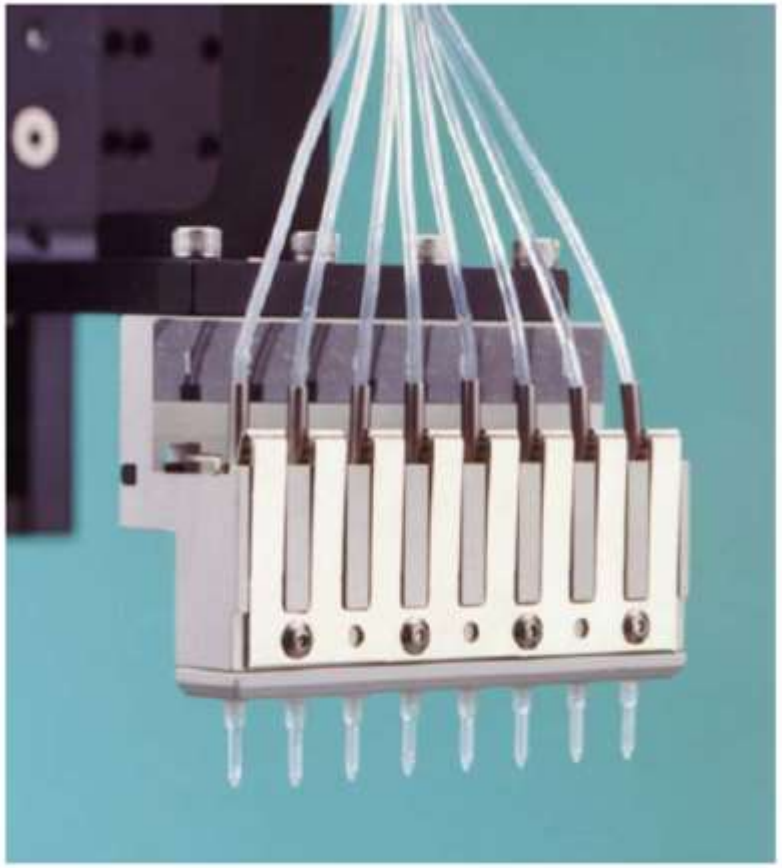
Commercially available microarray



Affymetrix
"Gene Chip"
500,000 probes
25 mer (represents a fragment of a gene)



Contact pins



Inkjet technology

Spotted Vs. Oligonucleotide array

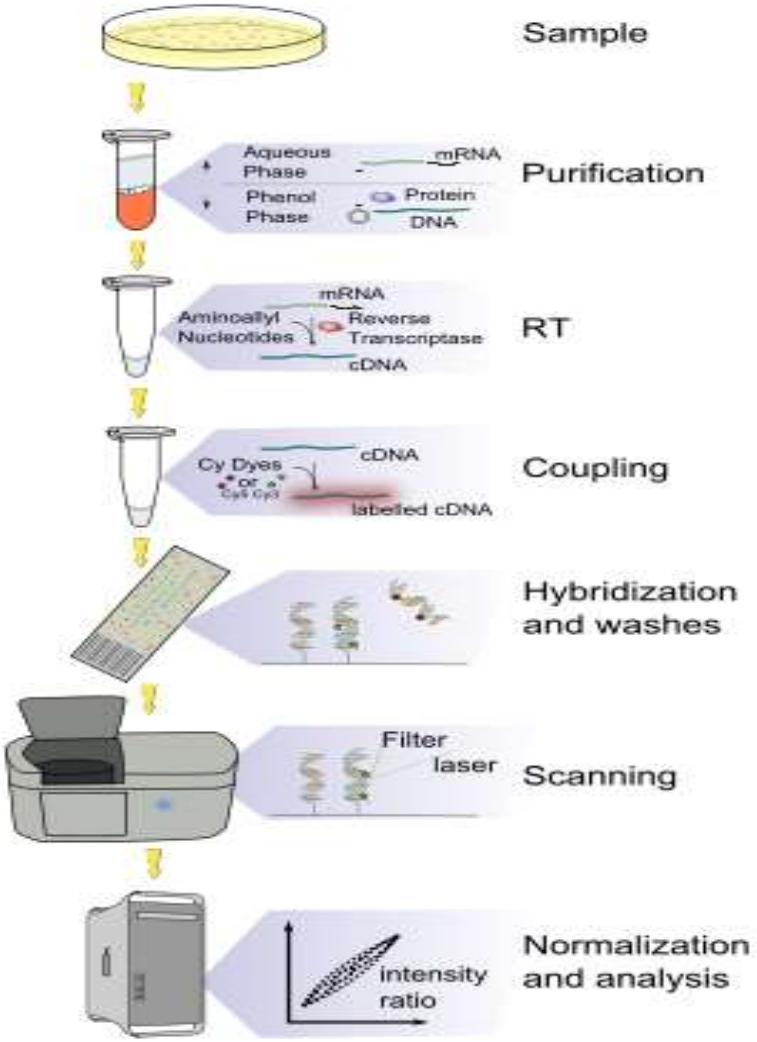
Spotted Arrays	Affy Gene Chips
❑ Relative cheap to make (~$10 slide) ❑ Flexible - spot anything you want ❑ Cheap so can repeat experiments many times ❑ Highly variable spot deposition ❑ Usually have to make your own	❑ Expensive ($500 or more) ❑ Limited types avail, no chance of specialized chips ❑ Fewer repeated experiments usually ❑ More uniform DNA features ❑ Can buy off the shelf

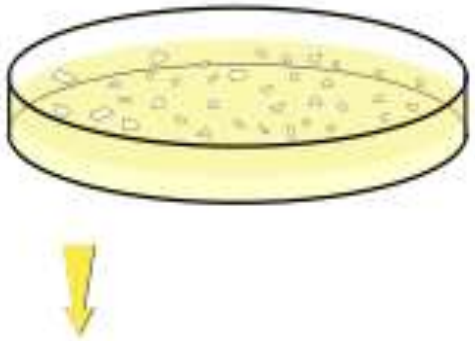
Companies manufacturing them

Agilent Technologies

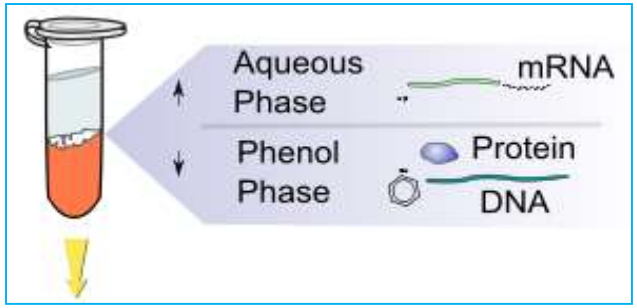
Experiment

- Microarrays use relative quantization in which the intensity of a spot is compared to the intensity of the same spot under a different condition Identity of the spot is known by its position.



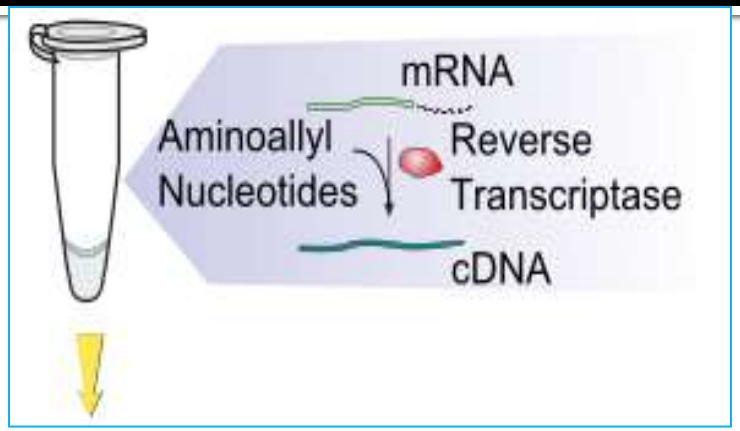


The two samples to be compared (pairwise comparison) are grown/acquired.

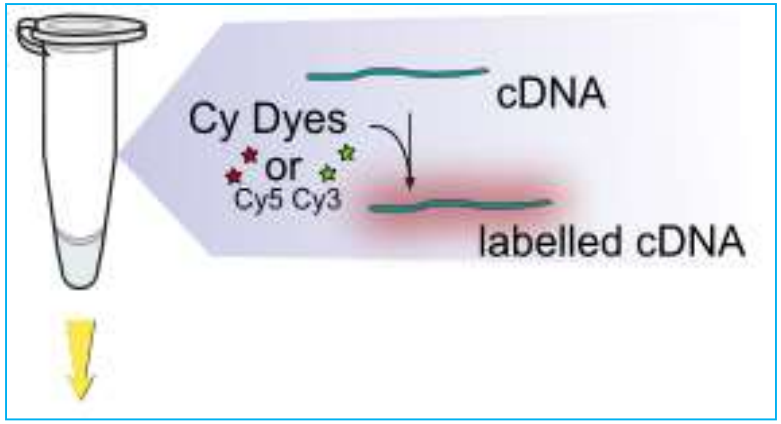


RNA
DNA
DNA/RNA bound to a protein

The purified RNA is analysed for quality (by capillary electrophoresis) and quantity (by using a nanodrop spectrometer)

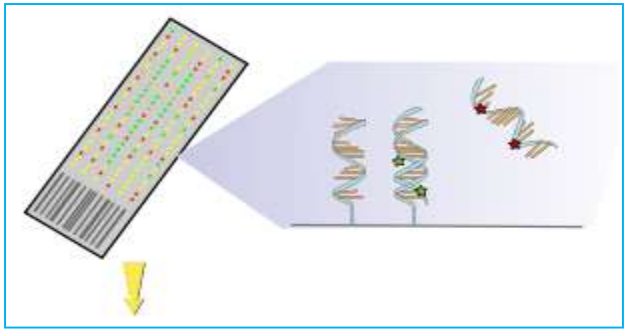


Optional PCR Amplification

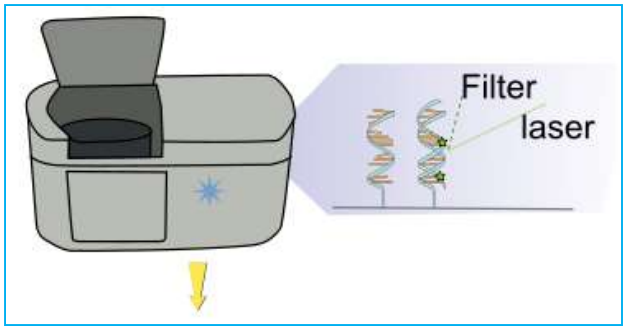


The label is added either in the RT step or in an additional step after amplification if present

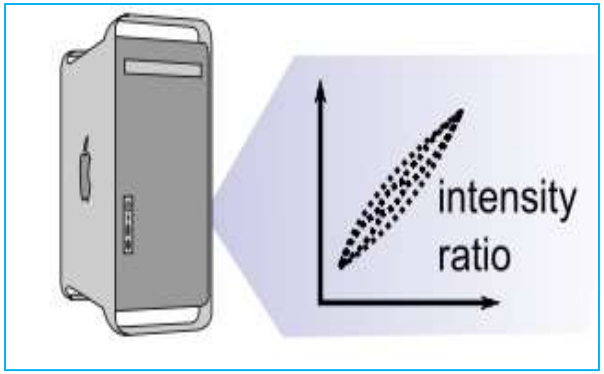
The labeled samples are then mixed with a propriety hybridization solution. SDS, SSC, dextran sulfate, a blocking agent, Denhardt's solution and formamine.



This mix is denatured and added to a pin hole in a microarray.



The holes are sealed and the microarray hybridized.



The microarray is dried and scanned in a special machine where a laser excites the dye and a detector measures its emission. After that the raw that is normalized for study

Applications

Gene expression analysis

- The process of measuring gene expression via cDNA is called expression analysis.
- Not all the genes in the human genome are active at all times.
- Used to detect DNA , or detect RNA that may or may not be translated into proteins.
- Thousand genes are simultaneously assessed.
- Study the effects of certain treatments, diseases, and developmental stages on gene expression.

- E.g.: identify genes expression changes due to pathogens or other organisms by comparing with uninfected cells or tissues.
- **Disease diagnosis**
  Help to investigate about different diseases
- E.g.: Earlier cancers classified on the basis of the organs in which the tumors develop
- Now, classify the types of car
  the patterns of gene activity i
  tumor cells.

Drug Discovery

Extensive application in *Pharmacogenomics*

- Comparative analysis of the genes.
- Help the identification of the specific proteins produce by diseased cells.
- Information used to synthesize drugs which combat with these proteins and reduce their effect.
- Help to produce very effective drugs.

Toxicological Research

- A rapid platform for the research of the impact of toxins on the cells and their passing on to the progeny.
- Important for Toxicogenomic studies

Gene ID

- Small microarrays to check IDs of organisms in food and feed (like GMO) and mycoplasms in cell culture
- Mostly combining PCR and microarray technology

Nutrigenomic research

Study variations in the genes related to the influence of diets.

- These variations, known as single nucleotide polymorphism.
- E.g.: Studies are followed to reveal,
  - Effects of calorie restriction on gene expression.
  - Obesity and high-fat diets.
  - Genes responds to gluten and soy protein.

Advantages

- *Provides data for thousands of genes.*
- *One experiment instead of many.*
- *Fast and easy to obtain results.*
- *Huge step closer to discovering cures for diseases and cancer.*
- *Different parts of DNA can be used to study gene expression.*

Disadvantages

- *Very little knowledge is available about many genes*
- *Just because mRNA is "turned on" doesn't mean proteins are made*
- *The findings may lead to unethical medical procedures*
- *Scientists have no standardized way to share results.*

CONCLUSION

DNA Microarrays are one of the most effective invention ever developed. A DNA Microarray is a test that allows for the comparison of thousands of genes at once. Microarray technology uses chips with attached DNA sequences as probes for gene expression. Any DNA in the sample that is complementary to a probe sequence will become bound to the chip. Microarray technology is most powerful when it used on species with a sequenced genome. The microarray chip can hold sequences from every gene in the entire genome and the expression of every gene can be studied simultaneously. Gene expression data can provide information on the function of previously uncharacterized genes.

Source:

- http://www.gene-chips.com/
- http://www.bio.davidson.edu/courses/genomics/chip/chip.html
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thank you