BHARATHIDASAN UNIVERSITY

Program: M.Sc., Biomedical Science

Course Code : 18BMS47C10

Course Title : Genetic Engineering

DNA chips

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DNA chips

Technologies and utility

Historical perspective

- DNA hybridization (1960s)
- Detection of hybrids
  - hydroxyapatite
  - radioactive labelling
  - enzyme-linked detection
  - fluorescent labelling
- Fixing sample on solid support
  - Southern blots (1970s)
  - Northern blots
  - Dot blots

Basic principles

- Main novelty is one of **scale**
  - hundreds or thousands of probes rather than tens
- **Probes** are attached to solid supports
- **Robotics** are used extensively
- **Informatics** is a central component at all stages

DNA array

- DNA array is much advanced method.
- Using known list of genes imprinted in a membrane or in a glass chip, total RNA were allowed to hybridized and compared the intensity of the radioactive spots in an autoradiograph.

  ex: Apoptosis array, cytokines array, growth arrest array, etc….

cDNA arrays on nylon and glass

- Nylon arrays
  - Up to about 1000 probes per filter
  - Use radiolabeled cDNA target
  - Can use phosphorimager or X-ray film
- Glass arrays
  - Up to about 40’000 probes per slide, or 10’000 per 2cm^2 area (limited by arrayer’s capabilities)
  - Use fluorescent targets
  - Require specialized scanner

Major technologies

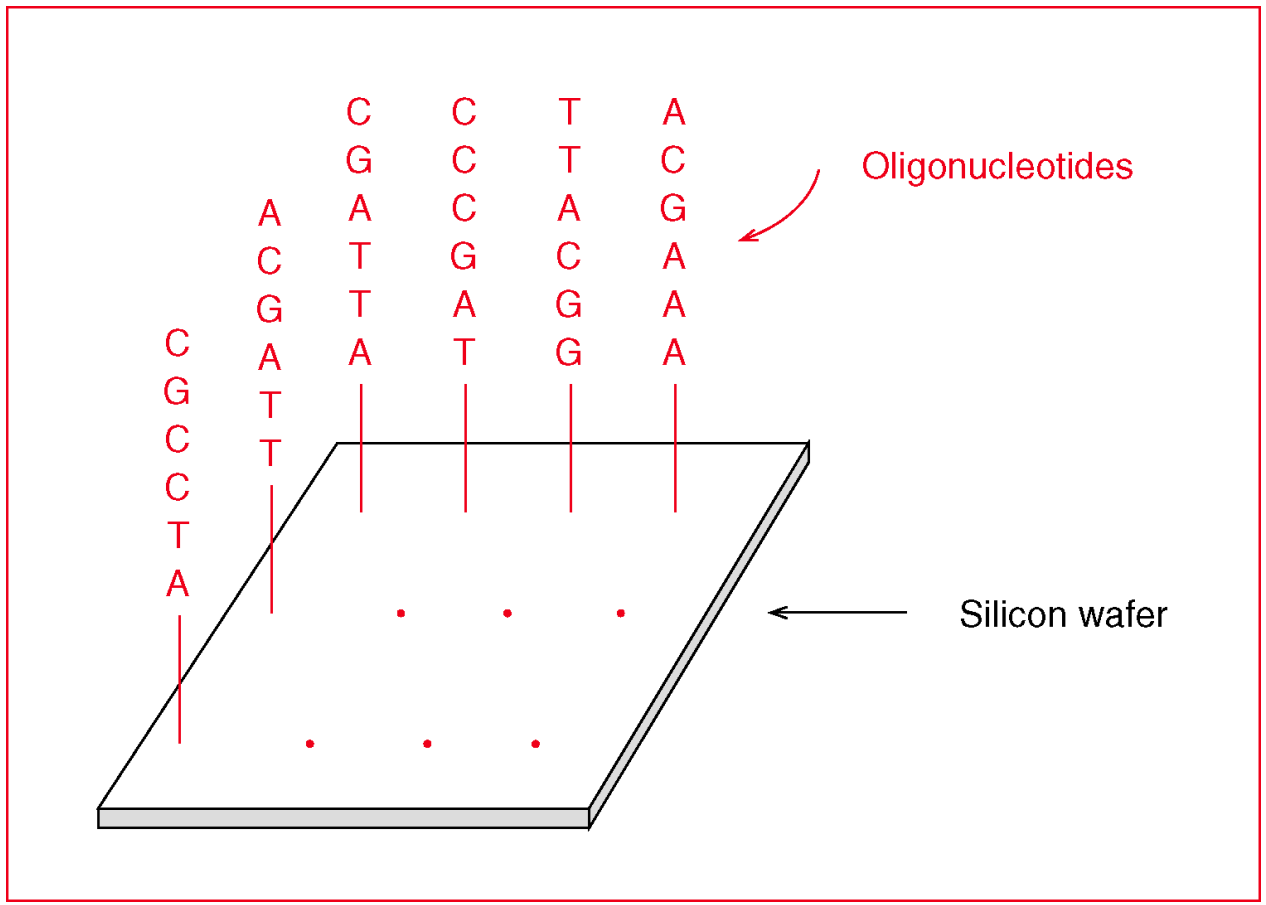
- cDNA probes (> 200 nt), usually produced by PCR, attached to either nylon or glass supports
- Oligonucleotides (25-80 nt) attached to glass support
- Oligonucleotides (25-30 nt) synthesized in situ on silica wafers (Affymetrix)
- Probes attached to tagged beads

cDNA chips

- Probes are cDNA fragments, usually amplified by PCR
- Probes are deposited on a solid support, either positively charged nylon or glass slide
- Samples (normally poly(A)+ RNA) are labelled using fluorescent dyes
- At least two samples are hybridized to chip
- Fluorescence at different wavelengths measured by a scanner

Glass chip manufacturing

- Choice of coupling method
  - Physical (charge), non-specific chemical, specific chemical (modified PCR primer)
- Choice of printing method
  - Mechanical pins: flat tip, split tip, pin & ring
  - Piezoelectric deposition (“ink-jet”)
- Robot design
  - Precision of movement in 3 axes
  - Speed and throughput
  - Number of pins, numbers of spots per pin load


C G C C T A
A C G A T T
C G A T T A
C C G A T
T T A C G G
A C G A A A
Oligonucleotides
Silicon wafer

An experiment on a microarray

In this schematic:

GREEN represents **Control DNA**

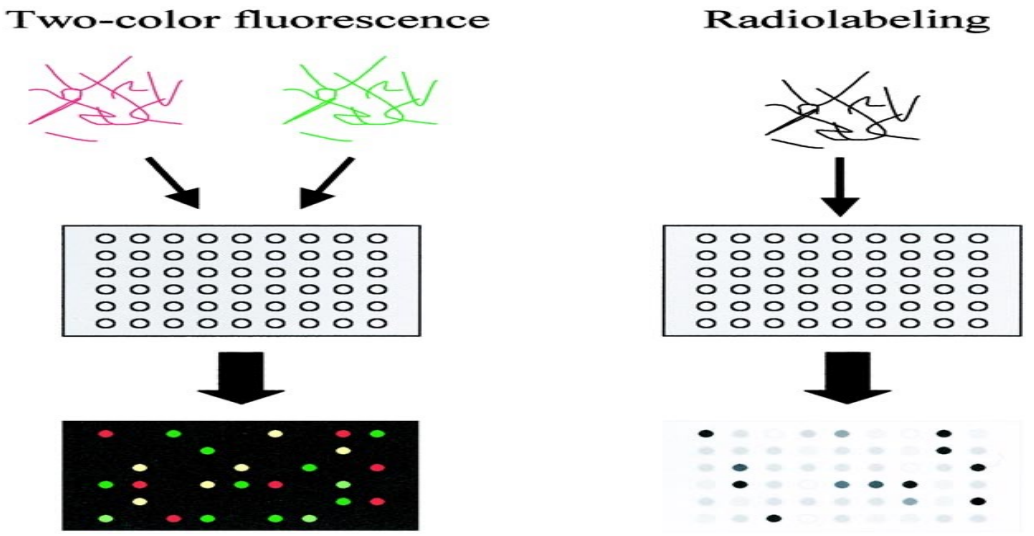
RED represents **Sample DNA**

YELLOW represents **a combination of Control and Sample DNA**

BLACK represents areas where **neither the Control nor Sample DNA**

Each color in an array represents either healthy (control) or diseased (sample) tissue. The location and intensity of a color tell us whether the gene, or mutation, is present in the control and/or sample DNA.

Labeling technique for DNA arrays

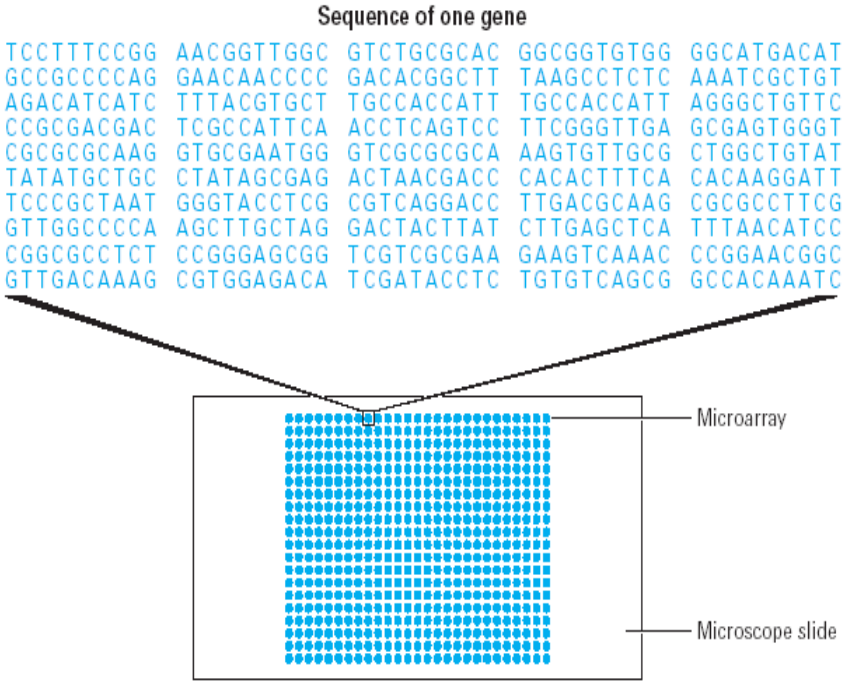


RNA samples are labeled using fluorescent nucleotides (***left***) or radioactive nucleotides (***right***), and hybridized to arrays. For fluorescent labeling, two or more samples labeled with differently colored fluorescent markers are hybridized to an array. Level of RNA for each gene in the sample is measured as intensity of fluorescence or radioactivity binding to the specific spot. With fluorescence labeling, relative levels of expressed genes in two samples can be directly compared with a single array.

DNA Microarray

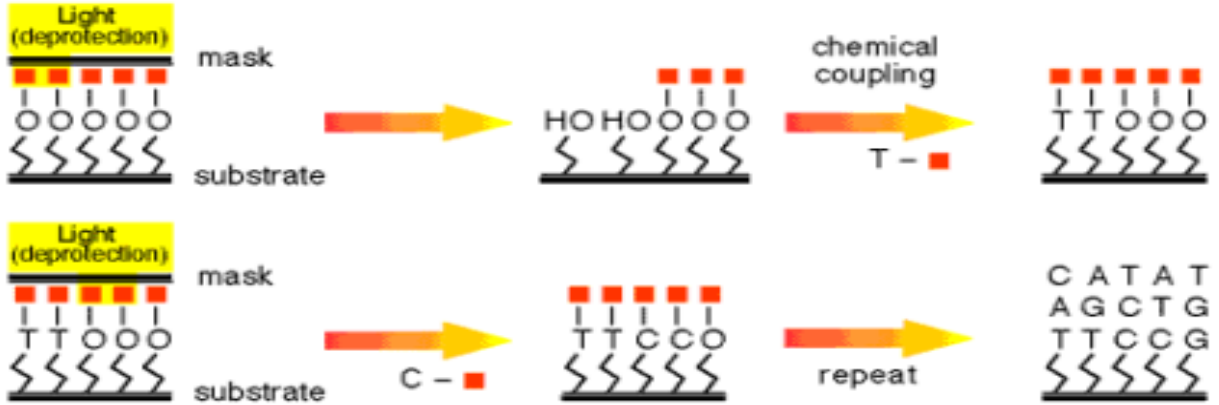
Affymetrix

Microarray is a tool for analyzing gene expression that consists of a glass slide.



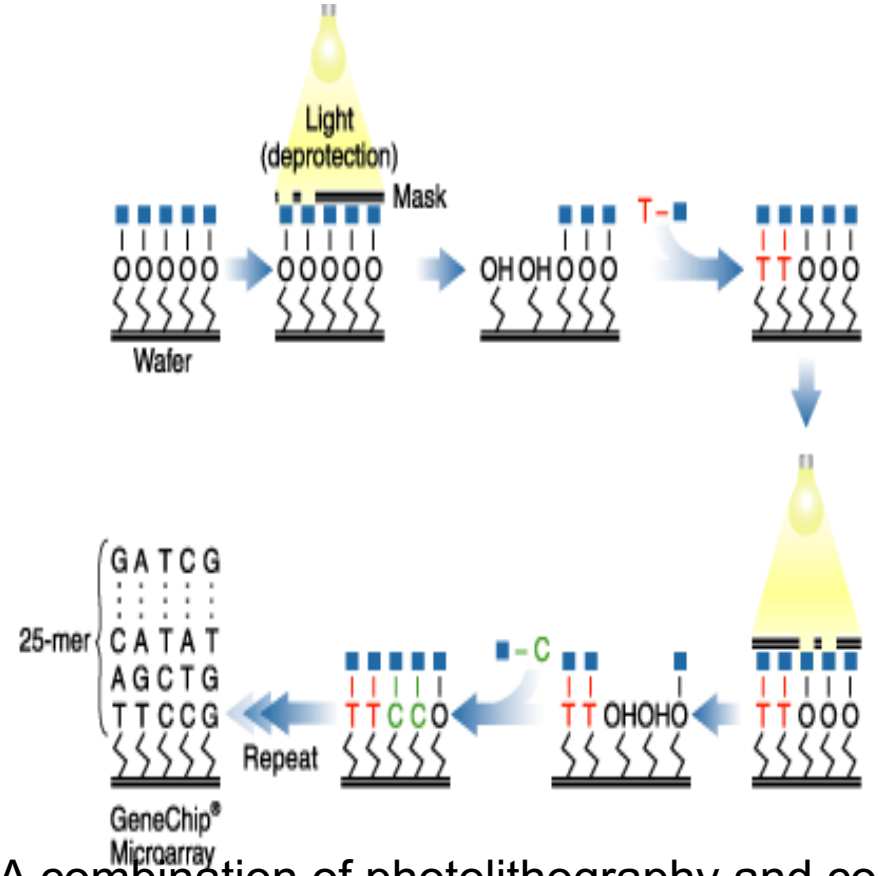
Each blue spot indicates the location of a PCR product. On a real microarray, each spot is about 100um in diameter.

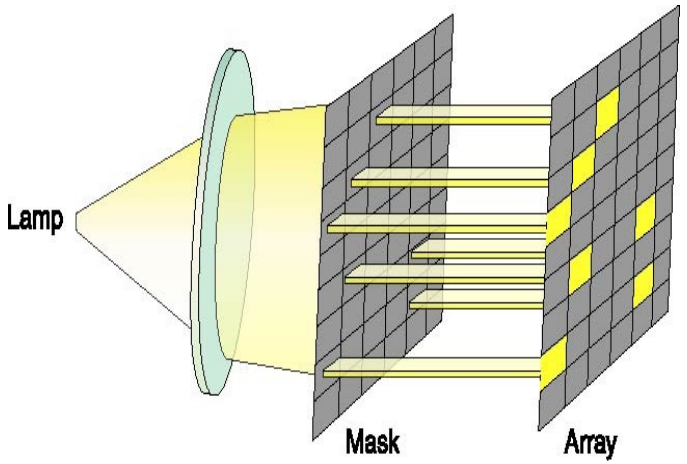
Photolithography



- Light directed oligonucleotide synthesis.
- A solid support is derivatized with a covalent linker molecule terminated with a photolabile protecting group.
- Light is directed through a mask to deprotect and activate selected sites, and protected nucleotides couple to the activated sites.
- The process is repeated, activating different set of sites and coupling different based allowing arbitrary DNA probes to be constructed at each site.

Affymetrix GeneChip® Arrays

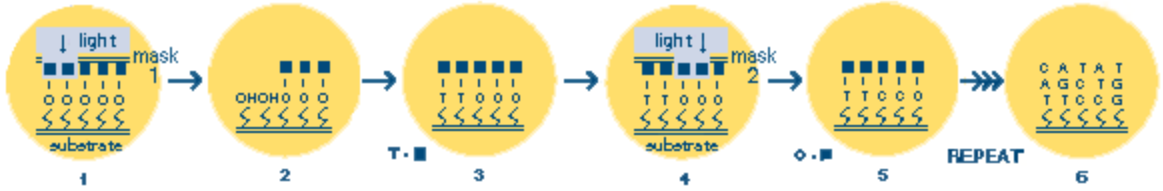




A combination of photolithography and combinatorial chemistry to manufacture GeneChip® Arrays. With a minimum number of steps, Affymetrix produces arrays with thousands of different probes packed at extremely high density. Enable to obtain high quality, genome-wide data using small sample volumes.

http://www.affymetrix.com/technology/manufacturing/index.affx

Affymetrix chip production



The Affymetrix approach

- Probes are oligos synthesized *in situ* using a photolithographic approach
- There are at least 5 oligos per cDNA, plus an equal number of negative controls
- The apparatus requires a fluidics station for hybridization and a special scanner
- Only a single fluorochrome is used per hybridization
- It is **very** expensive !

Commercial chips

- Clontech, Incyte, Research Genetics - filter-based arrays with up to about 8000 clones
- Incyte / Synteni - 10’000 probe chips, not distributed (have to send them target RNA)
- Affymetrix - oligo-based chips with 12’000 genes of known function (16 oligos/gene) and 4x10’000 genes from ESTs