DNA MICROARRAY ANALYSIS

B.Sc. 4th Semester

Introduction:

Definition: DNA microarrays are solid supports, usually of glass or silicon, upon which DNA is attached in an organized grid fashion. Each spot of DNA, called a probe, represents a single gene.

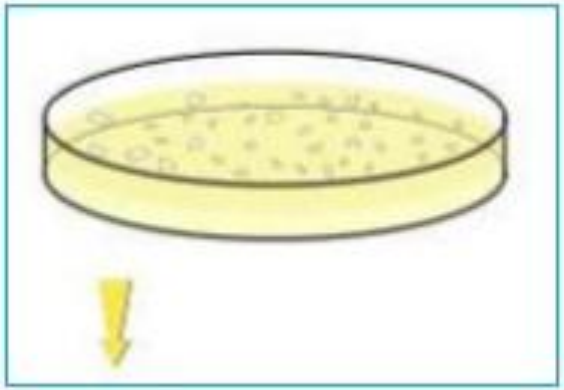
There are several synonyms of DNA microarrays such as DNA chips, gene chips, DNA arrays, gene arrays and biochips.

History: Microarray technology evolved from Southern blotting, where fragmented DNA is attached to a substrate and then probed with a known DNA sequence.

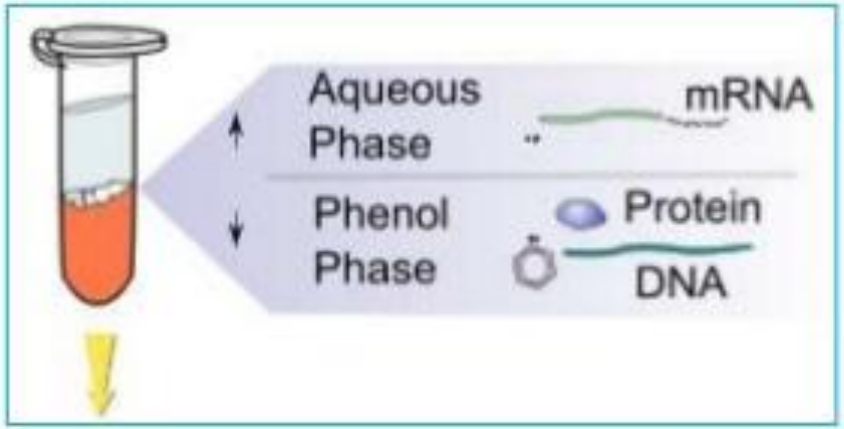
The use of miniaturized microarrays for gene expression profiling was first reported in 1995, and a complete eukaryotic genome (*Saccharomyces cerevisiae*) on a microarray was published in 1997.

[Ref: Presscot (Book for Microbiology), www.wikipedia.org]

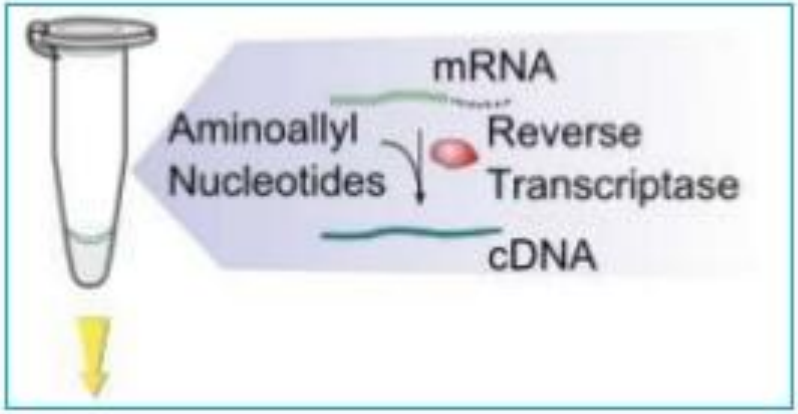
1. Sample preparation



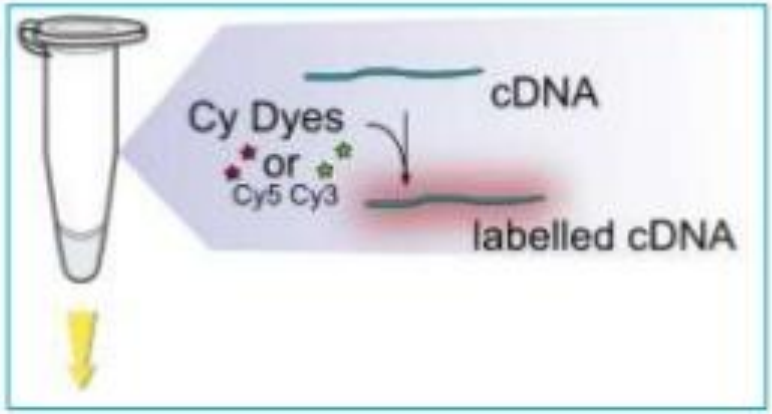
2. Purification



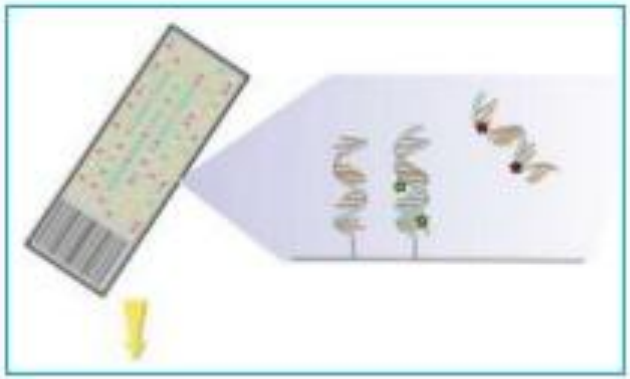
3. Reverse Transcription



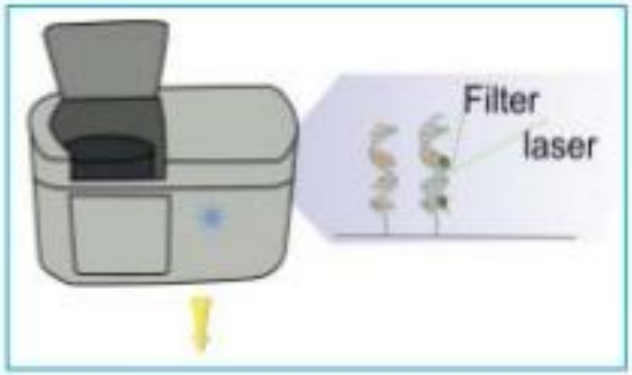
4. Labelling



5.Hibridization



6. Scanning



7.Normalization and analysis

Types of DNA chips

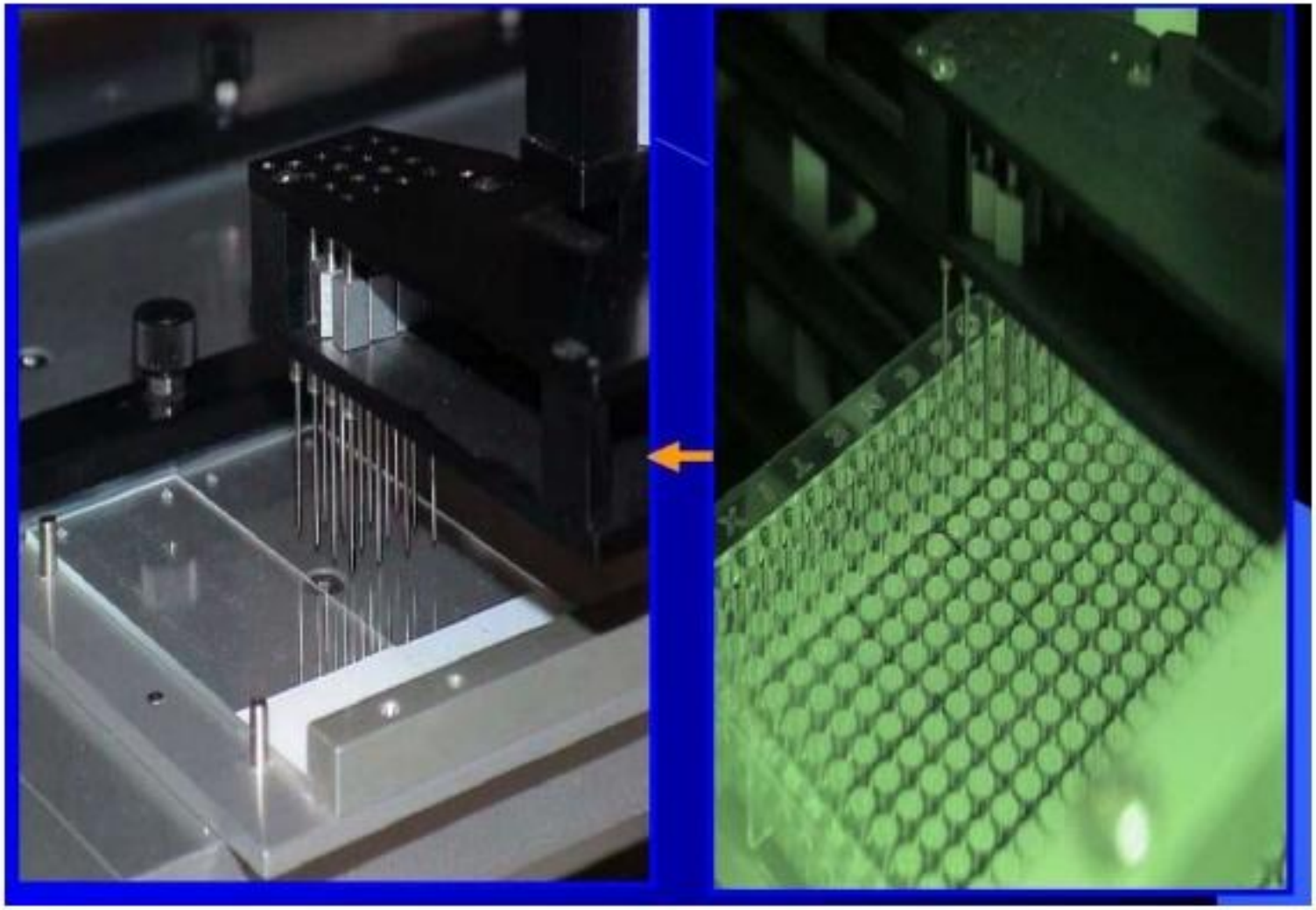
There are 2 types of DNA Chips/Microarrays:

1. cDNA based microarray
2. Oligonucleotide based microaaray

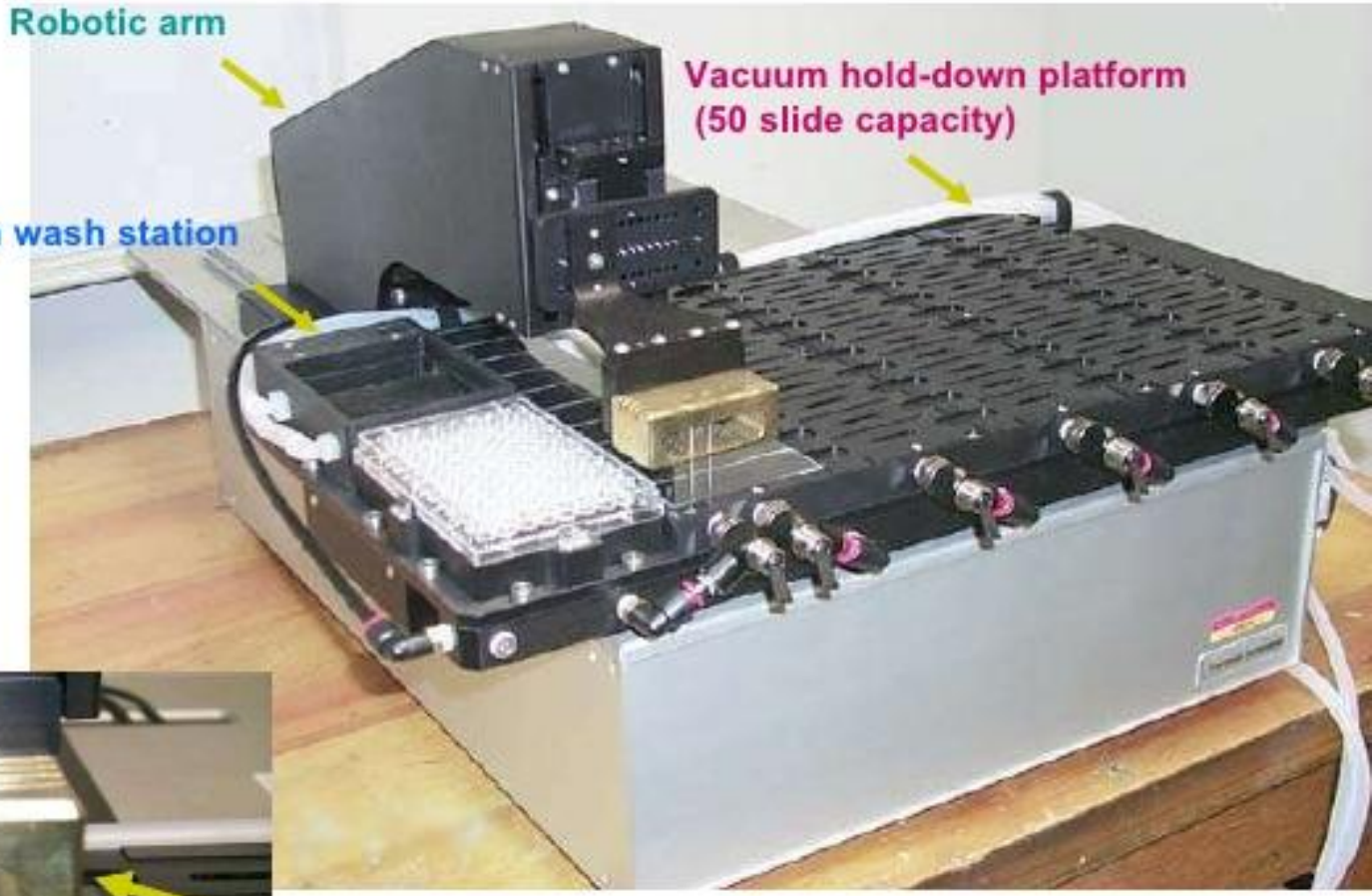
How do we manufacture a microarray?

- Start with individual genes, e.g. the ~6,200 genes of the yeast genome
- Amplify all of them using polymerase chain reaction (PCR)
- "Spot" them on a medium, e.g. an ordinary glass microscope slide
- Each spot is about 100 μm in diameter
- Spotting is done by a robot
- Complex and potentially expensive task

Robotic spotting



The PixSys 5500 Arraying Robot (Cartesian Technologies)

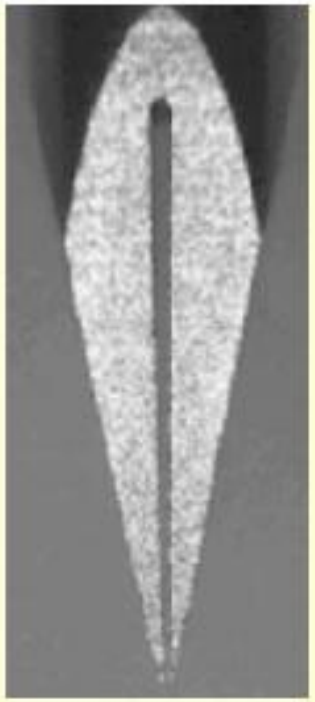


The print head holds up to 32 pins
in a 8x4 format

24

Contact Printing

- **pins**
  - Uptake 0.25 ul
  - Dispense 0.6 nl
  - (approximately 1-10ng per spot)
  - 100 um feature size

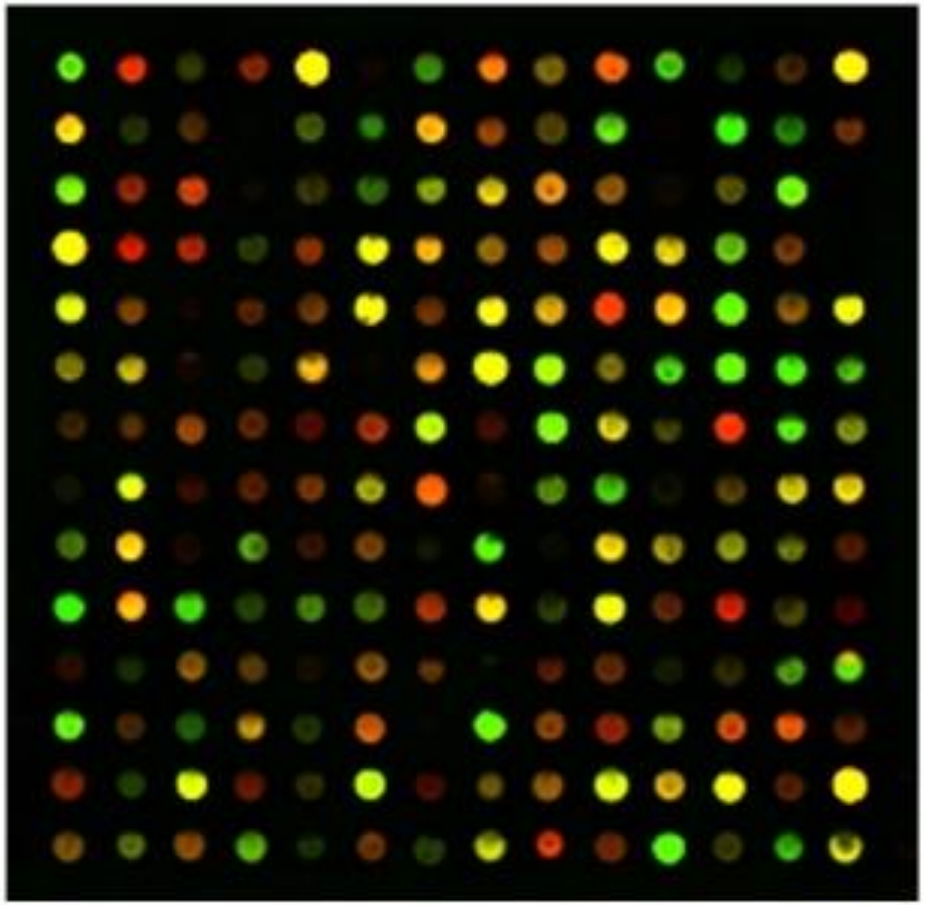




Affymetrix Arrays

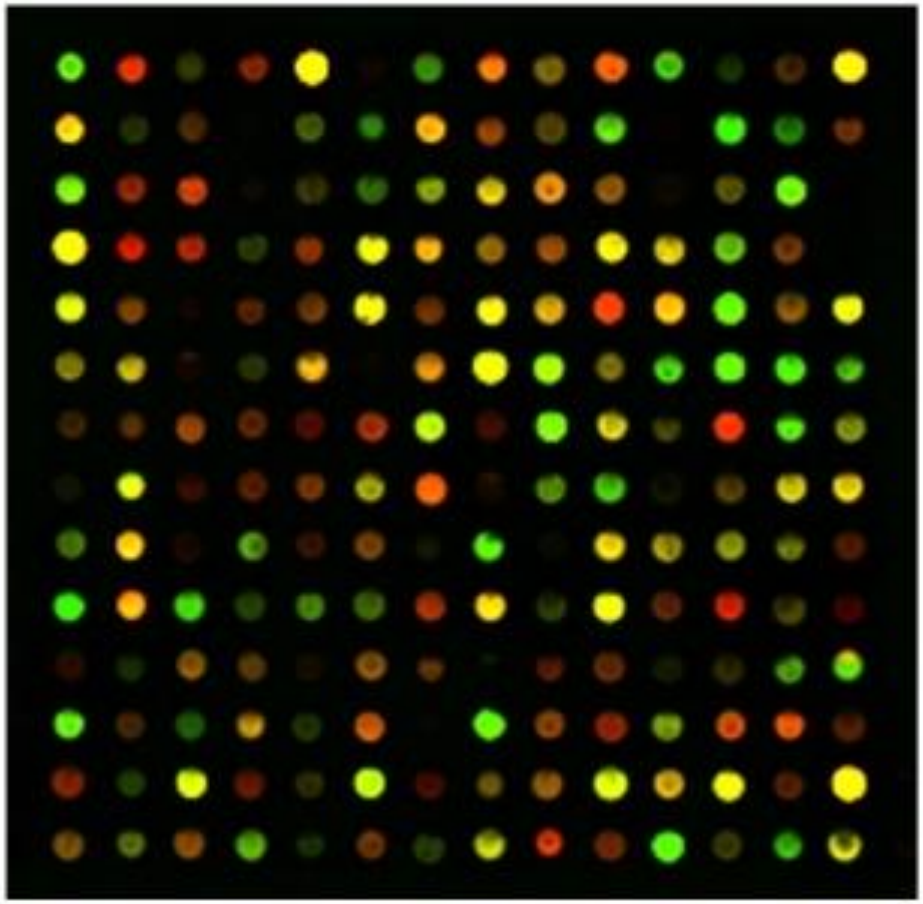
Microarray

- **Green**: mRNA produced in control sample (wild type, normal, untreated)
- **Red**: mRNA produced in test sample (treated)
- **Yellow**: both samples produce the same amount of mRNA

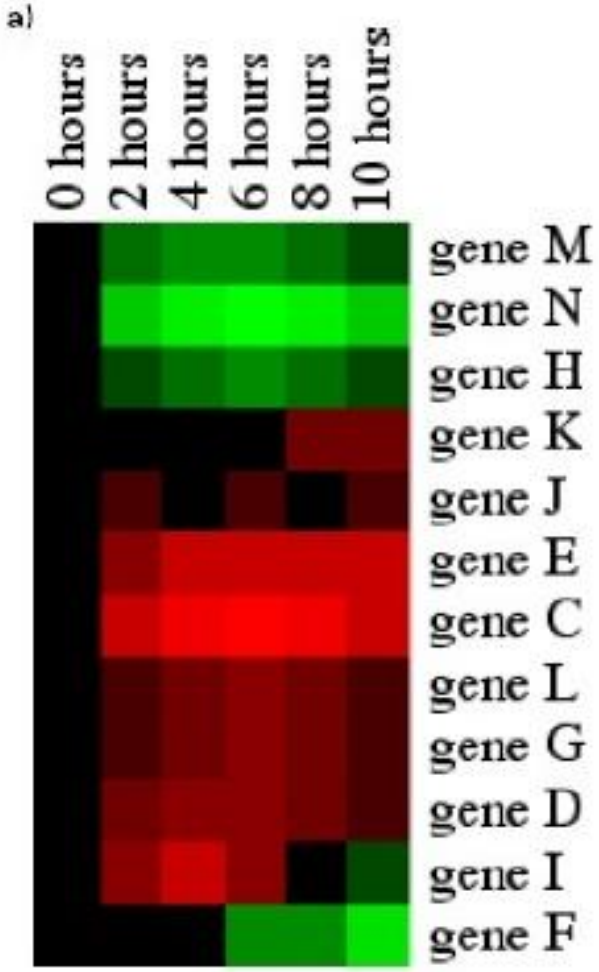


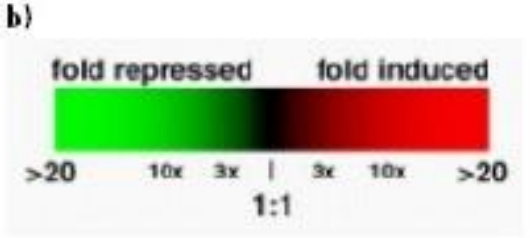
Gene Expression

- **Green**: signifies down regulation of a gene in testing conditions
- **Red**: signifies up regulation of a gene in testing conditions
- **Yellow**: no changes in gene expression when testing conditions are changed

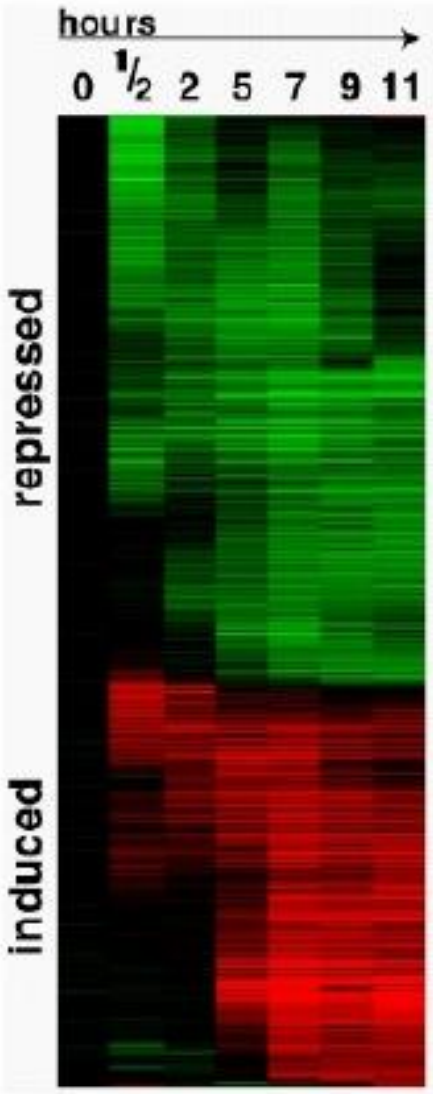


Clustering of example





Clustering of entire yeast genome



Campbell & Heyer 2003

Applications

The DNA chips are used in many areas as given below:

- Gene expression profiling
- Discovery of drugs
- Diagnostics and genetic engineering
- Alternative splicing detection
- Proteomics
- Functional genomics
- DNA sequencing
- Toxicological research (Toxicogenomics)

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 expresion.

[Ref: www.biotechnologyforums.com, www.ehow.com]

Disadvantages:

- The biggest disadvantage of DNA chips is that they are expensive to create.
- The production of too many results at a time requires long time for analysis, which is quite complex in nature.
- The DNA chips do not have very long shelf life, which proves to be another major disadvantage of the technology.

[Ref: www.biotechnologyforums.com, www.ehow.com]