Expressed genes profiling (Microarrays)

Overview Of Gene Expression Control

Profiling Of Expressed Genes

Genes can be regulated at many levels

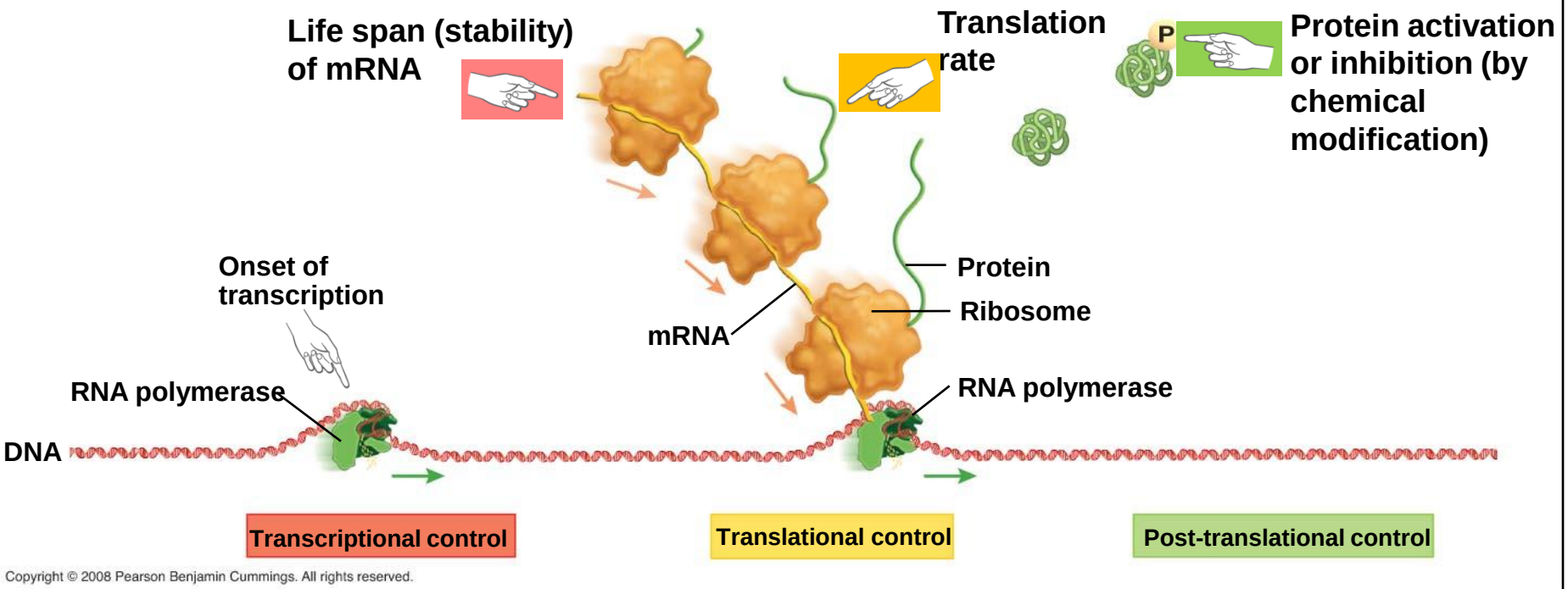
- Usually, gene regulation, are referring to **transcriptional regulation.**
- The complete set of all genes being transcribed are referred to as the ***"transcriptome."***

- **transcription**
- **post transcription (RNA stability)**

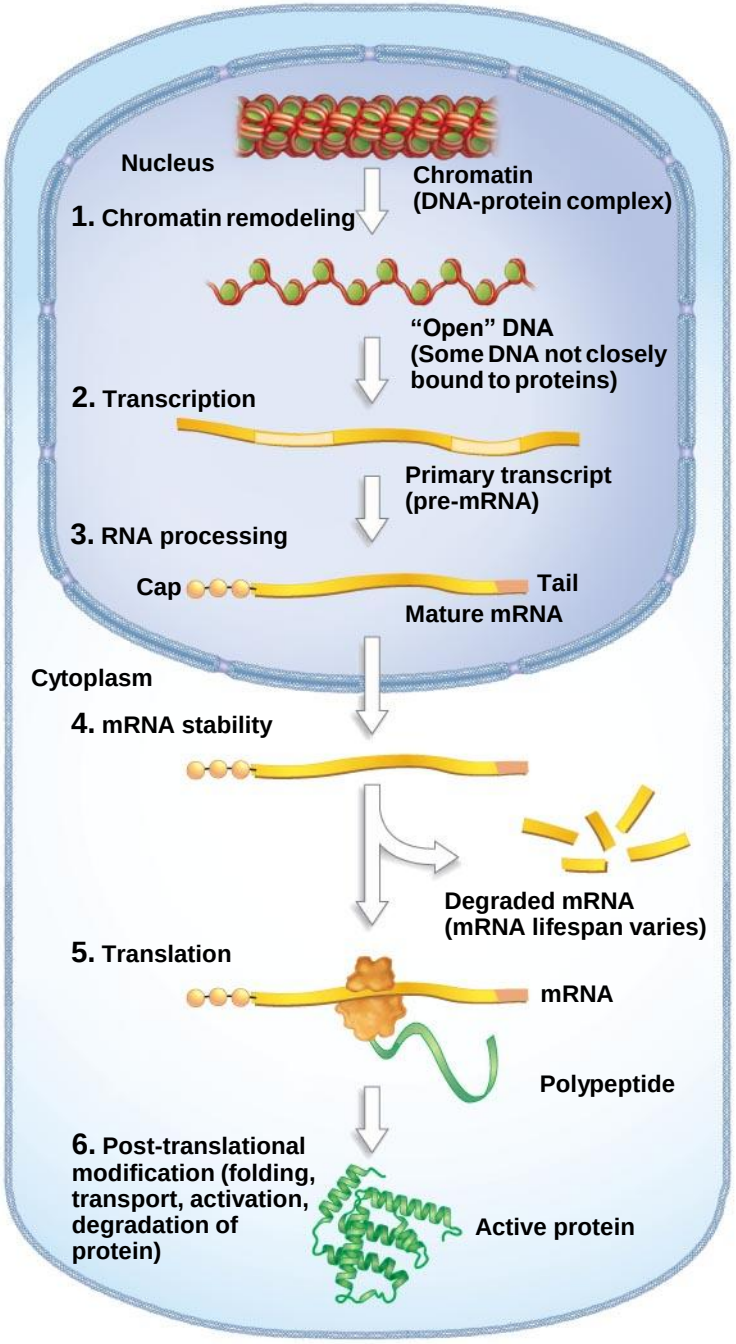
} **the "transcriptome"**

- **post transcription (translational control)**
- **post translation (not considered gene regulation)**

- **In prokaryotes**,
  - gene expression is controlled by the external environment (i.e. fuel availability) and occurs at 3 levels:



- **In eukaryotes**,
  - gene expression is controlled by the internal environment (i.e. hormones) and occurs at these same 3 levels + 2 other levels:
    1. Chromosome remodeling
    2. Transcription
    3. Post-transcription
    4. Translation
    5. Post-translation



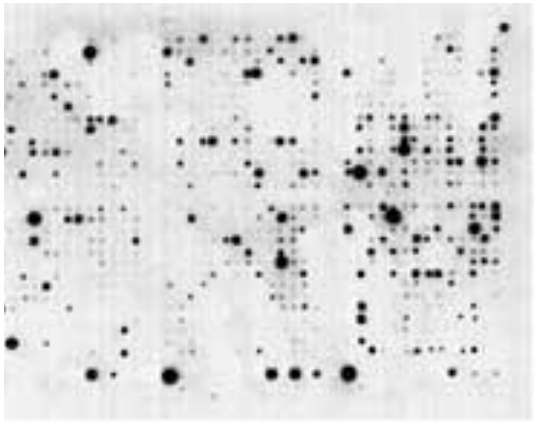
DNA Microarrays

- A technology that **can measure the expression level** of thousands of genes at once
- A large number of DNA fragments (‘**probes**’) are attached in a systematic way (**array**) to a solid substrate
- A test solution is prepared, **polynucleotides being labeled**
- The test solution is applied and allowed to **hybridize** with the (immobilized) probes
- The solid support is washed and the bound (hybridized), labeled, nucleic acids are **determined**.

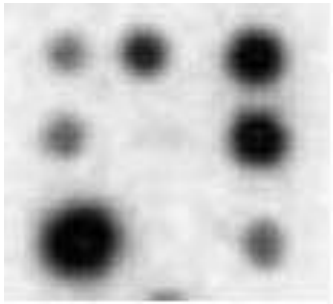
Microarray Production Process

- Slide preparation is achieved by blocking the polylysine not fixed to DNA in order to avoid target binding.
- Prior to hybridization, DNA is denatured to obtained a single strand DNA on the microarray, this will allow the probe to bind to the complementary strand from the target.
- Apart from glass slide microarray other types of chips exist:
  - High density filters (macroarrays)
  - **Glass slides (microarray)**
  - Oligonucleotides chips

High density filters(macroarrays)



Detail:

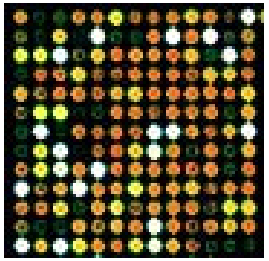


Size: 12cm x 8cm

- 2400 clones by membrane
- radioactive labelling
- 1 experimental condition by membrane

Glass slides (microarrays)

Detail:

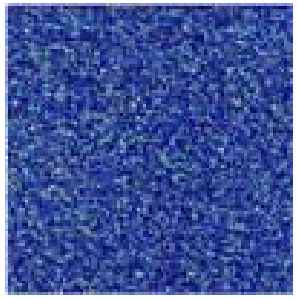


Size: 5,4cm x 0,9cm

- 10000 clones by slide
- fluorescent labelling
- 2 experimental conditions by slide

Oligonucleotides chips

Detail:



Size: 1,28cm x 1,28cm

- 300000 oligonucleotides by slide
- fluorescent labelling
- 1 experimental condition by slide

How Microarrays can be done?

- **DNA samples are prepared** from the cells or tissues of interest.
  - For genotyping analysis, the sample is genomic DNA.
  - For expression analysis, the sample is cDNA, DNA copies of mRNA.
- **The DNA samples are tagged** with a radioactive or fluorescent label and applied to the array.
- Single stranded DNA will bind to a complementary strand of DNA at positions on the array where the immobilized DNA recognizes a complementary DNA in the sample, **binding or hybridization occurs**.
- The labeled sample DNA marks the exact positions on the array where binding occurs, **allowing automatic detection**.

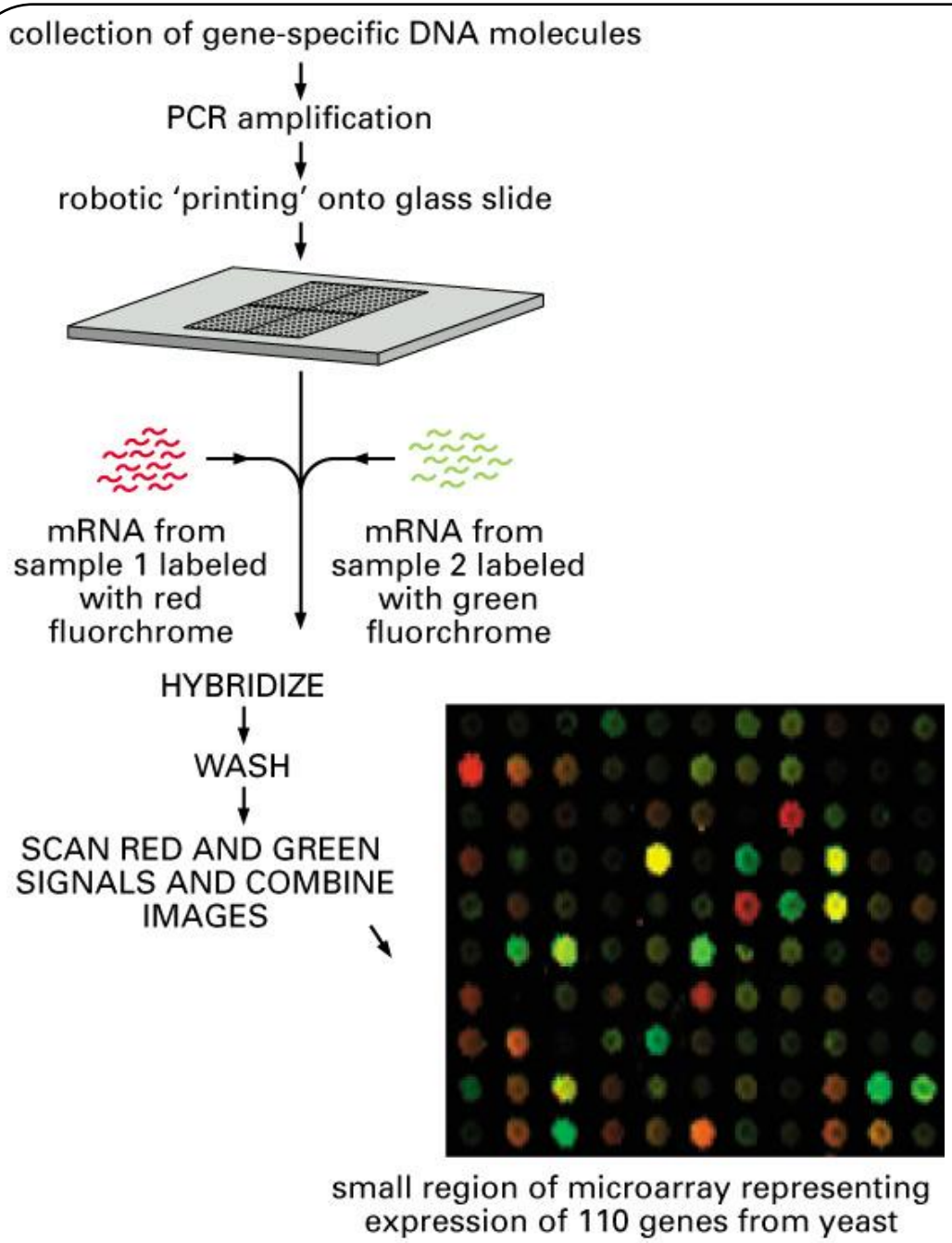
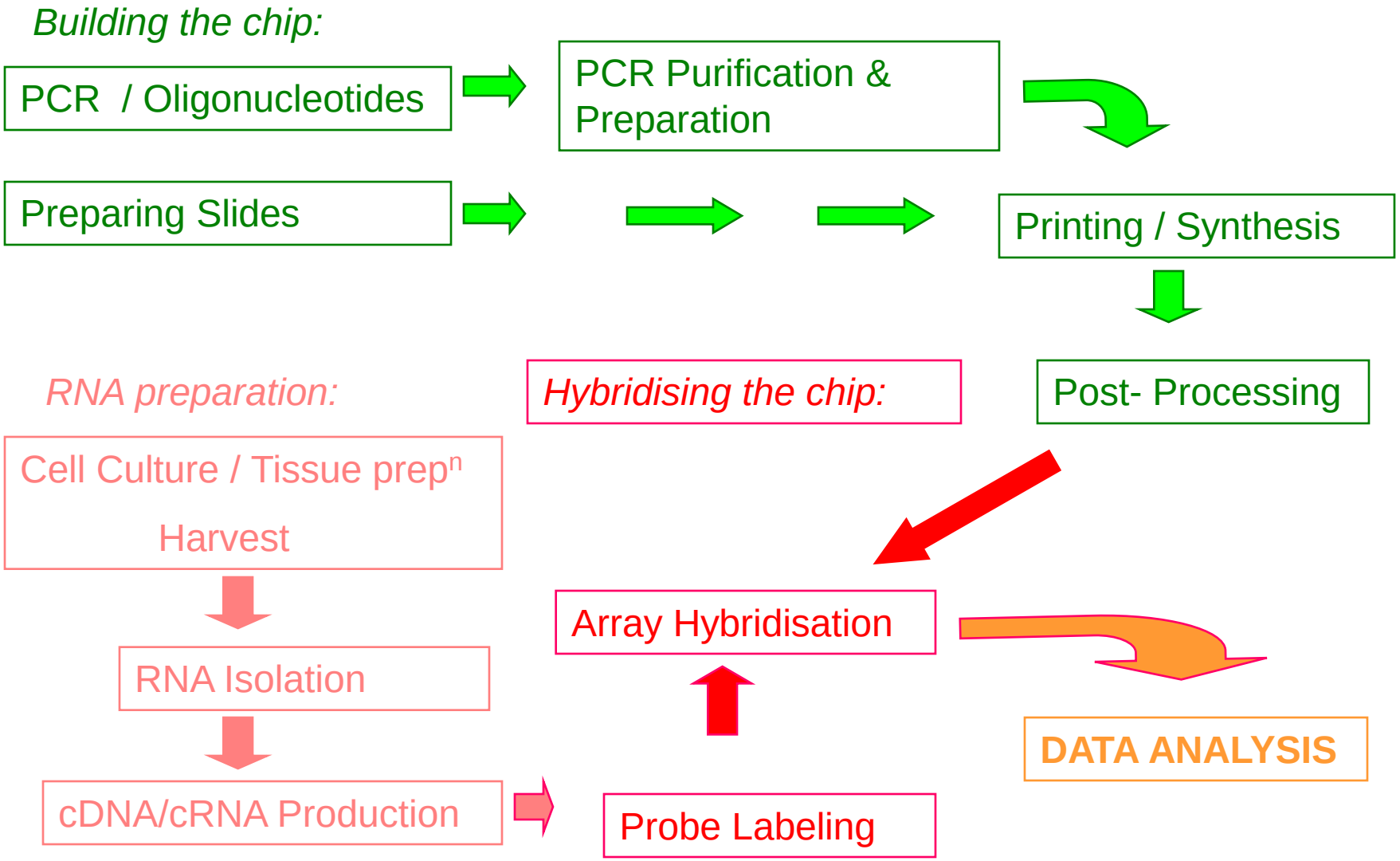


Figure 8–62. Molecular Biology of the Cell, 4th Edition.

MICROARRAY

Using microarray to monitor the expression of thousands of genes simultaneously

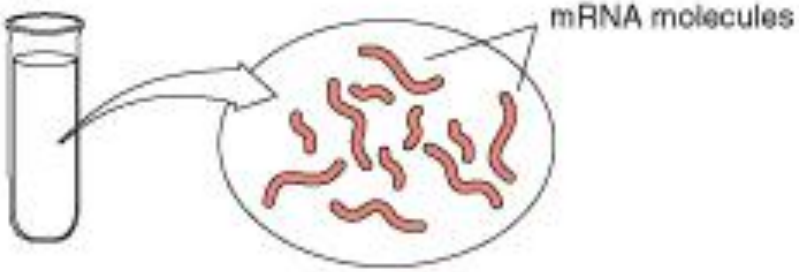
The process


Building the chip:
PCR / Oligonucleotides
PCR Purification & Preparation
Preparing Slides
Printing / Synthesis
Post- Processing
RNA preparation:
Hybridising the chip:
Cell Culture / Tissue prepn
Harvest
Array Hybridisation
RNA Isolation
DATA ANALYSIS
cDNA/cRNA Production
Probe Labeling

Expressed Genes MICROARRAY

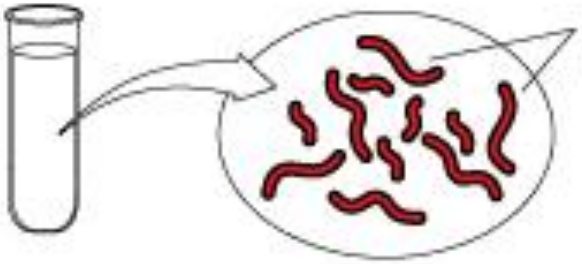
Tissue sample

1) Isolate mRNA.



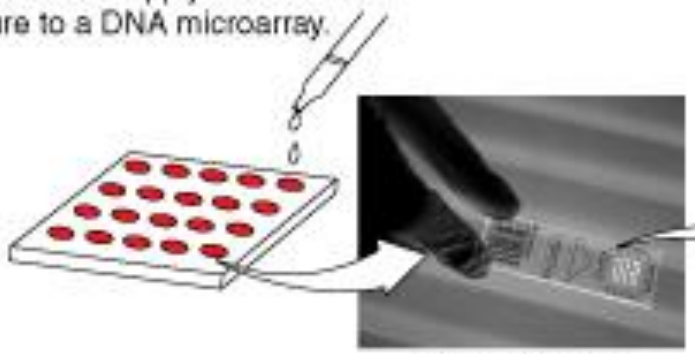
mRNA molecules

2) Make cDNA by reverse transcription, using fluorescently labeled nucleotides, red in this example.



Labeled cDNA molecules (single strands)

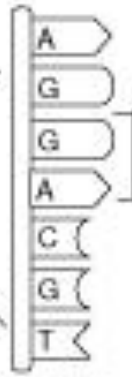
3) Hybridization: Apply the cDNA mixture to a DNA microarray.



Microarray (chip)

Segment of a chip

Spot containing copies of a single DNA molecule



DNA bases

Fixed to each spot on a microscope slide are millions of copies of short single-stranded DNA molecules, a different gene in each spot

Profiling Of Expressed Genes MICROARRAY

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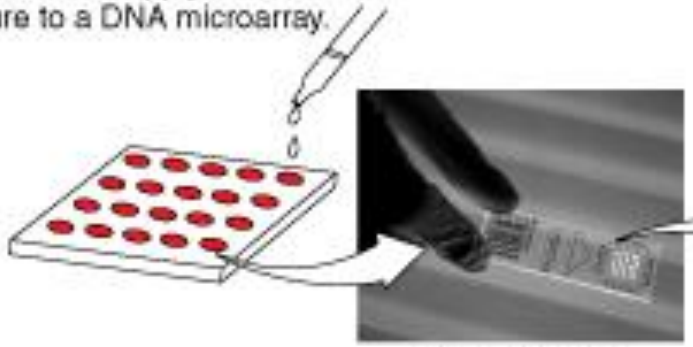

Microarray (chip)

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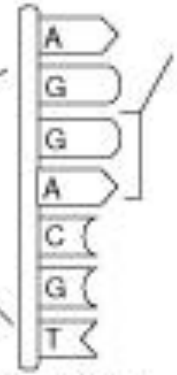
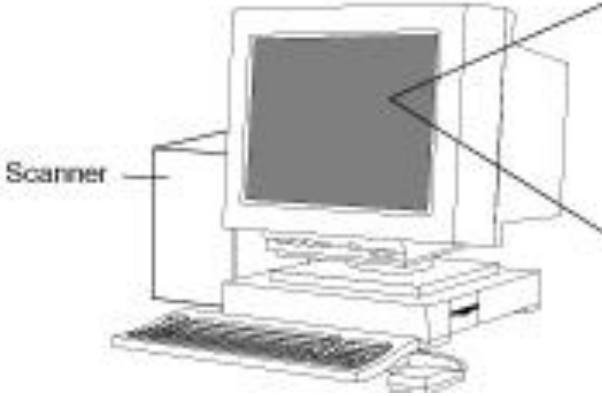


DNA bases

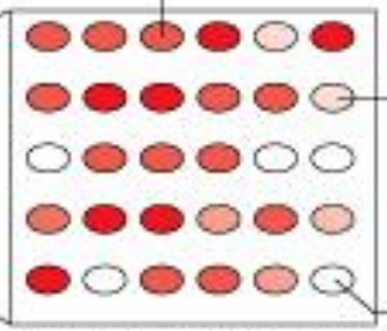
Fixed to each spot on a microscope slide are millions of copies of short single-stranded DNA molecules, a different gene in each spot

Part of one DNA strand

4) Rinse off excess cDNA, put the chip in a scanner to measure fluorescence of each spot. Fluorescence intensity indicates the amount of gene expressed in the tissue sample.



Scanner



Readout

Bright fluorescence: highly expressed gene in tissue sample

Moderate fluorescence: low gene expression

Light: no fluorescence, gene not expressed in tissue sample

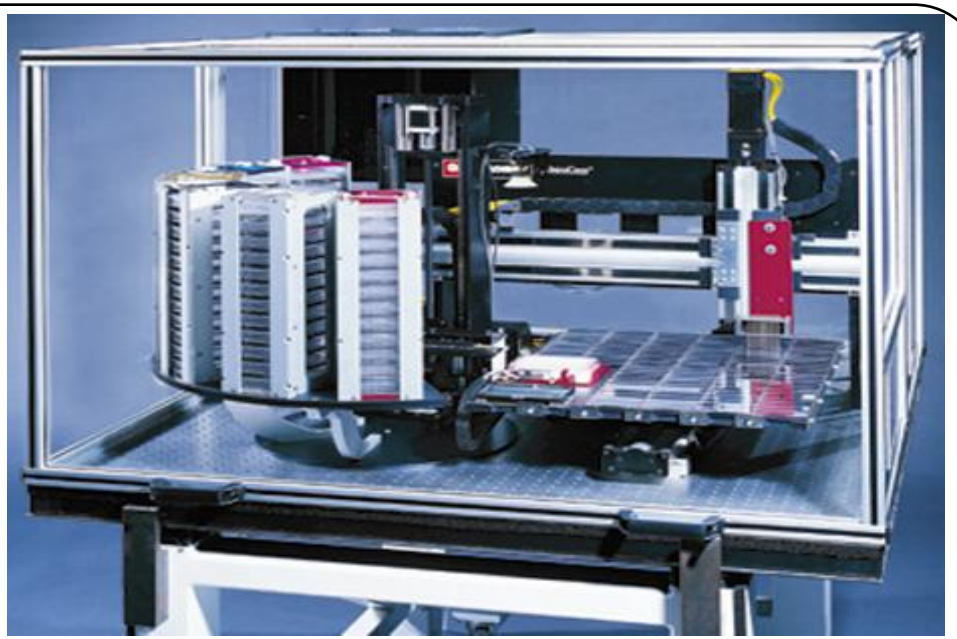
In this image red spots indicate bright fluorescence and white spots indicate no fluorescence.

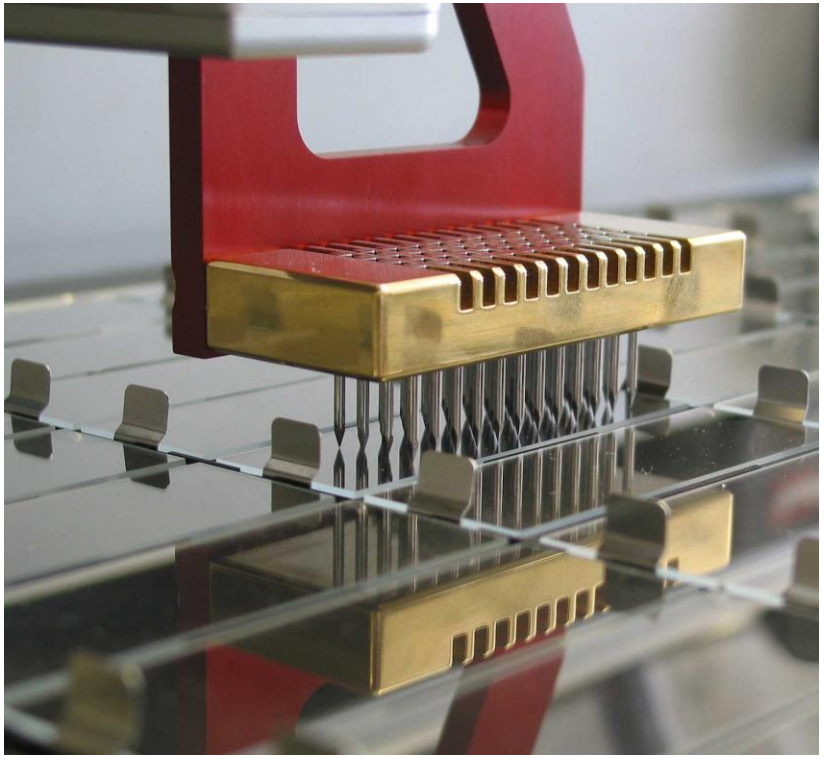
Slide-based DNA microarrays

- For **slide-based microarrays**, the probe DNA is affixed directly to the surface of a glass microscope slide.
- The probe DNA can range from a ***medium-length oligonucleotide (e.g., 60 nt)*** to ***an entire cDNA clone or larger.***
- Oligonucleotide arrays have become more common and can be obtained from several different commercial vendors.
- The DNA is deposited on the slide by any of a number of methods, including "**printing**" with what is essentially an ink-jet printer and spotting using a robotically controlled set of fine-tipped

DNA spotting (printing)

- DNA spotting usually uses multiple pins
- DNA in microtiter plate
- DNA usually PCR amplified
- Oligonucleotides can also be spotted

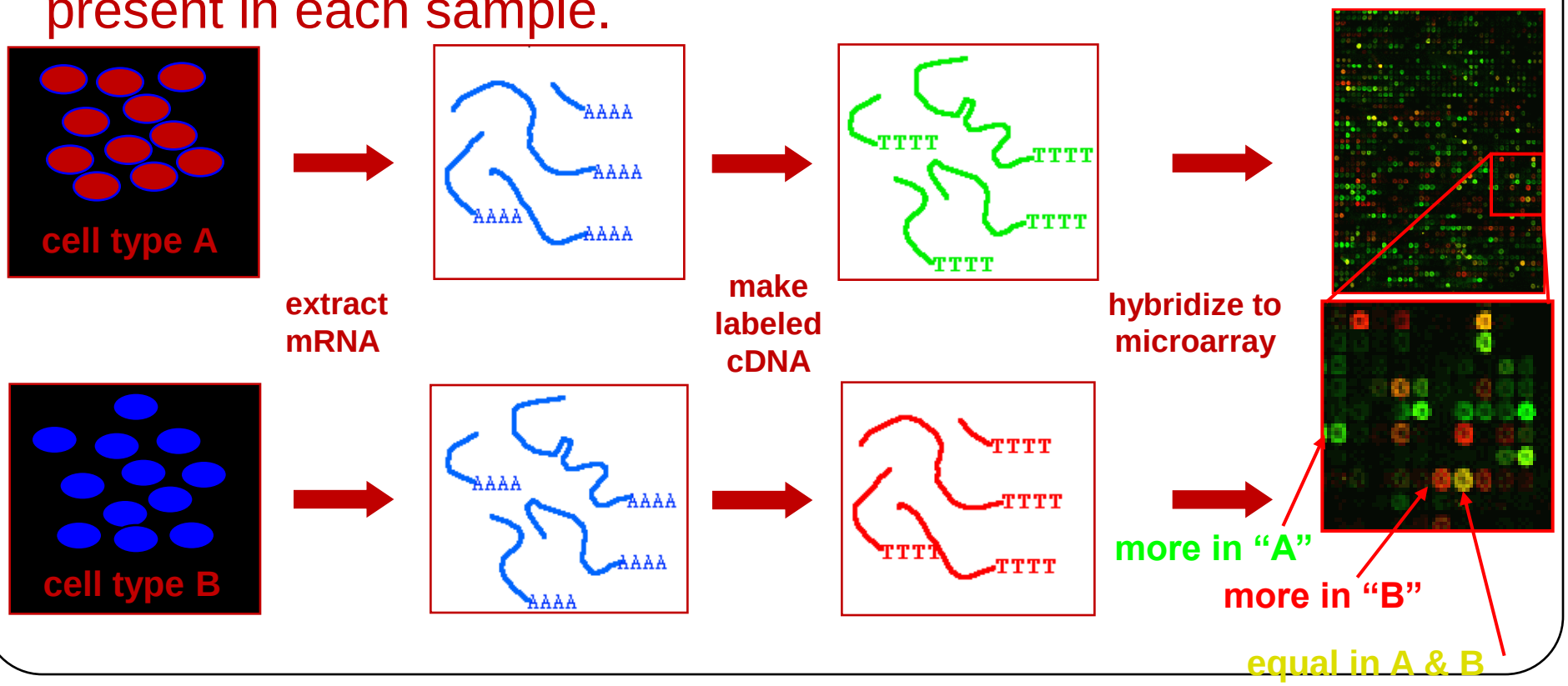




Slide-based DNA microarrays

In general, slide-based arrays are used to make a *direct comparison* between two different RNA samples.

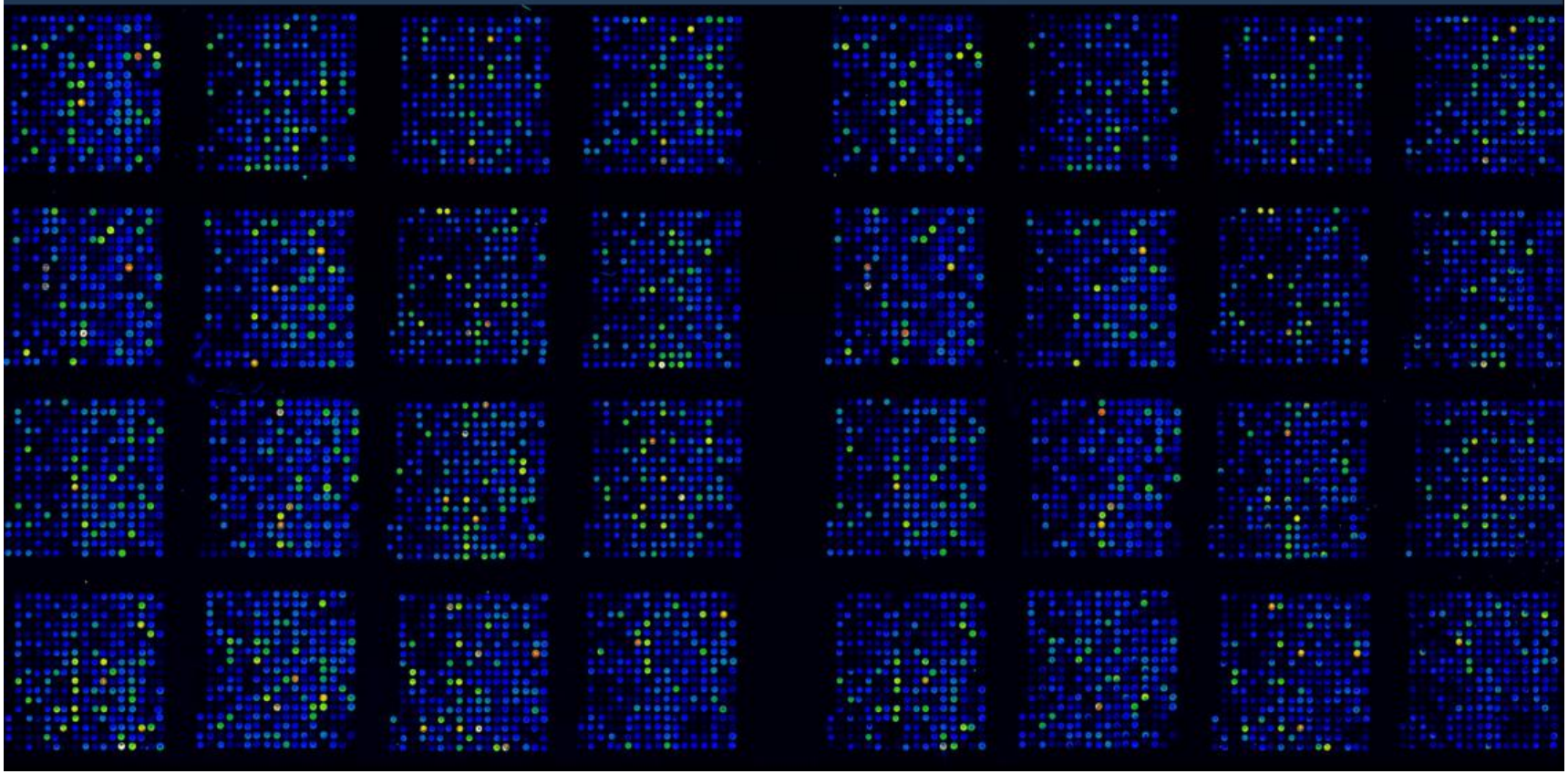
These can be a tissue sample vs. a reference, mutant vs. wild type, treated vs. control, etc. The microarray provides a readout of the *relative differences in abundance* of the RNAs present in each sample.



- A light source (laser) scans the gene chip array, causing the dyes to fluoresce
- The chip can be scanned at multiple wavelengths (green / red)
- The fluorescence is detected by the sensor (photo-multiplier tube) and is used to determine the relative abundance of the RNA
- Compare nucleic acid isolated from control state (e.g. labeled ‘green’) with nucleic acid from test state (e.g. labeled ‘red’)
- This information must be then processed to determine the level of activity for each expressed gene

gene array scanner

Array analysis – 5,000 mouse genes



Applications of microarrays

- Alterations in gene expression patterns or in a DNA sequence can have profound effects on biological functions.
  - These variations in gene expression are at the core of altered physiologic and pathologic processes.
  - DNA array technologies provide rapid and cost-effective methods of identifying gene expression and genetic variations.

- Discovery of Therapeutic Targets.
  - The identification of signature genes or biomarkers indicative of a disease process can identify candidate targets for therapeutic intervention

Applications of microarrays

- Predict drug/toxin activity.
  - Arrays assist in the identification of sentinel genes that demonstrate altered expression in a given cell or tissue type in response to drug or toxin exposure.
  - Creating profiles of sentinel genes associated with drugs sharing a common mechanism allows potential new therapies to be rapidly screened for similar activities. This facilitates the selection of compounds for further investigation and may reduce the need for animal testing. An in vitro screen for potential toxicity has the potential to reduce drug-

Applications of microarrays

- Determination of Pharmacologic Mechanism
  - Analysis of sentinel genes can assist in determining the mechanism of action of a drug or toxin.
  - Given that there is a multitude of events triggered by the initial action of a drug, screening thousands of genes at one time can identify multiple potential drug effectors.
  - This allows robust hypotheses of drug mechanism to be formed and tested in subsequent investigations.

Other Applications of microarrays

- Measuring transcript abundance (cDNA arrays)
- Genotyping
- Diagnosis
- Estimating DNA copy number
- Determining identity by descent
- Measuring mRNA decay rates
- Identifying protein binding sites
- The repertoire of genes expressed in a certain differentiation state
- The repertoire of genes expressed in a certain disease state
- The repertoire of genes expressed in a certain developmental stage