



Enzyme-based molecular techniques (part III)

Analysis of gene expression and RNA levels

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Analysis of gene expression

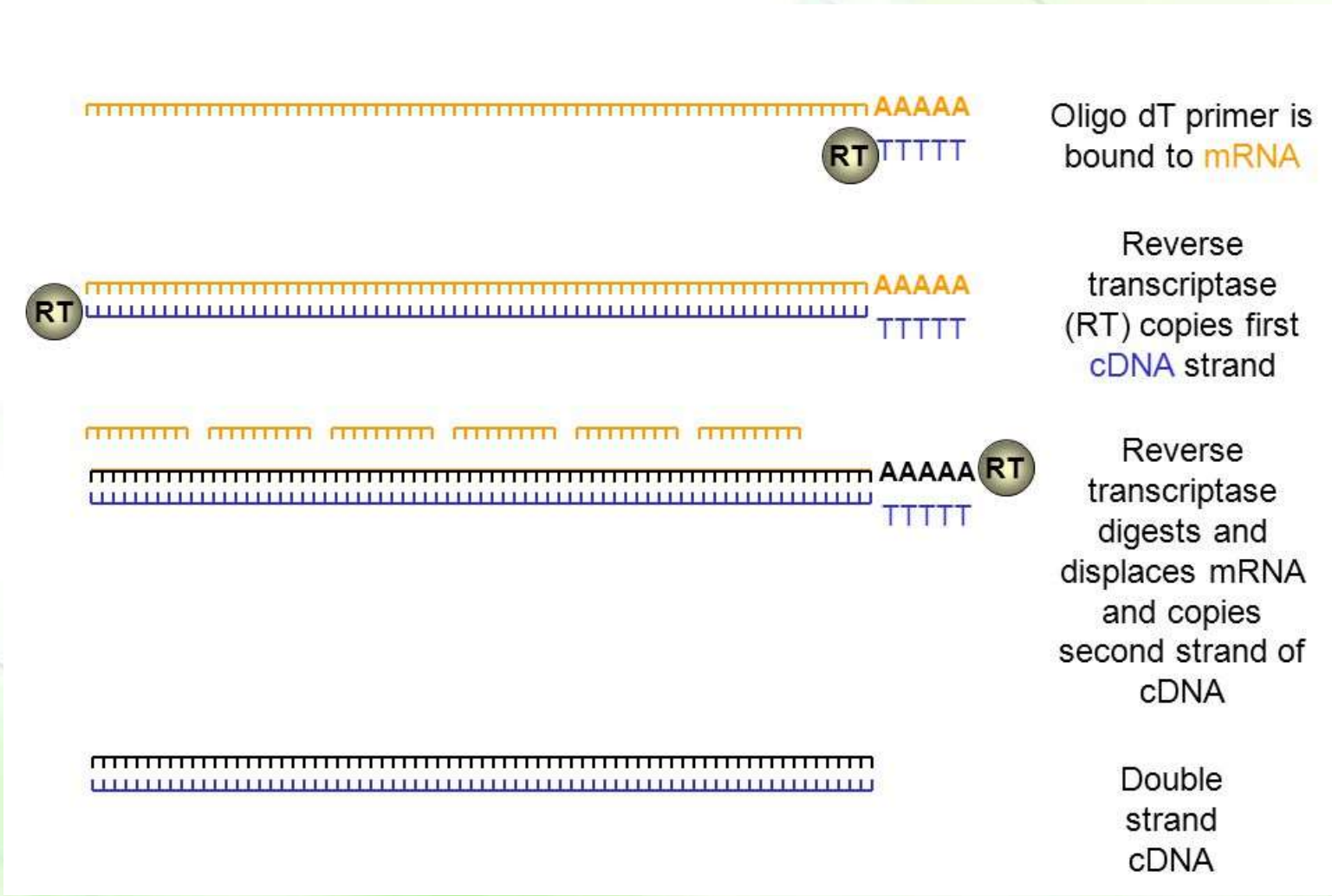
RNA level

Basic methods: Northern blotting, in situ hybridization

Advanced methods: real-time PCR, DNA microarray

Very advanced methods: RNA-seq

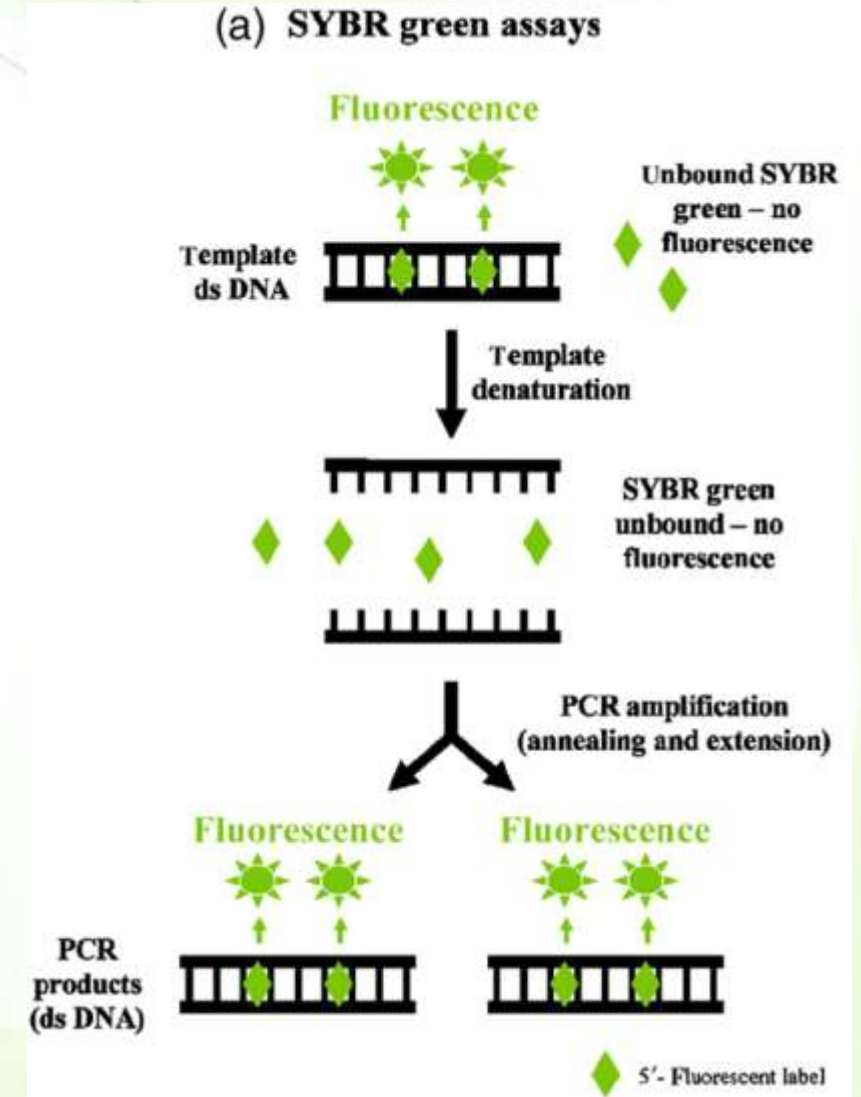
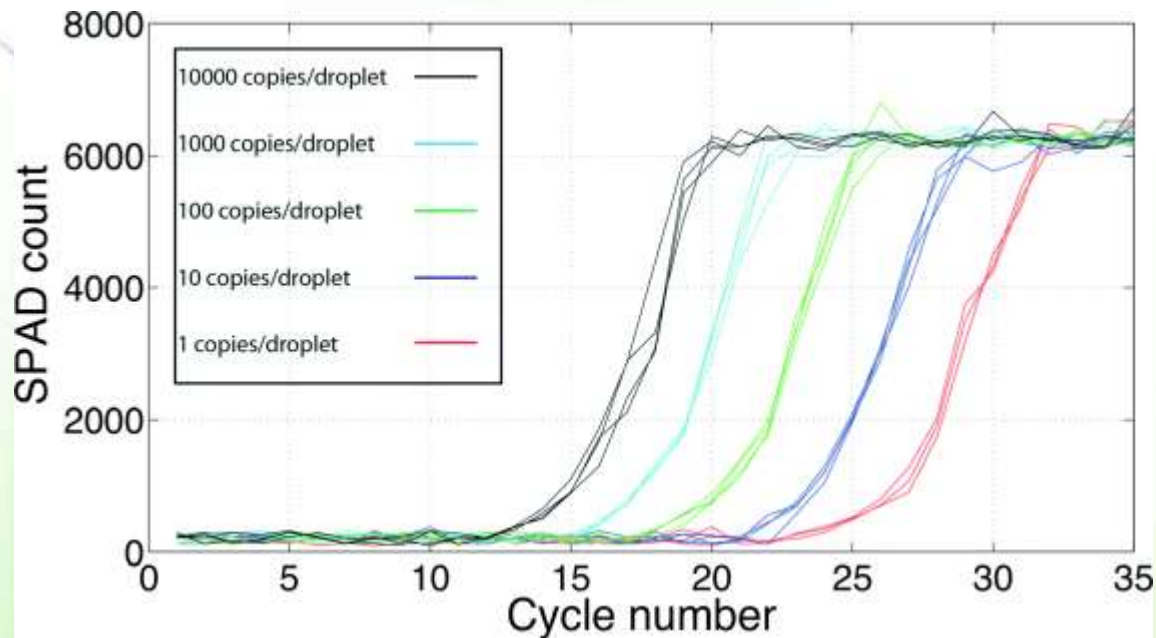
First make a complementary DNA (cDNA)



Quantitative reverse transcriptase real-time qPCR of mRNA



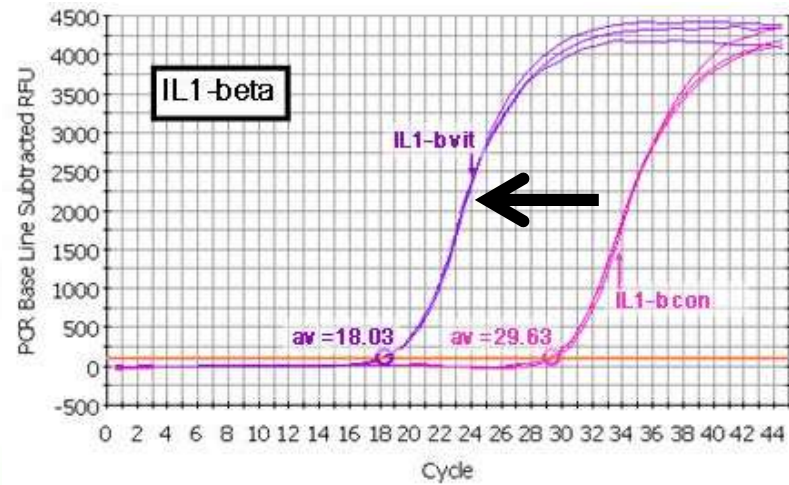
- Another way of relative quantitation of RNA expression is by converting RNA into cDNA followed by PCR in the presence of SYBR green.
- The higher the amount of RNA (cDNA), the sooner it is detected.



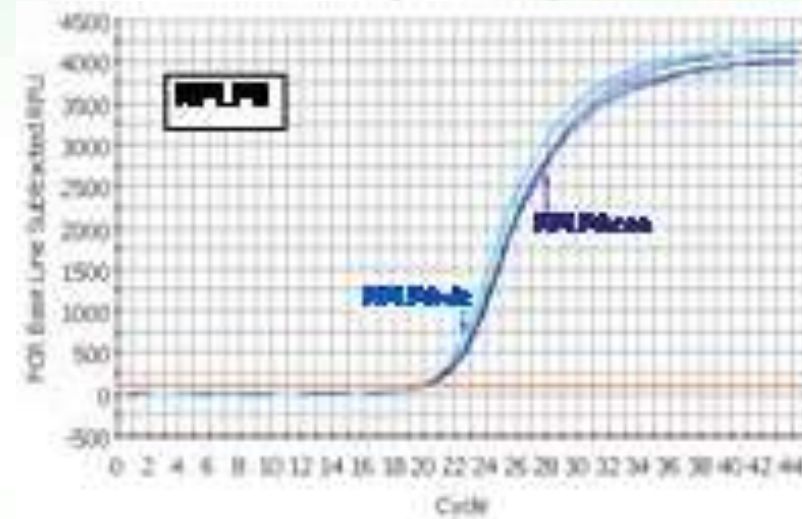
Example



A gene of interest



Housekeeping gene



Unaltered expression

Detection of SARS-Co-2



Tube with inactivated nasopharyngeal swabs



200 μ L aliquot



Automated-based extraction & one-step RT-qPCR

RNA extraction

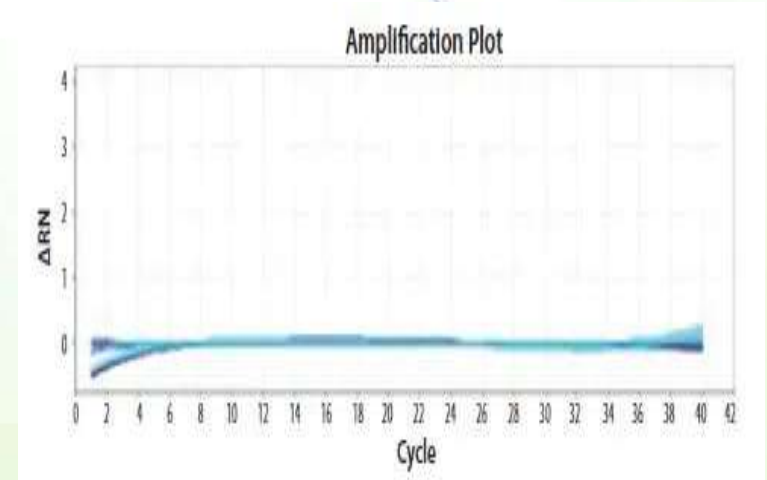
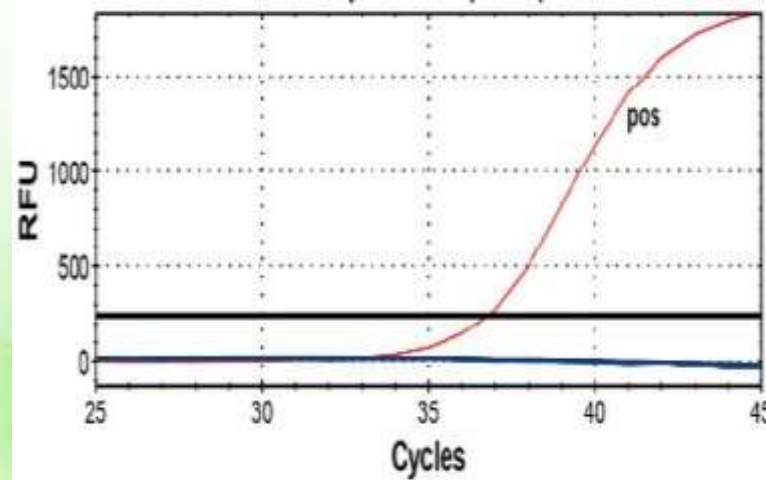
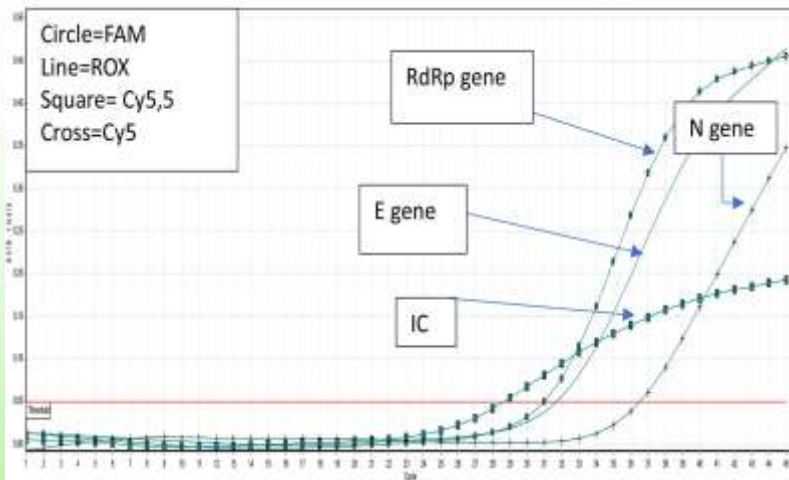


Viral RNA

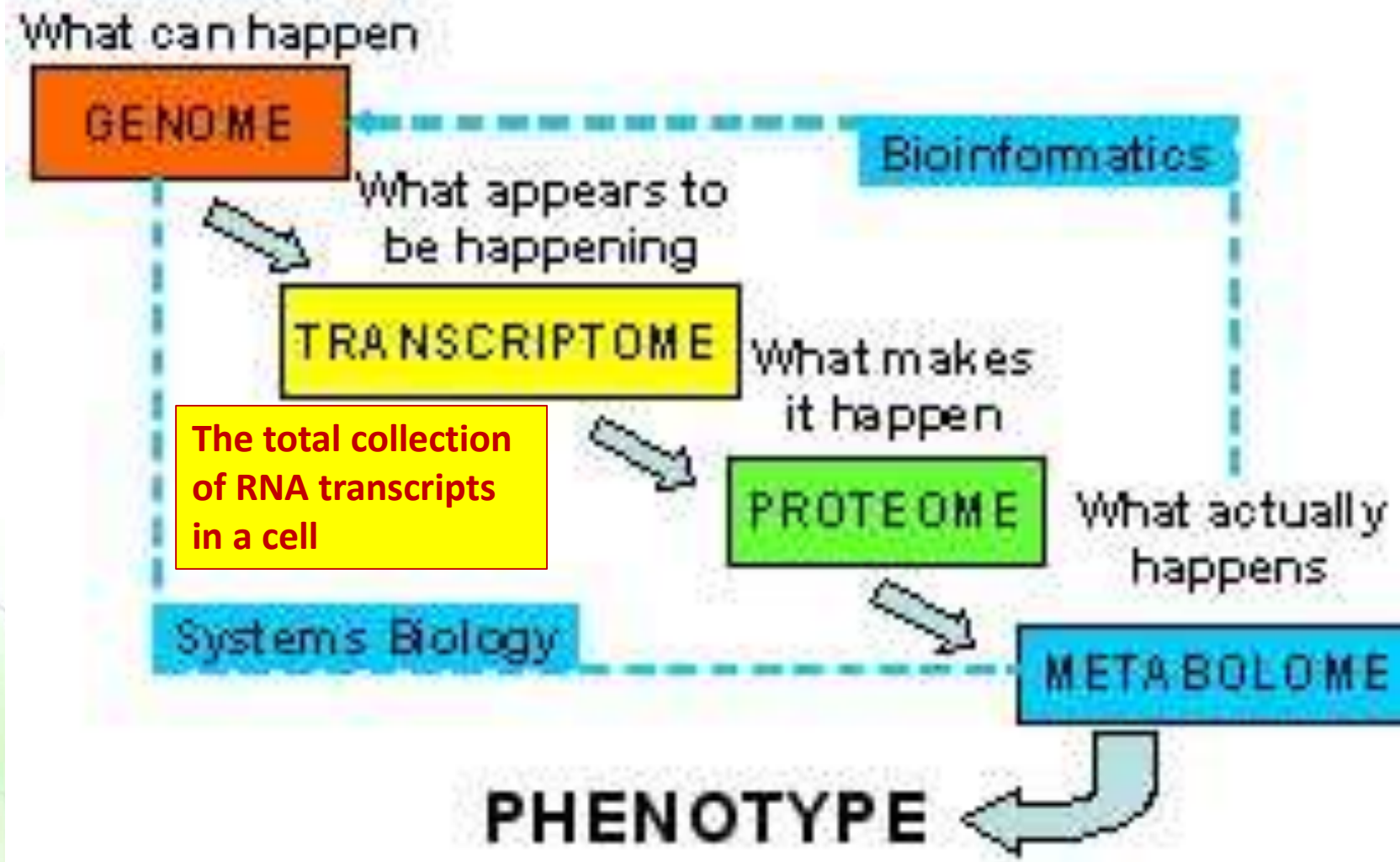
Amplified E-gene DNA



Quantitative detection of E-gene DNA*



The science of -omics



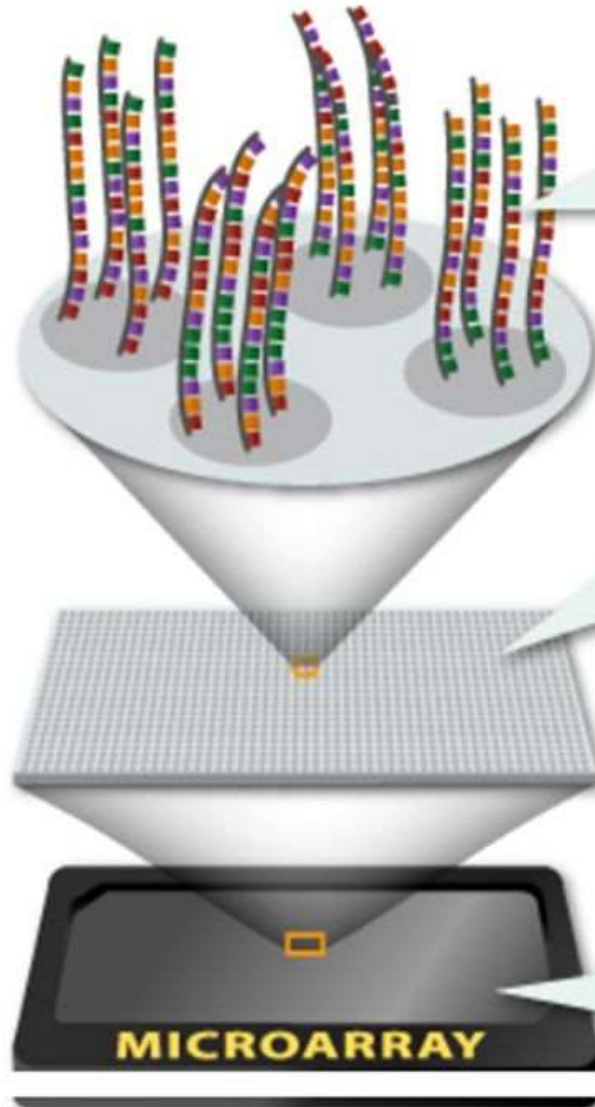
Studying the transcriptome



- One such method in studying transcriptomes is DNA microarrays, which allow the analysis of the RNA products of thousands of genes all at once.
- By examining the expression of so many genes simultaneously, we can understand gene expression patterns in physiological and pathological states.



- DNA microarrays are solid surfaces (glass microscope slides or chips) spotted with up to tens of thousands of DNA fragments in an area the size of a fingernail.
- The exact sequence and position of every DNA fragment on the array is known.
- <http://learn.genetics.utah.edu/content/labs/microarray/>
- <http://www.sumanasinc.com/webcontent/animations/content/dnachips.html>



A DNA microarray allows scientists to perform an experiment on thousands of genes at the same time.

Each spot on a microarray contains multiple identical strands of DNA.

The DNA sequence on each spot is unique.

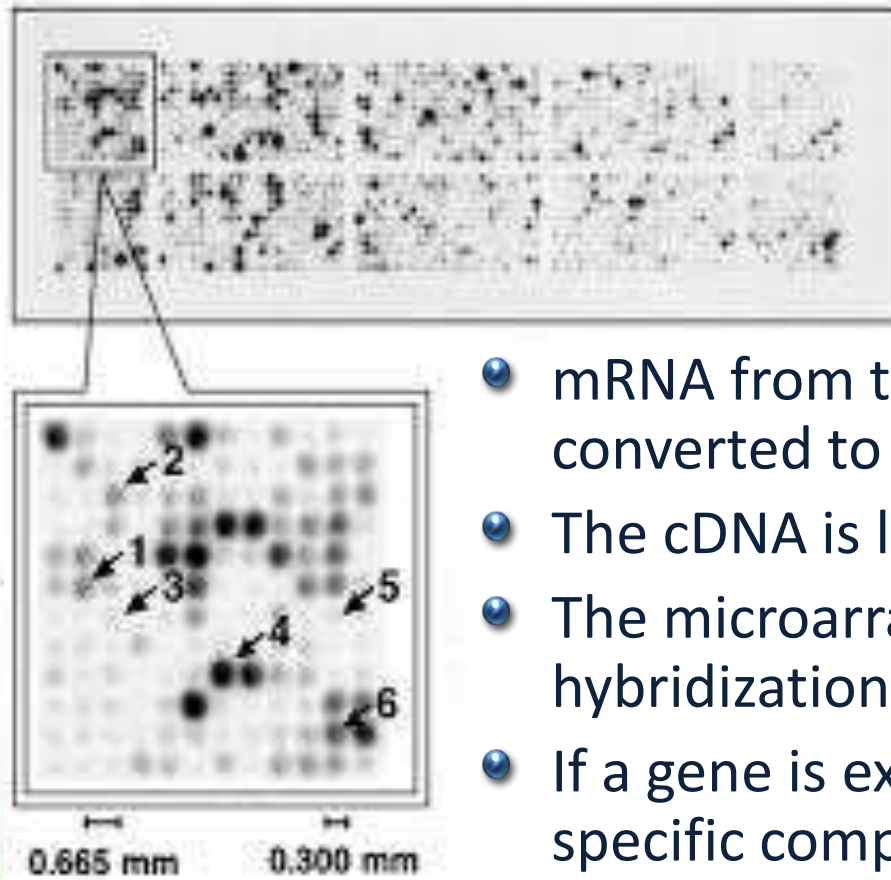
Each spot represents one gene.

Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass).

The precise location and sequence of each spot is recorded in a computer database.

Microarrays can be the size of a microscope slide, or even smaller.

A DNA microarray



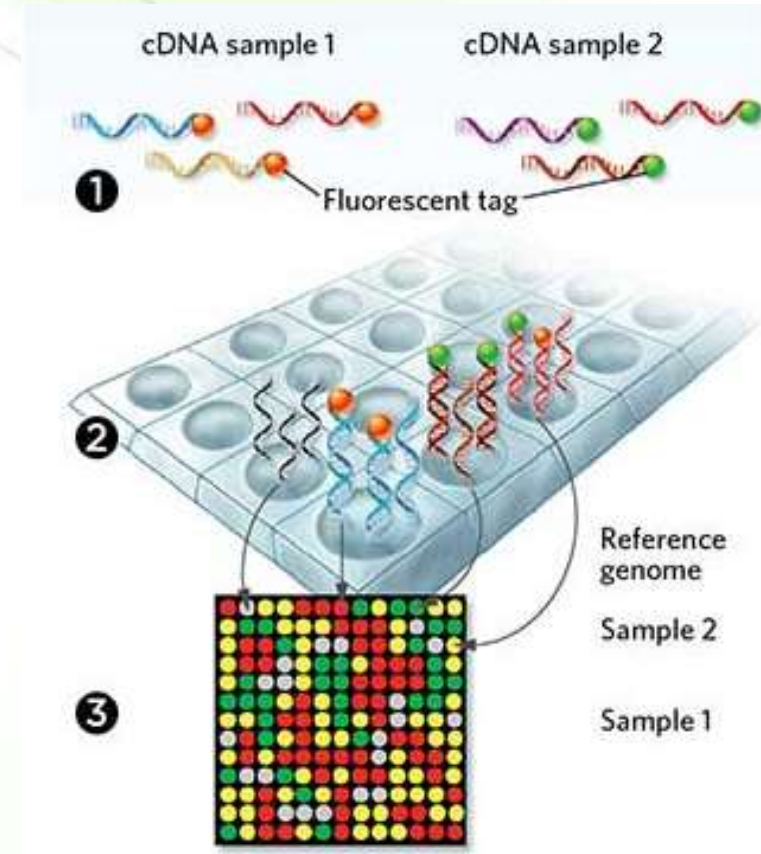
This is done for a single sample using radioactively labeled cDNA.

- mRNA from the cells being studied is first extracted and converted to cDNA.
- The cDNA is labeled with a radioactive probe.
- The microarray is incubated with the labeled cDNA sample for hybridization to occur.
- If a gene is expressed, then the cDNA will exist and bind to a specific complementary DNA fragment on the microarray.
- Binding can be detected since the cDNA is labeled and expression is determined.

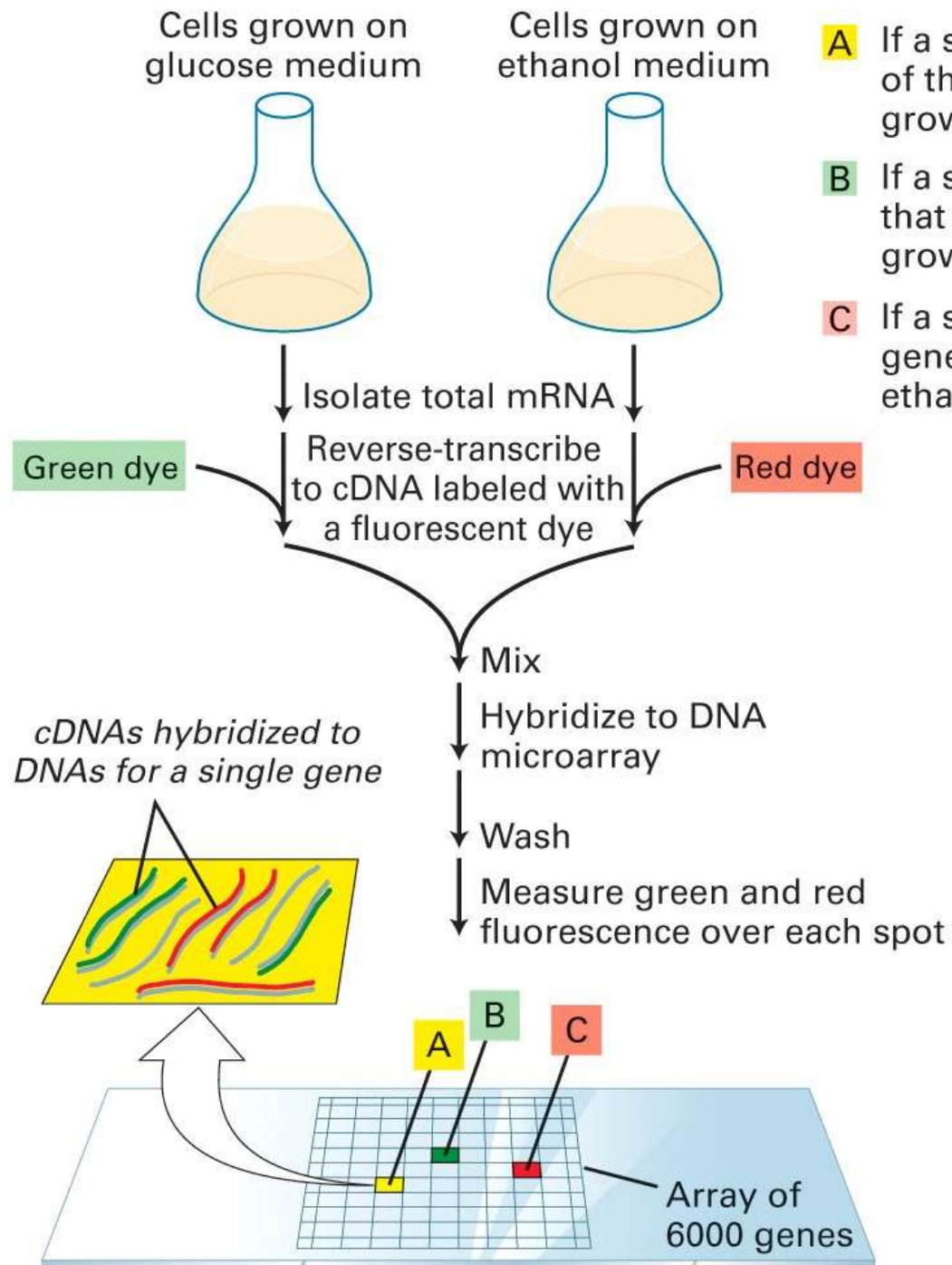
Comparative expression



- In order to compare expression of genes two different samples, the cDNA molecules are fluorescently labeled with different colors (green and red) and added to the array.
- An increase in the amount of a RNA molecule in one sample versus the other is reflected by an increase in the amount of produced cDNA and an increase in fluorescence in the bound spot.



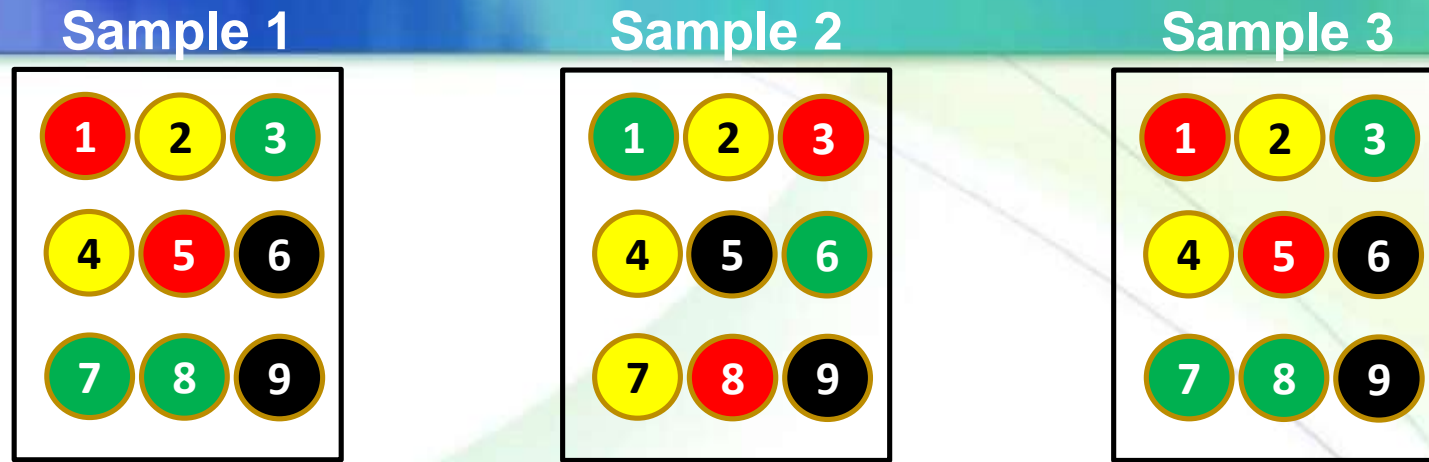
- ● Not expressed
- Sample 1 > sample 2
- Sample 2 > sample 1
- Sample 2 = sample 1



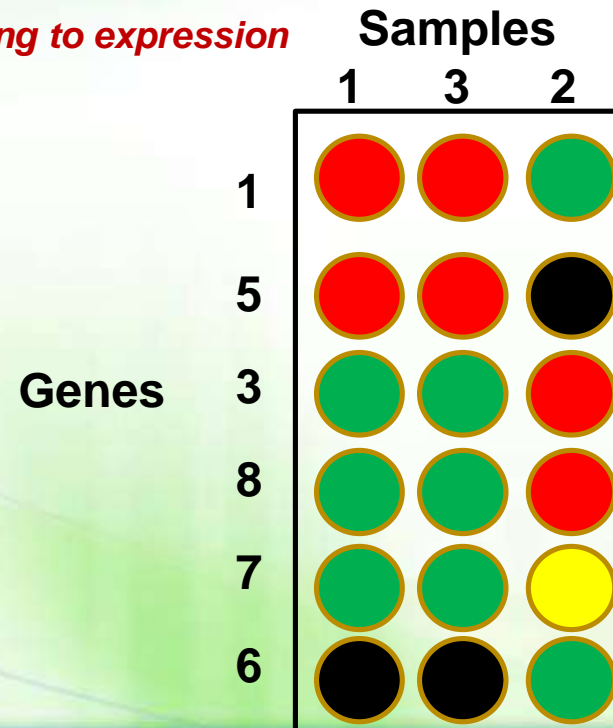
A If a spot is yellow, expression of that gene is the same in cells grown either on glucose or ethanol

B If a spot is green, expression of that gene is greater in cells grown in glucose

C If a spot is red, expression of that gene is greater in cells grown in ethanol

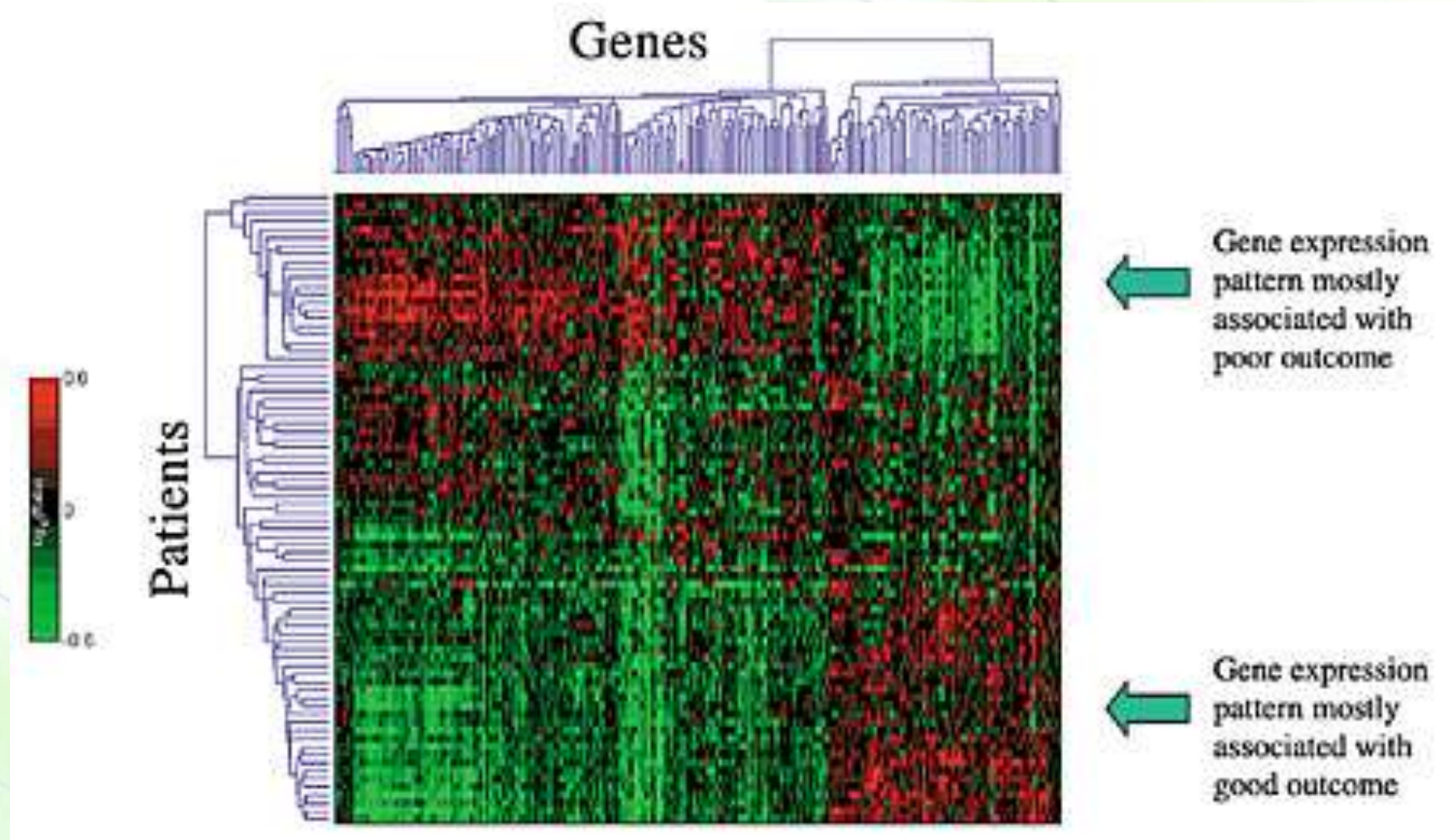


Combine results
Eliminate genes 2, 4, & 9
Cluster samples according to expression



This can be done at a larger scale whereby samples are compared to the same control sample and a computer program combines all data illustrating differences in expression among the samples and classifying them into different groups.

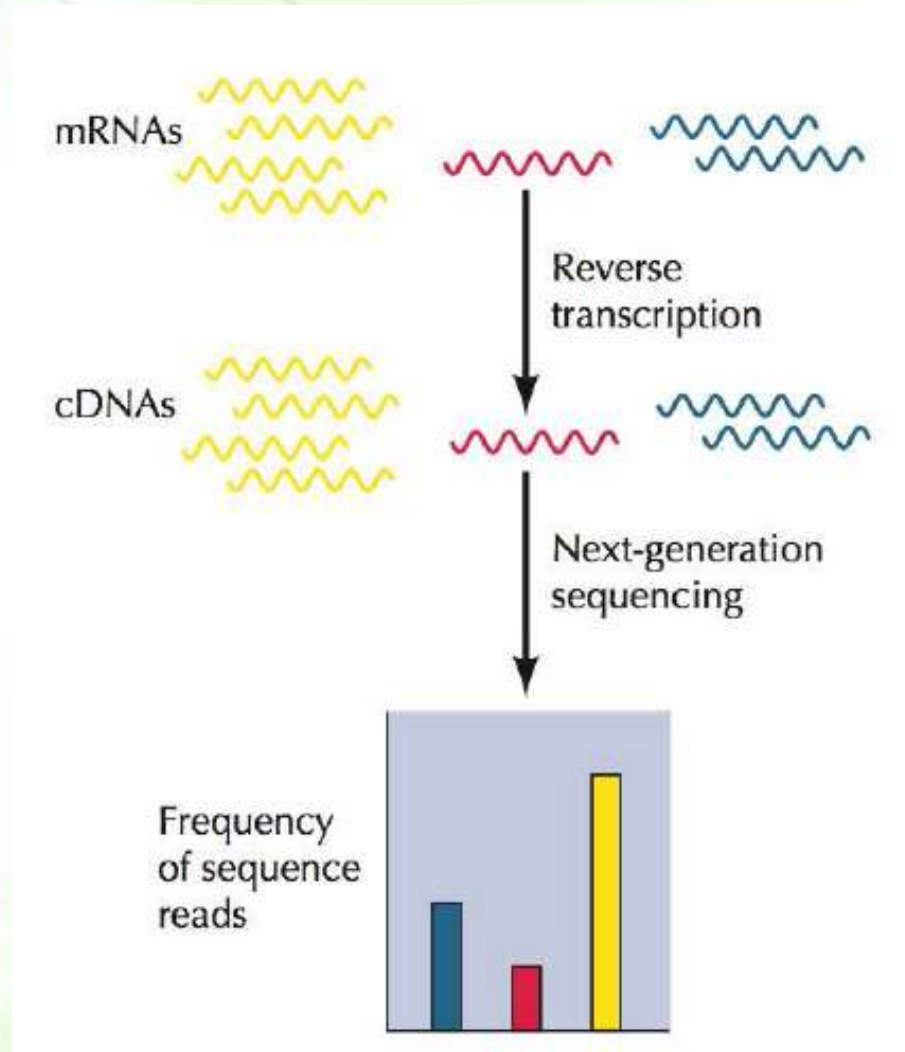
DNA microarrays and breast cancer



RNA sequencing (RNA-seq)



- Cellular RNA is reverse transcribed to cDNAs, which are subjected to next-generation sequencing.
- The relative amount of each cDNA (mRNA) is indicated by the frequency at which its sequence is represented in the total number of sequences read.



RNA-seq vs. microarray



- RNA-seq can be used to
 - characterize novel transcripts
 - Identify splicing variants
 - profile the expression levels of all transcripts
- Microarrays are limited to detect transcripts corresponding to known genomic sequences.