



Molecular Biology (8)

Transcription-related techniques

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Resources



- This lecture
- Cooper, pp. 124--132

How do different cells appear?

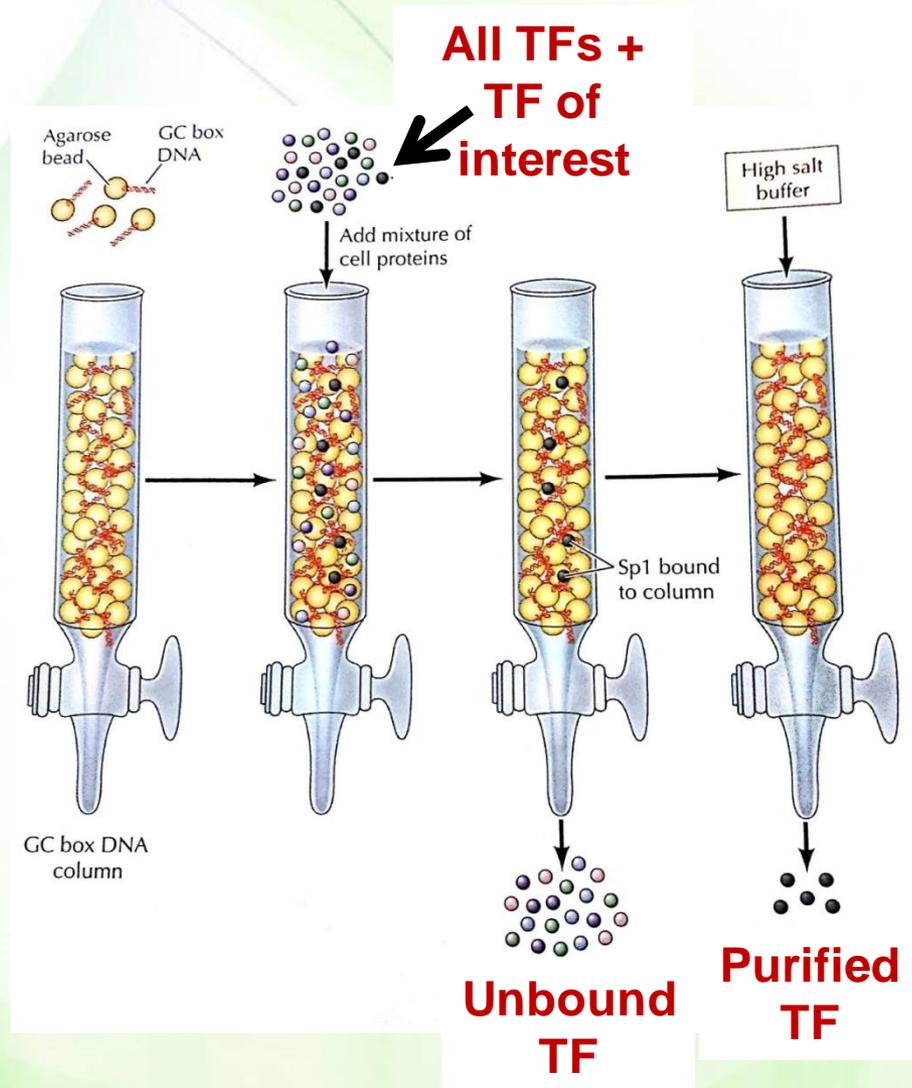
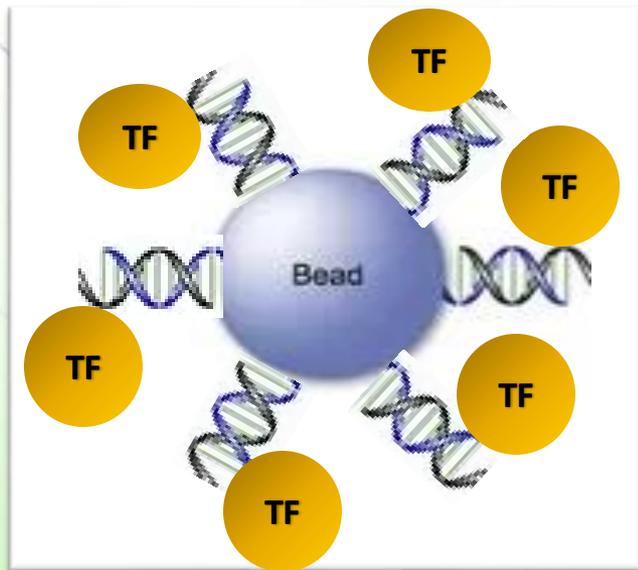


- All cells in one body have the same genome, but cell types in a multicellular organism become different from one another because they synthesize different sets of RNA and protein molecules.
- The patterns of mRNA can differentiate cell types from each other.
- How can we investigate RNA expression and function?

Isolation of transcription factors using affinity chromatography



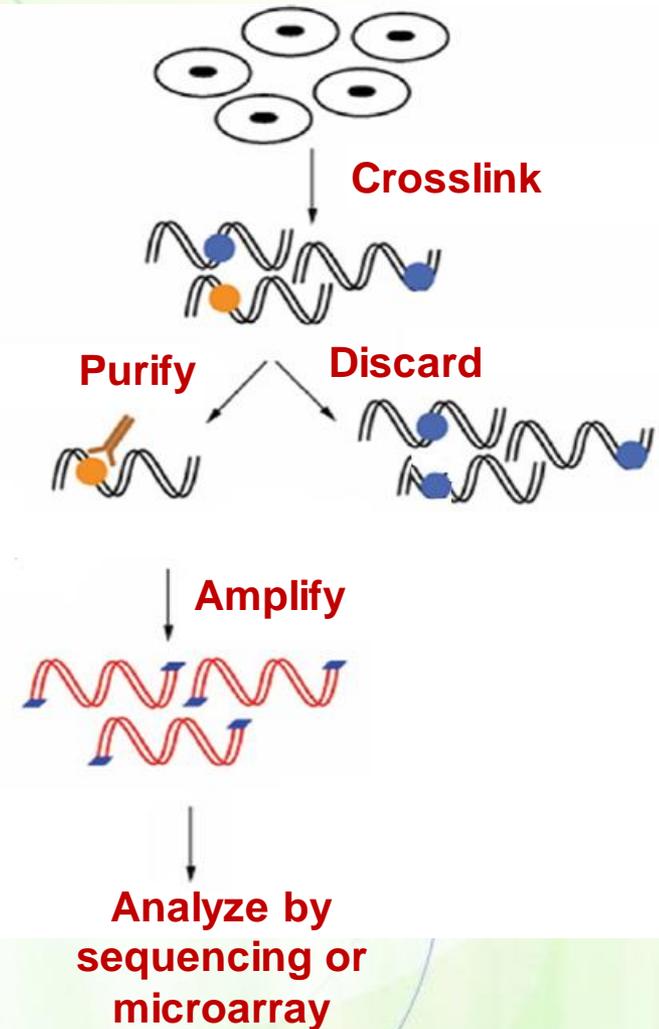
- Attach a DNA fragment with a specific sequence to a bead.
- Pass through nuclear fraction of a cell.
- Elute the bound proteins.



Chromatin immunoprecipitation



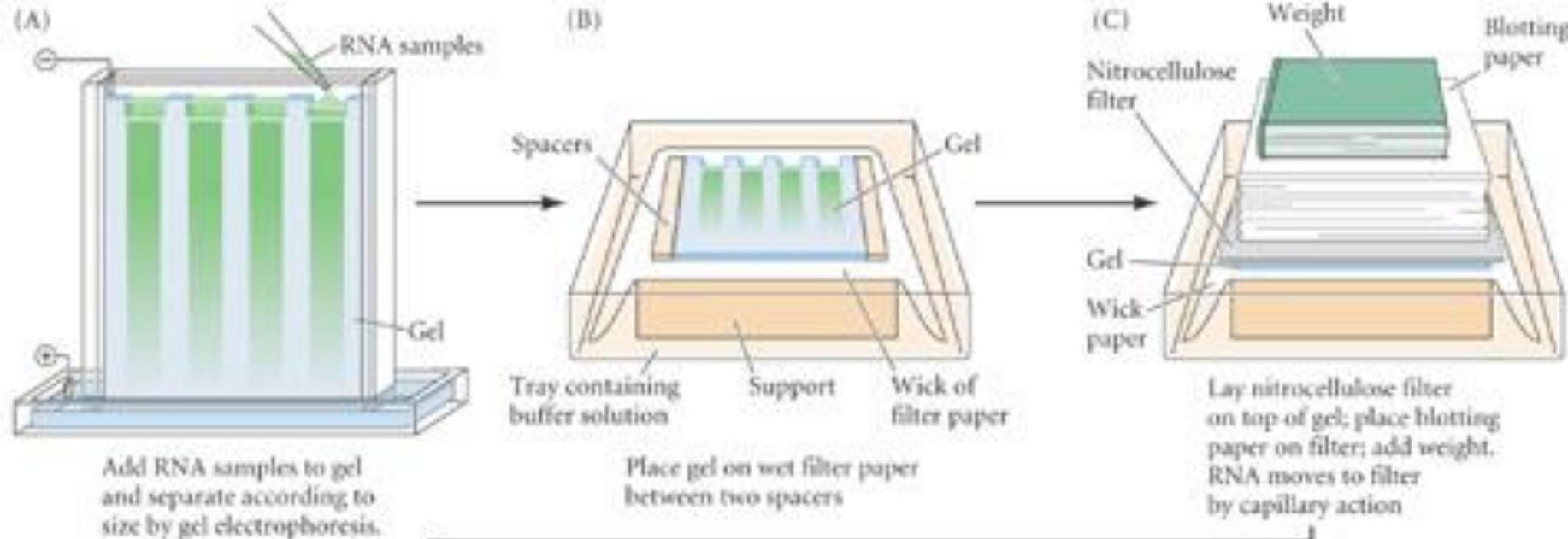
- Purpose: identify the DNA sequence to which a transcription factor binds.
- Crosslink proteins bound to DNA.
- Fragments the DNA into smaller pieces.
- Isolate a specific transcription factor by immunoprecipitation.
- Sequence the purified DNA fragment or identify by DNA microarray.



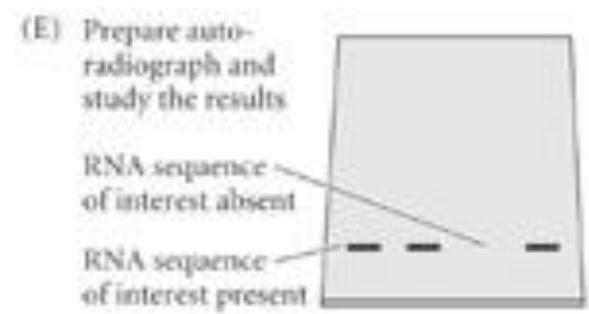
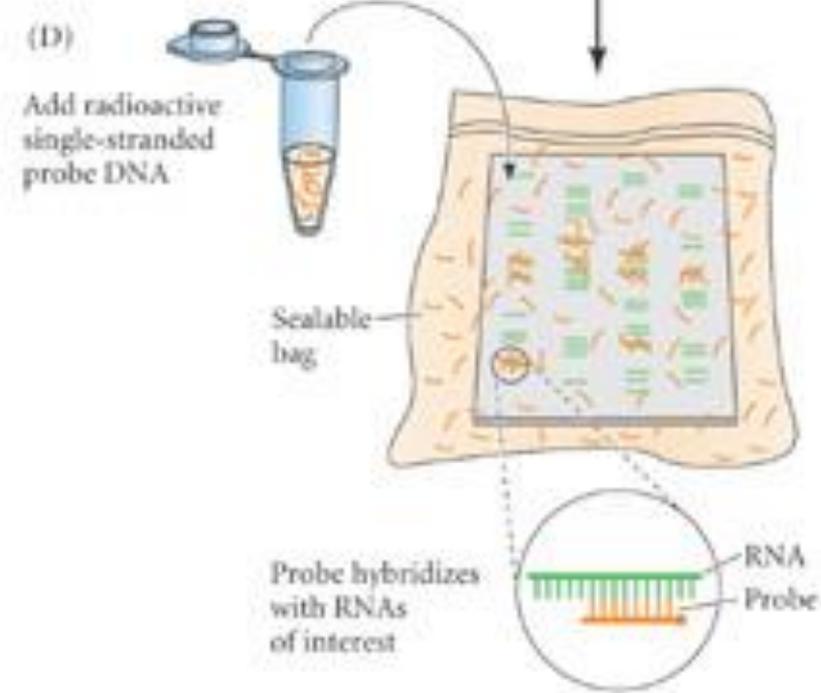
Northern blotting

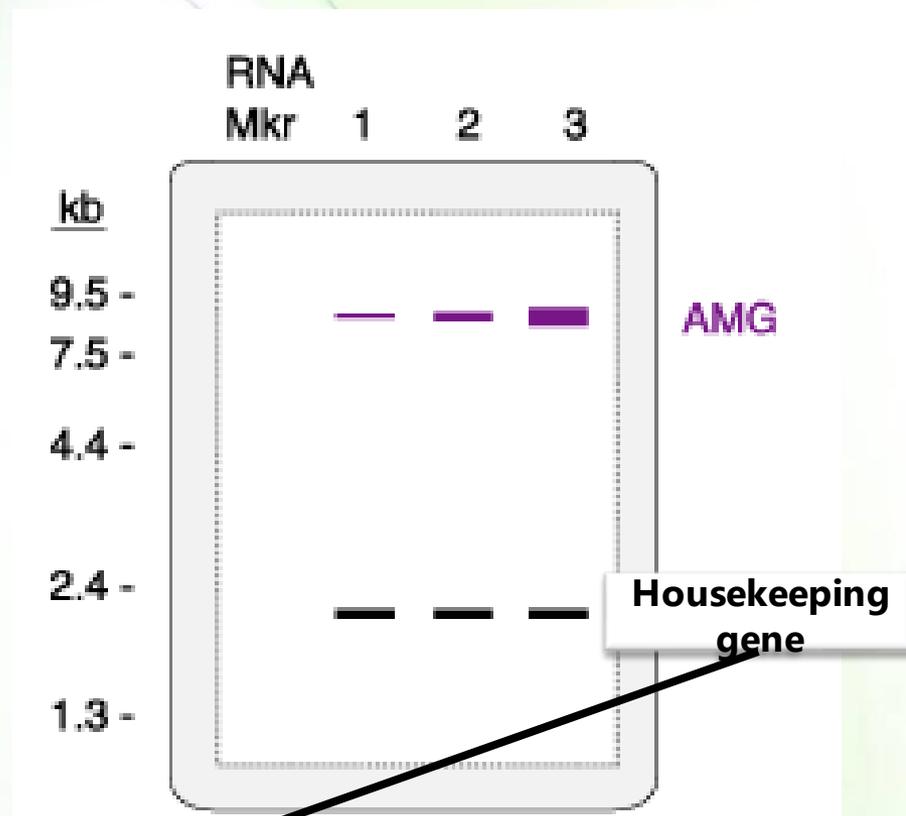
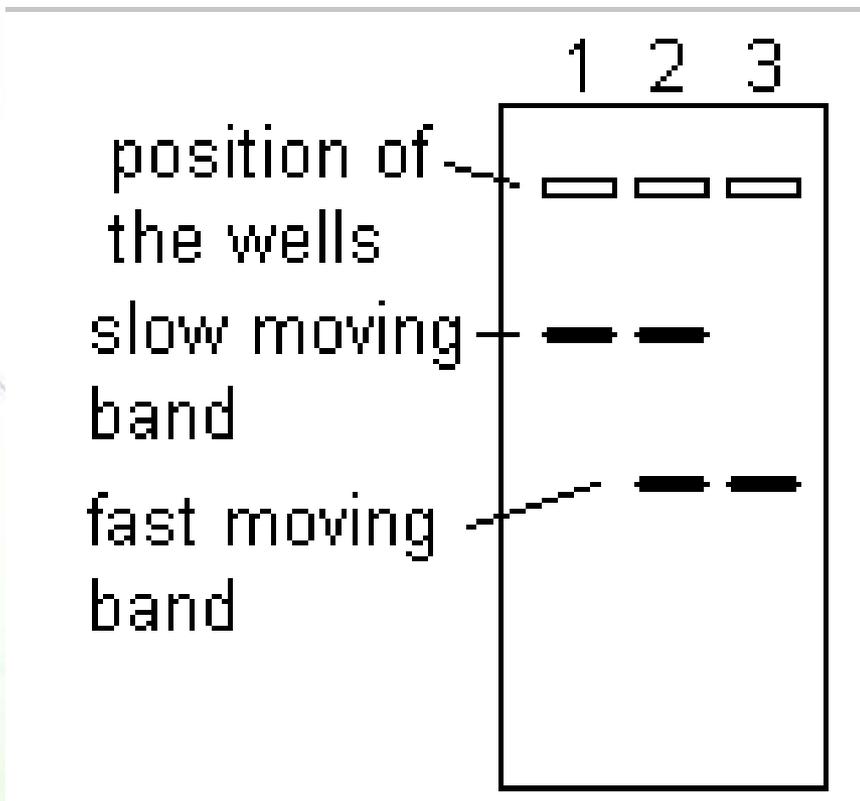


- This is done exactly like Southern blotting except that RNA from cells is isolated instead of DNA.
- RNA molecules are fractionated based on size by gel electrophoresis.
- The fractionated RNA molecules are transferred onto a membrane.
- RNA molecules are targeted by a labeled DNA probe with sequence that is complementary to a specific RNA molecule.
- **What information can you deduce from it?**



Lay nitrocellulose filter on top of gel; place blotting paper on filter; add weight. RNA moves to filter by capillary action





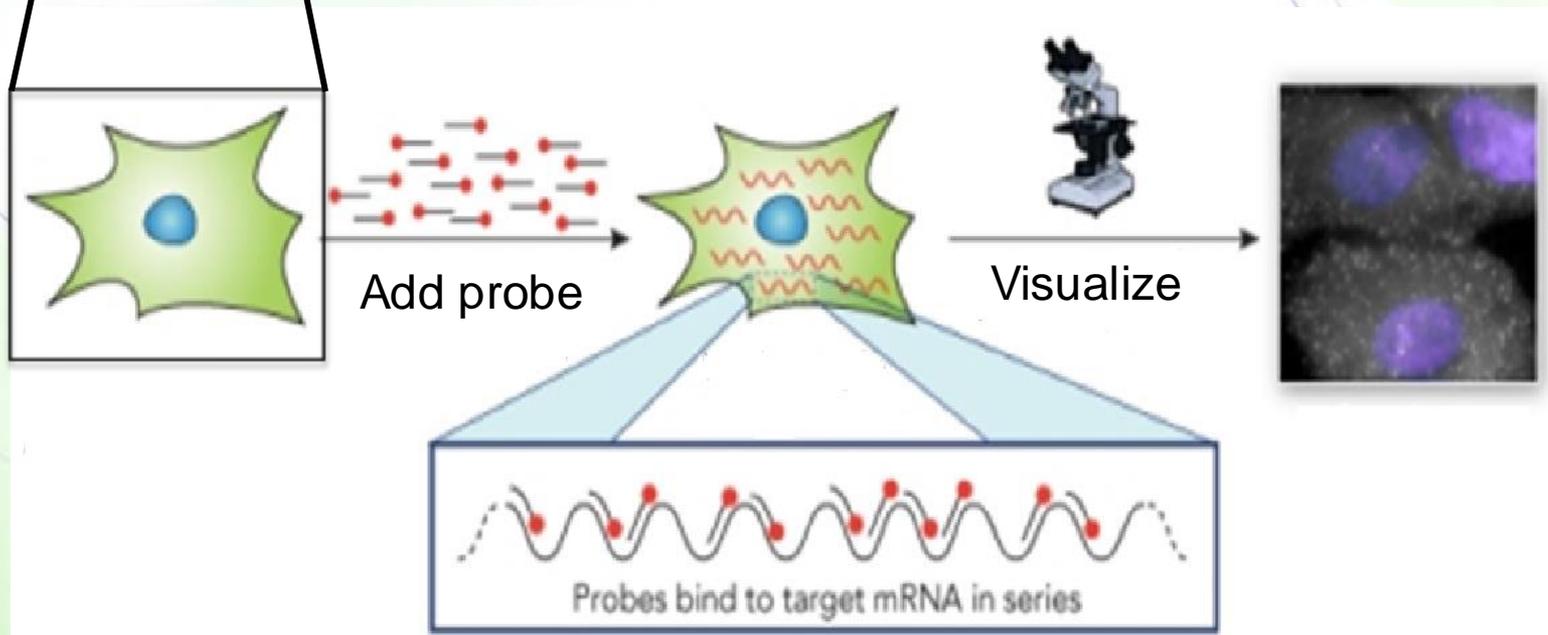
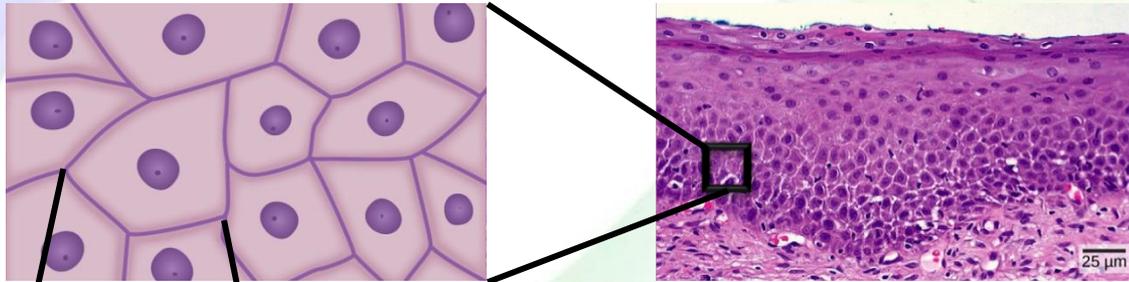
A gene with constant expression
(examples: actin, tubulin)

In situ hybridization

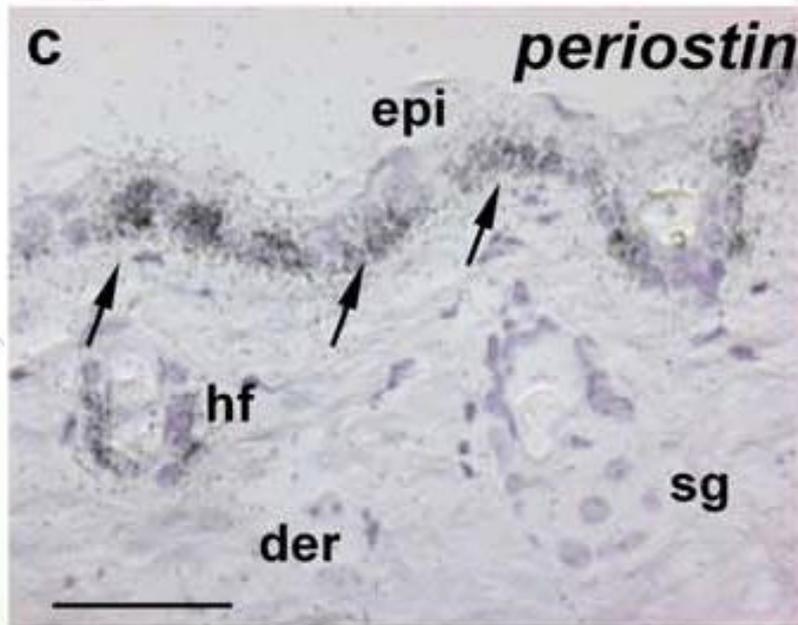


- In situ hybridization methods reveals the distribution of specific RNA molecules in cells in tissues.
- RNA molecules can hybridize when the tissue is incubated with a complementary DNA or RNA probe.
- In this way the patterns of differential gene expression can be observed in tissues, and the location of specific RNAs can be determined in cells.

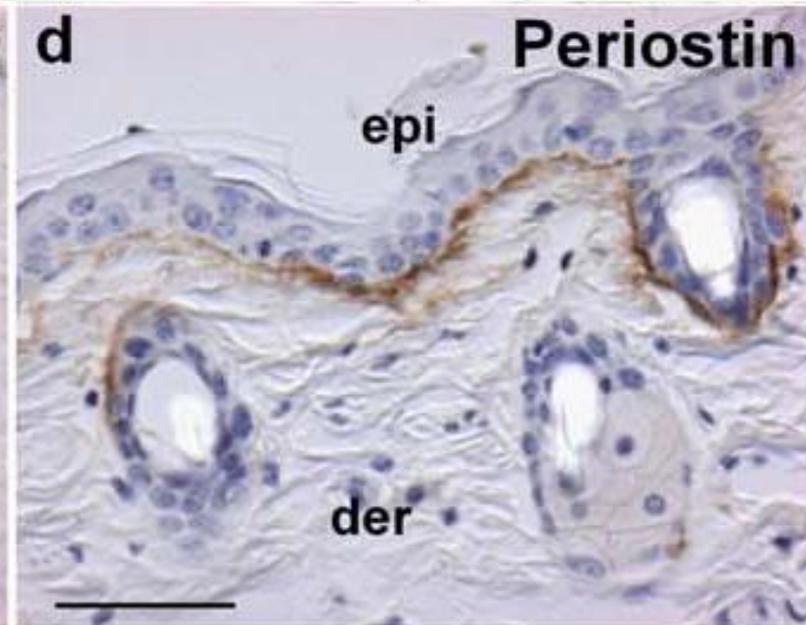
Procedure of in situ hybridization



ISH (RNA)



IHC (protein)

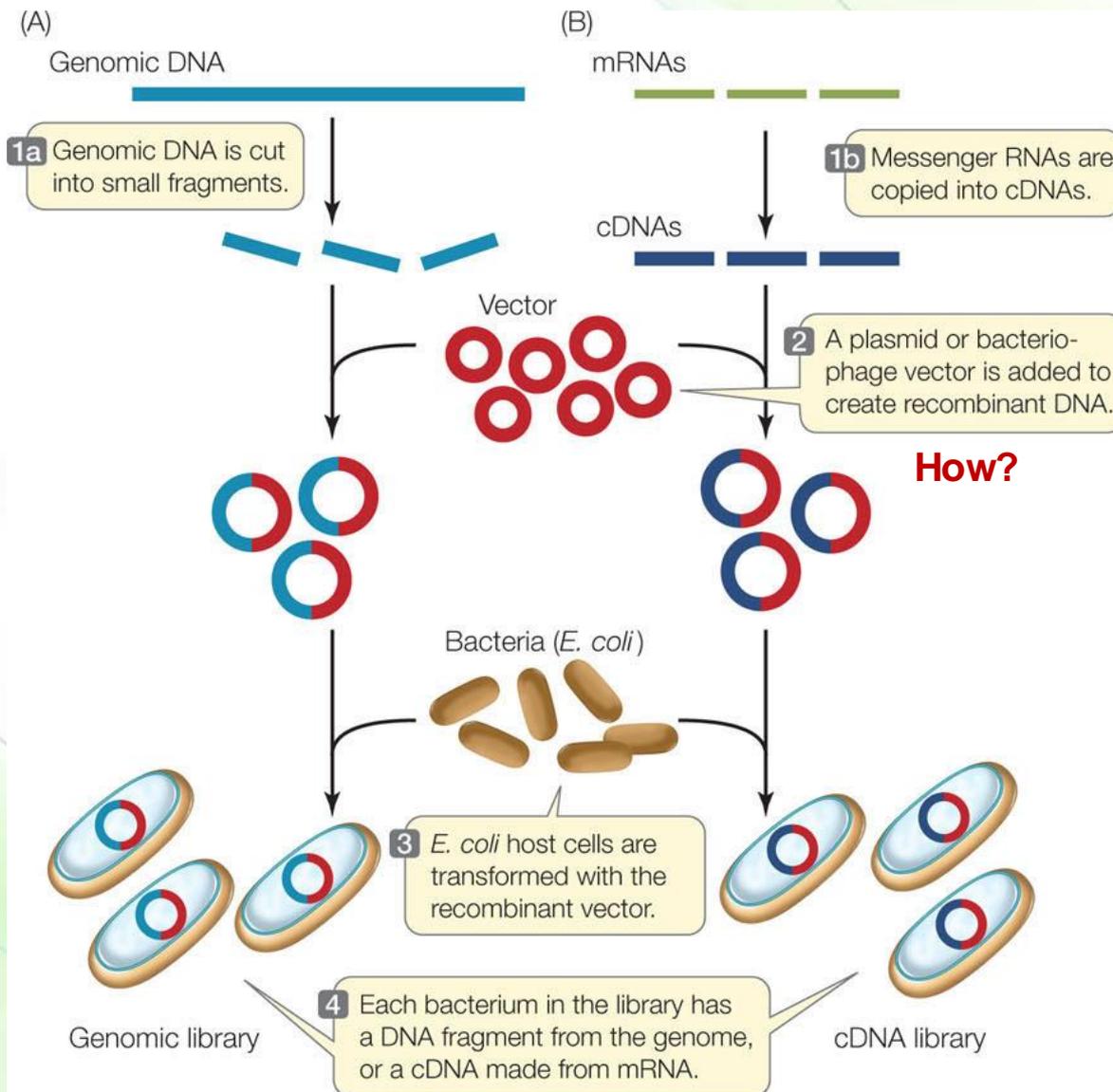


What is a DNA library?

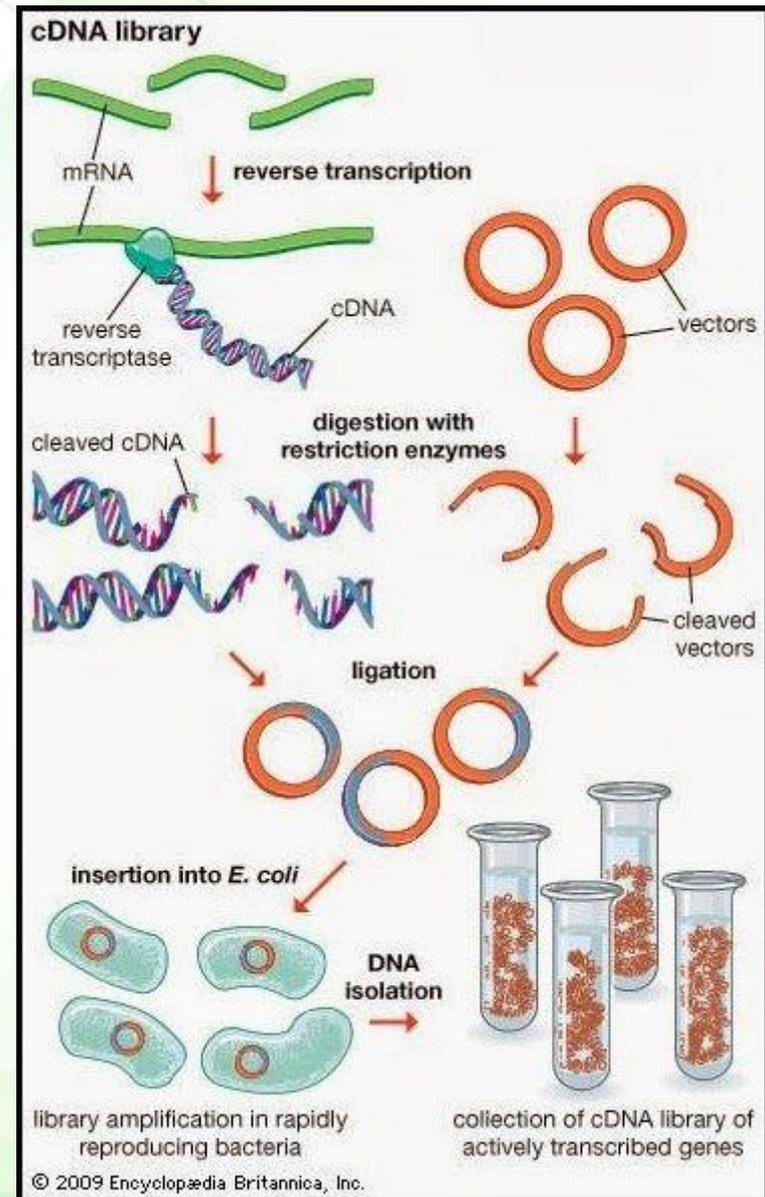
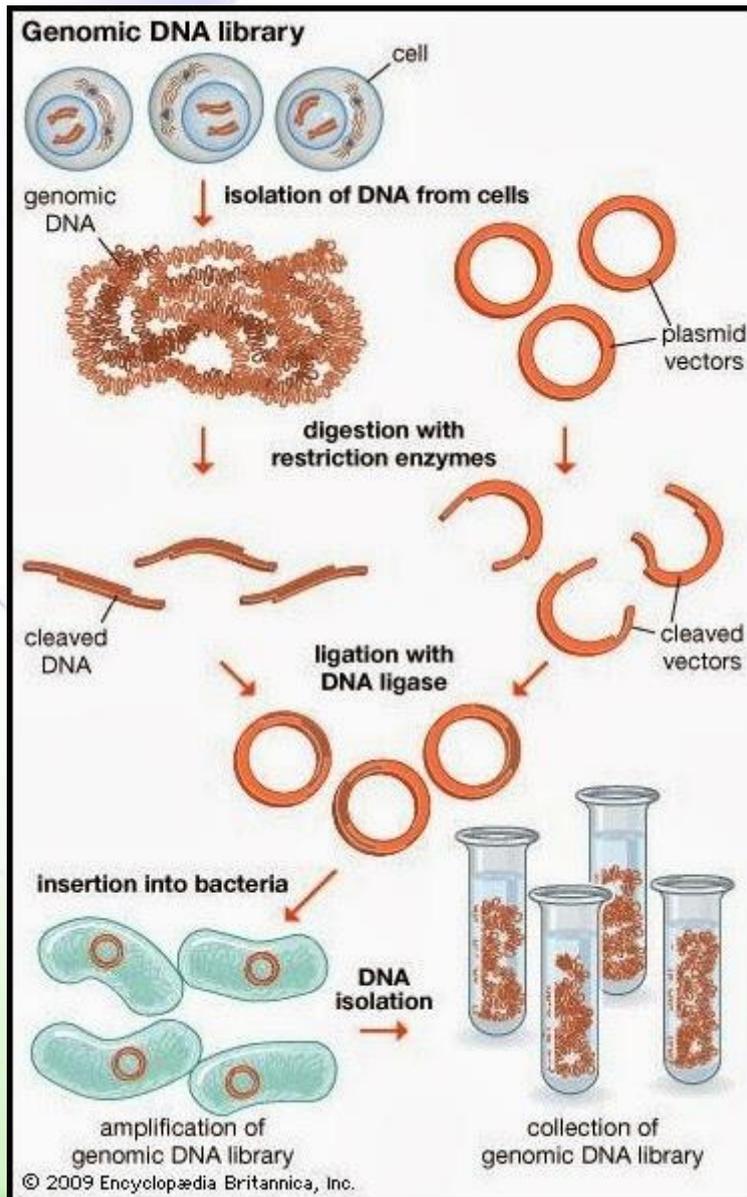


- A library can be created for DNA fragments just like book libraries.
- You can have clones of bacteria each containing a specific piece of DNA.
- You can save these clones in the freezer and take whichever clone you want to study.
- <http://www.sumanasinc.com/webcontent/animations/content/dnalibrary.html>

Genomic vs. cDNA library



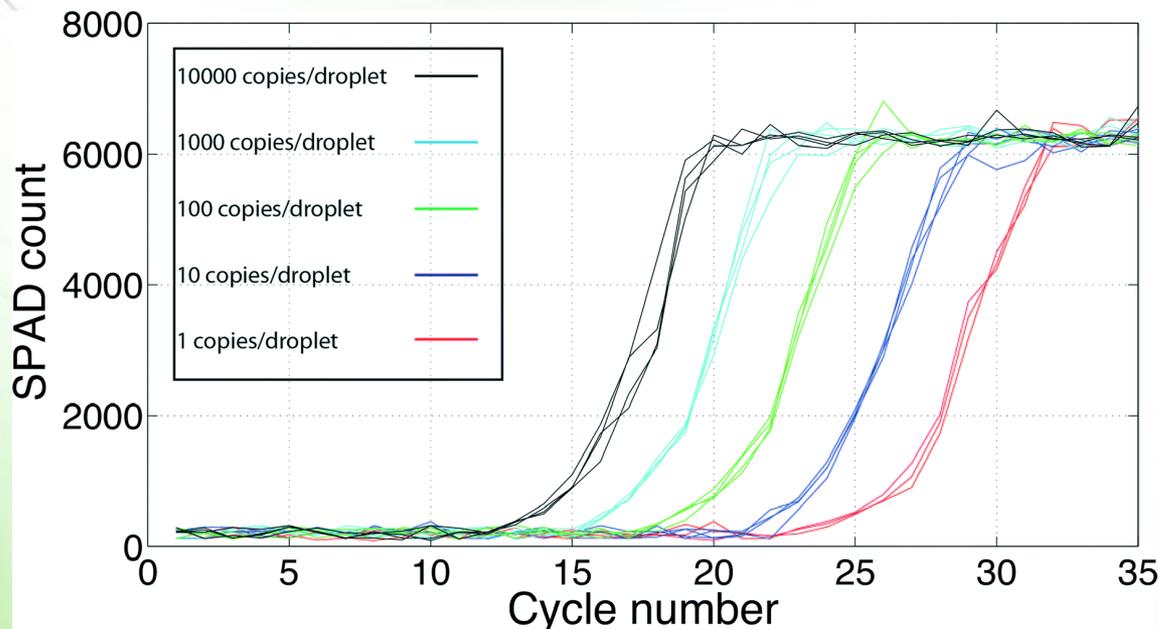
Genomic vs. cDNA library



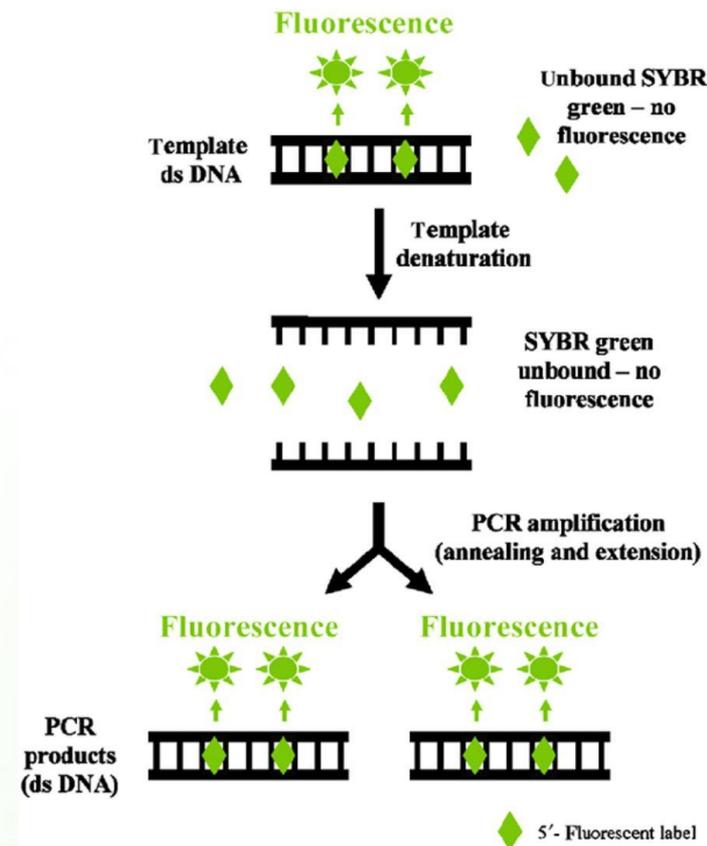
Quantitative real-time PCR of mRNA



- 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.



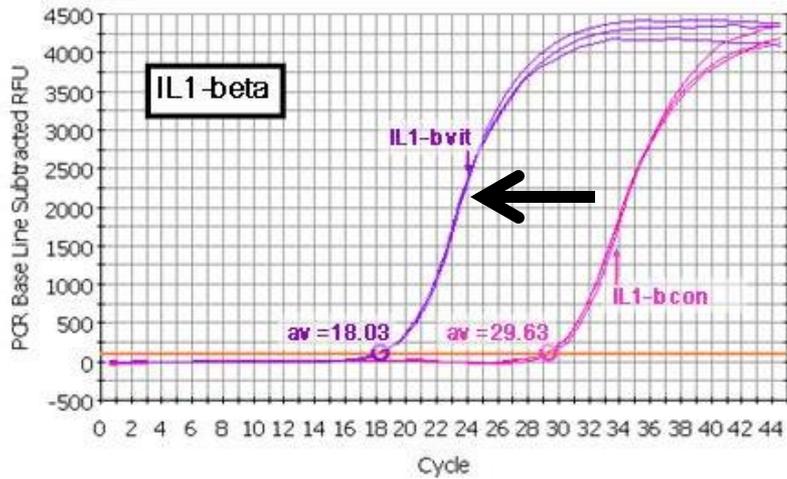
(a) SYBR green assays



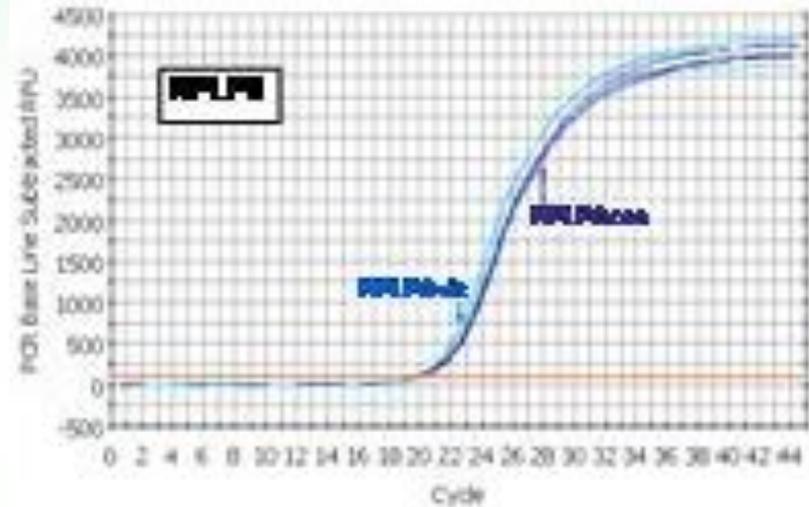
Example



A gene of interest

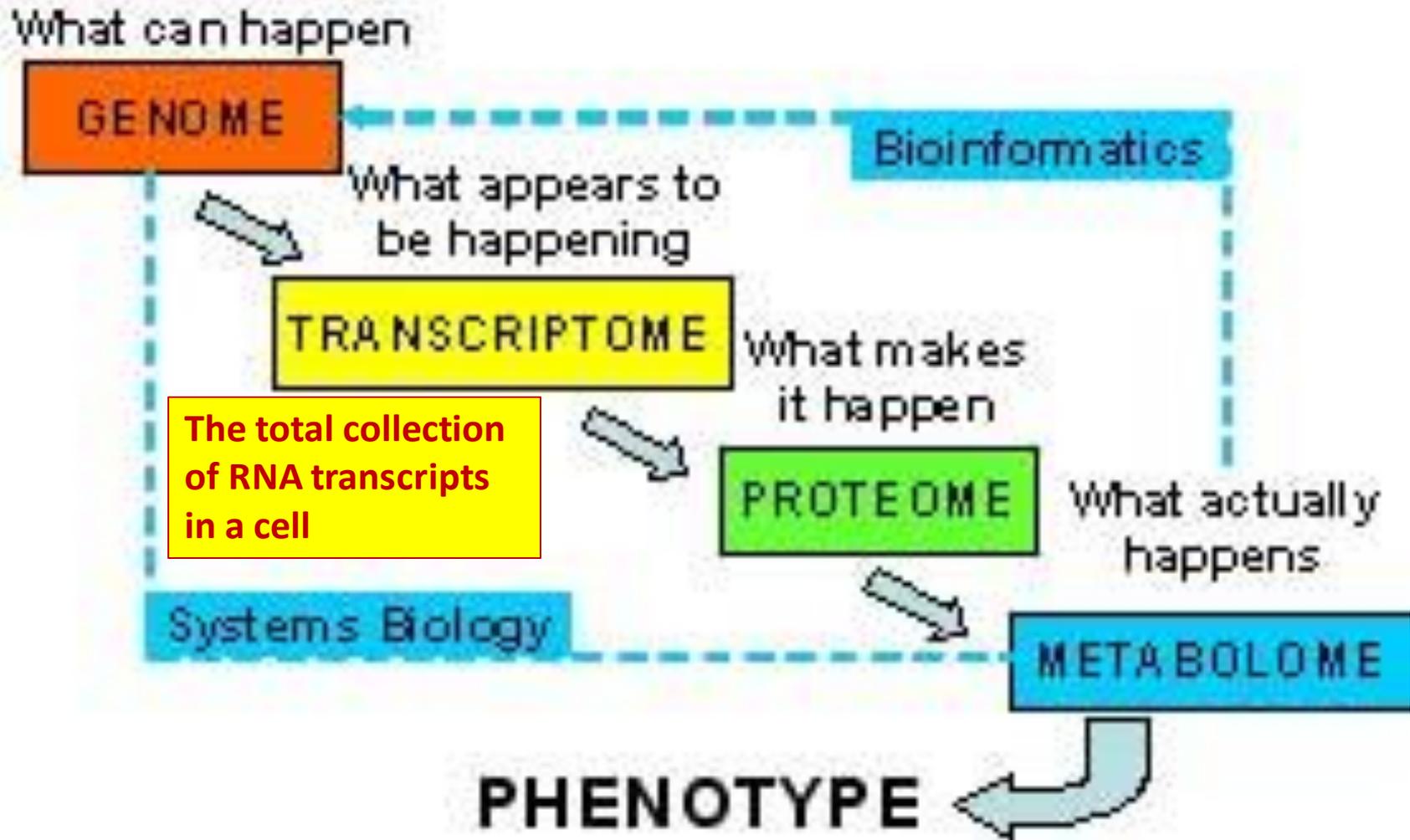


Housekeeping gene



Unaltered expression

The science of -omics



Studying the transcriptome

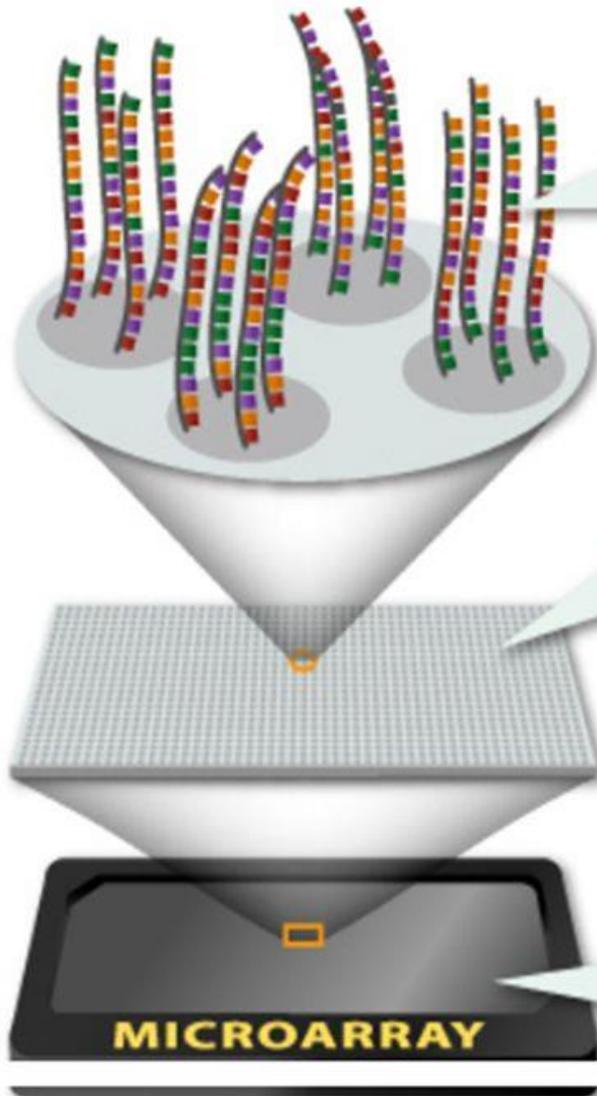


- One such method in studying transcriptomes is DNA microarrays, which allow the analysis of the RNA products of thousands of genes all at once.
- By examining the expression of so many genes simultaneously, we can understand gene expression patterns in physiological and pathological states.

DNA microarrays



- DNA microarrays are glass microscope slides 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.

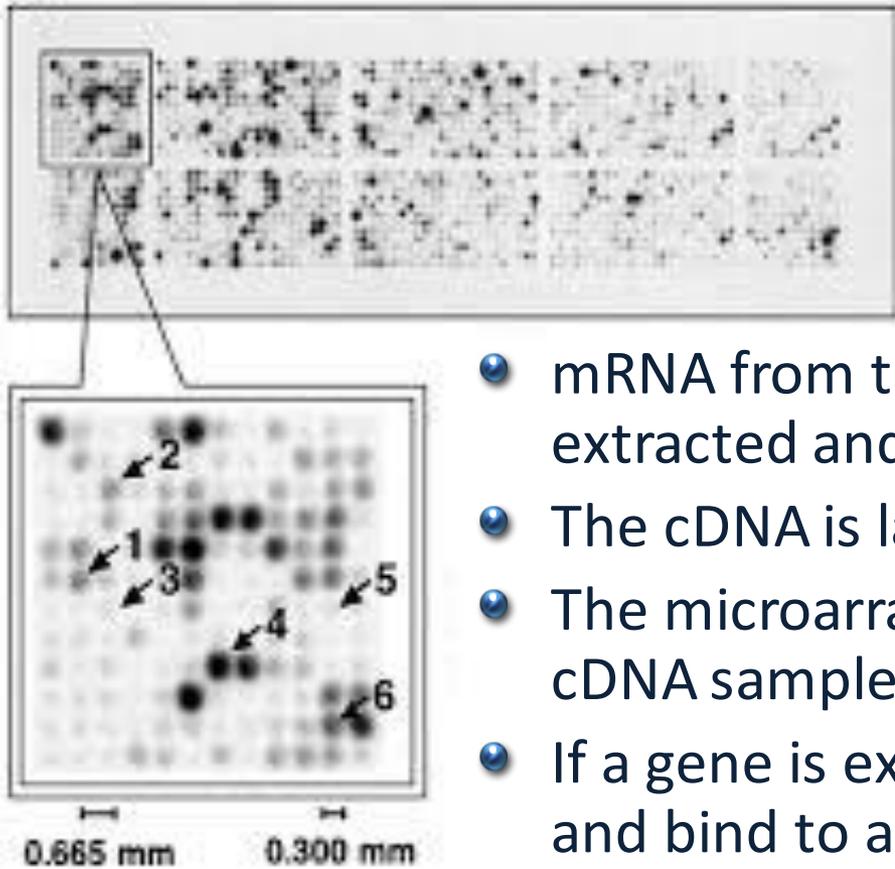
Each spot represents one gene.

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



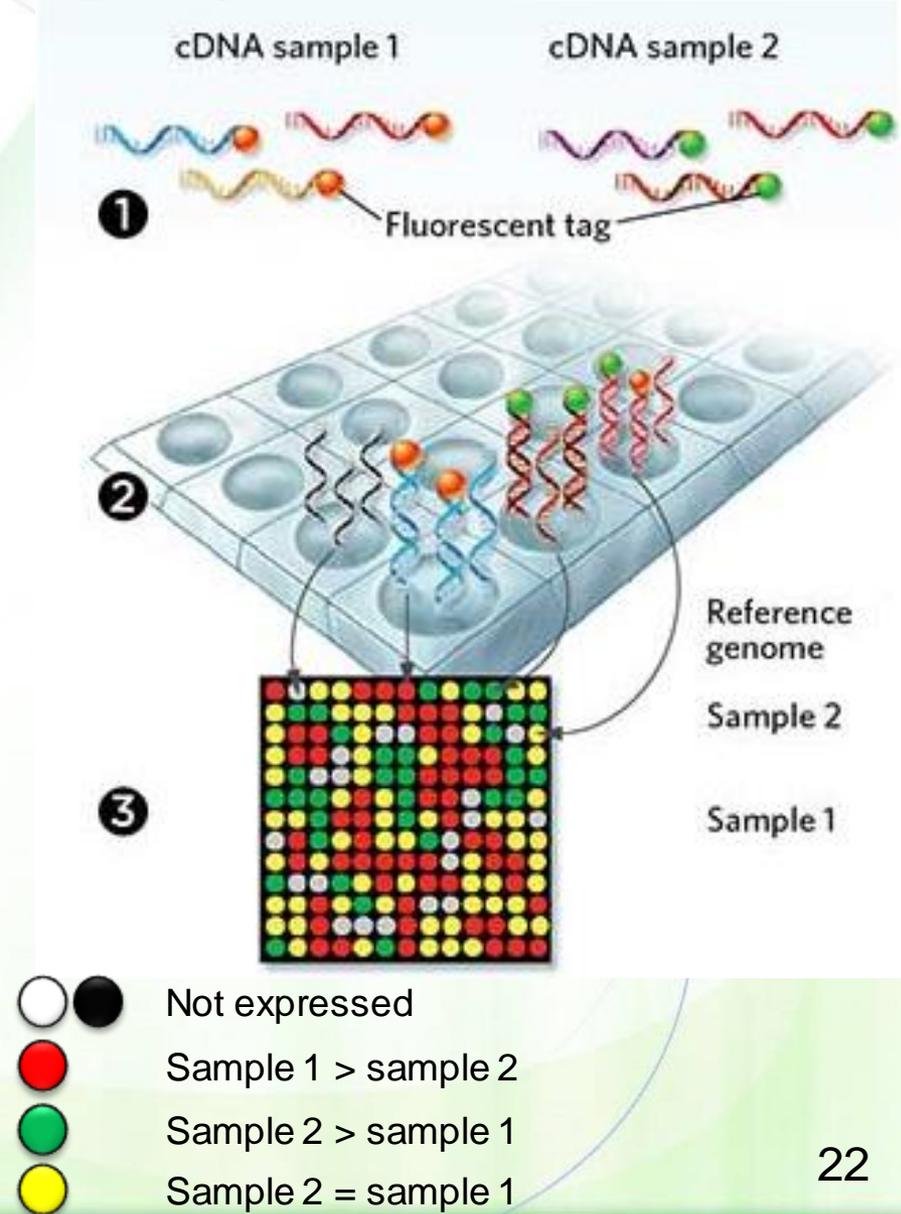
This is done for a single sample using radioactively labeled cDNA.

- 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



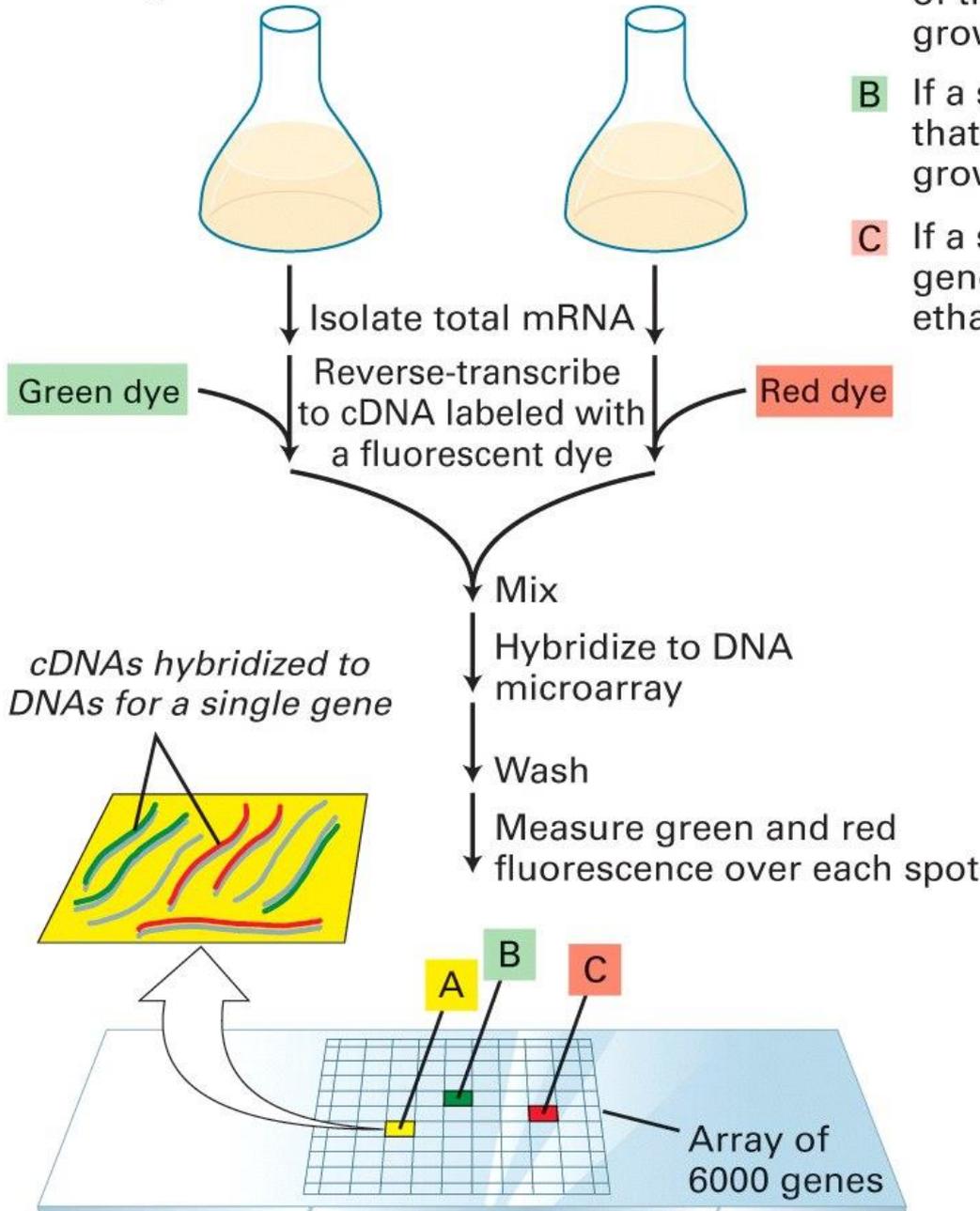
- In order to compare expression of genes two different samples, the cDNA molecules are fluorescently labeled with different colors (green and red) and added to the array.
- An increase in the amount of a RNA molecule in one sample versus the other is reflected by an increase in the amount of produced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



Cells grown on glucose medium

Cells grown on ethanol medium

- 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





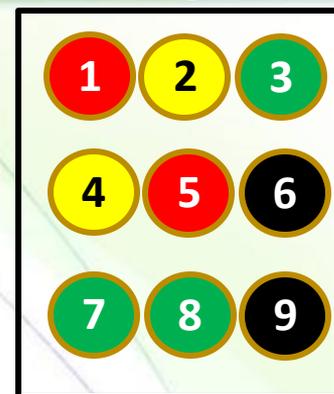
Sample 1



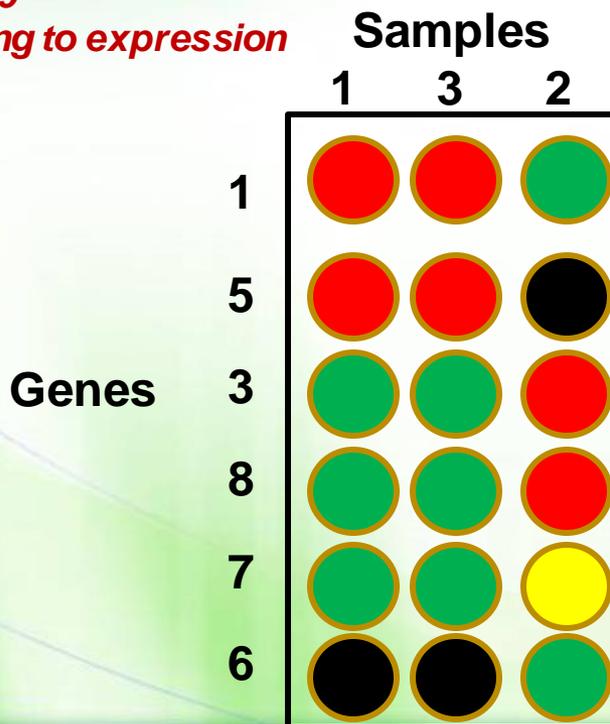
Sample 2



Sample 3



Combine results
Eliminate samples 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

