Molecular Biology (10)

Analysis of gene expression and RNA levels

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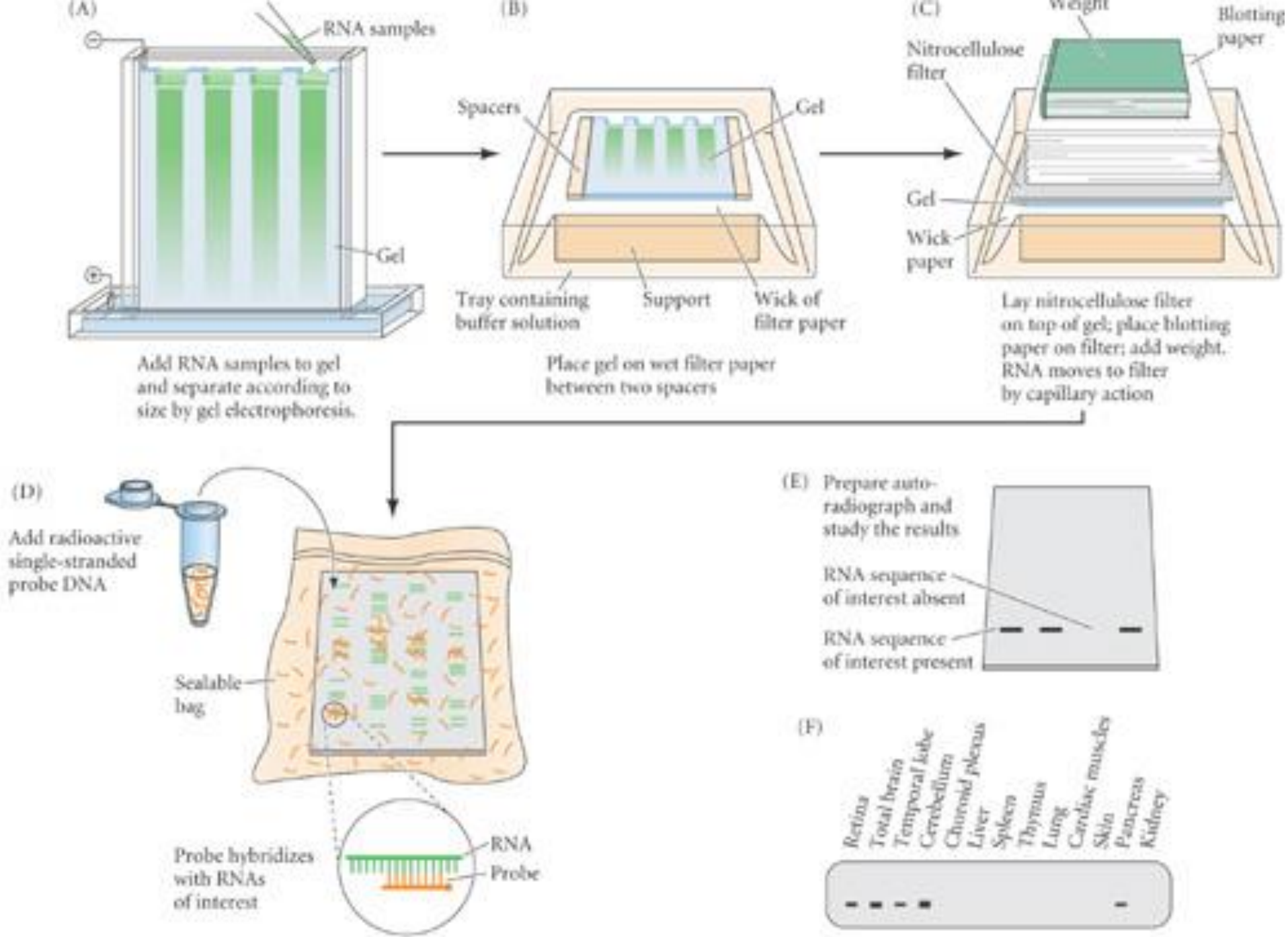
Analysis of gene expression
RNA level

Basic methods: Northern blotting, in situ hybridization
Advanced methods: real-time PCR, DNA microarray
Very advanced methods: RNA-seq

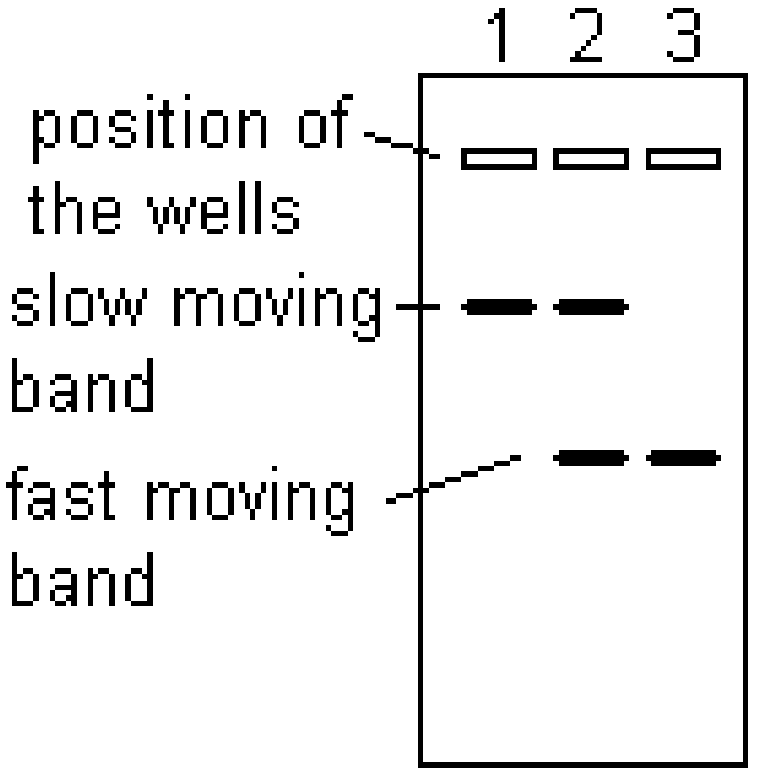
Northern blotting

- 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 a sequence that is complementary to a specific RNA molecule.

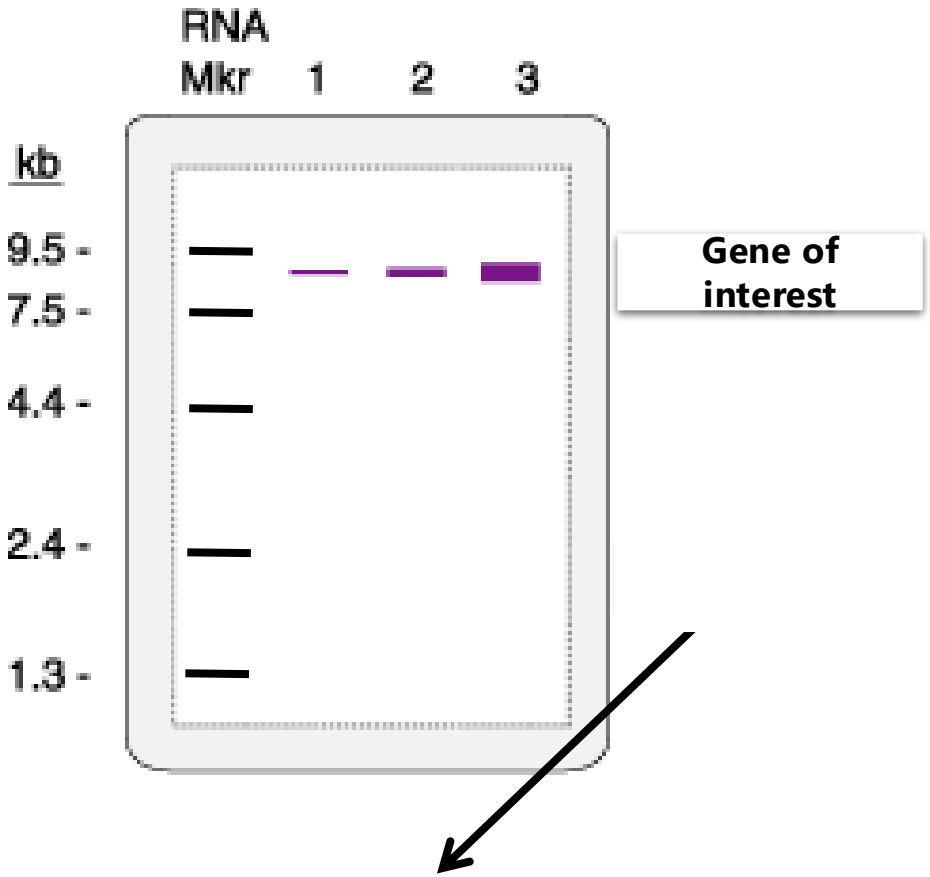
- What information can you deduce from it?


(A)
RNA samples
Gel
Add RNA samples to gel and separate according to size by gel electrophoresis.
(B)
Spacers
Gel
Tray containing buffer solution
Support
Wick of filter paper
Place gel on wet filter paper between two spacers
(C)
Weight
Blotting paper
Nitrocellulose filter
Gel
Wick paper
Lay nitrocellulose filter on top of gel; place blotting paper on filter; add weight. RNA moves to filter by capillary action
(D)
Add radioactive single-stranded probe DNA
Sealable bag
Probe hybridizes with RNAs of interest
RNA
Probe
(E) Prepare auto-radiograph and study the results
RNA sequence of interest absent
RNA sequence of interest present
(F)
Retina
Total brain
Temporal lobe
Cerebellum
Choroid plexus
Liver
Spleen
Thymus
Lung
Cardiac muscle
Skin
Pancreas
Kidney

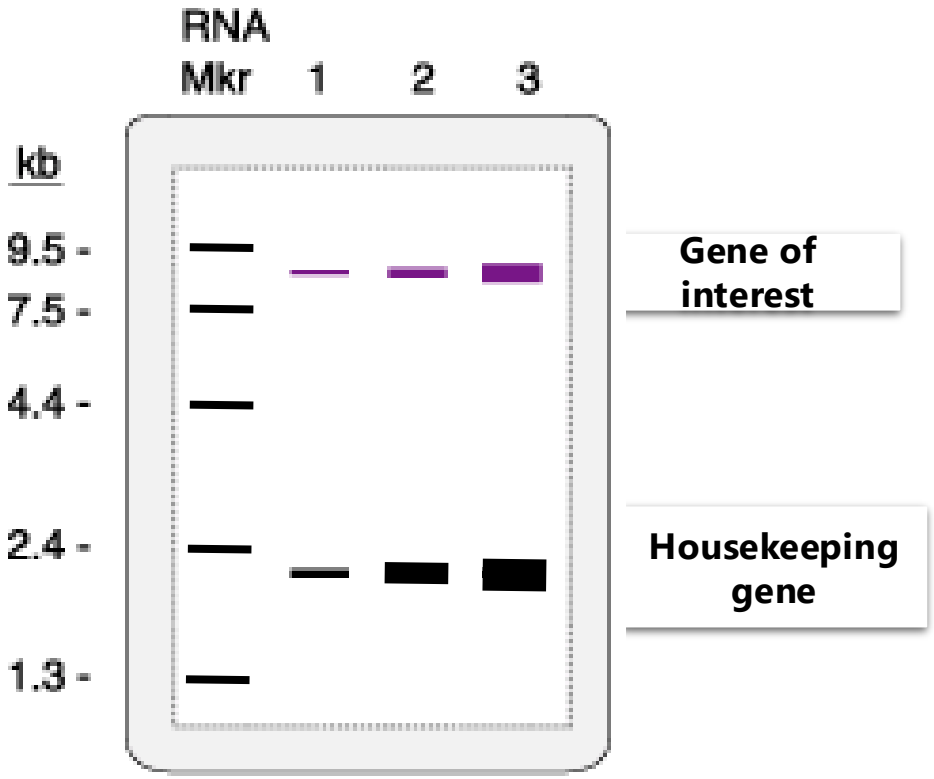
What are your interpretations?

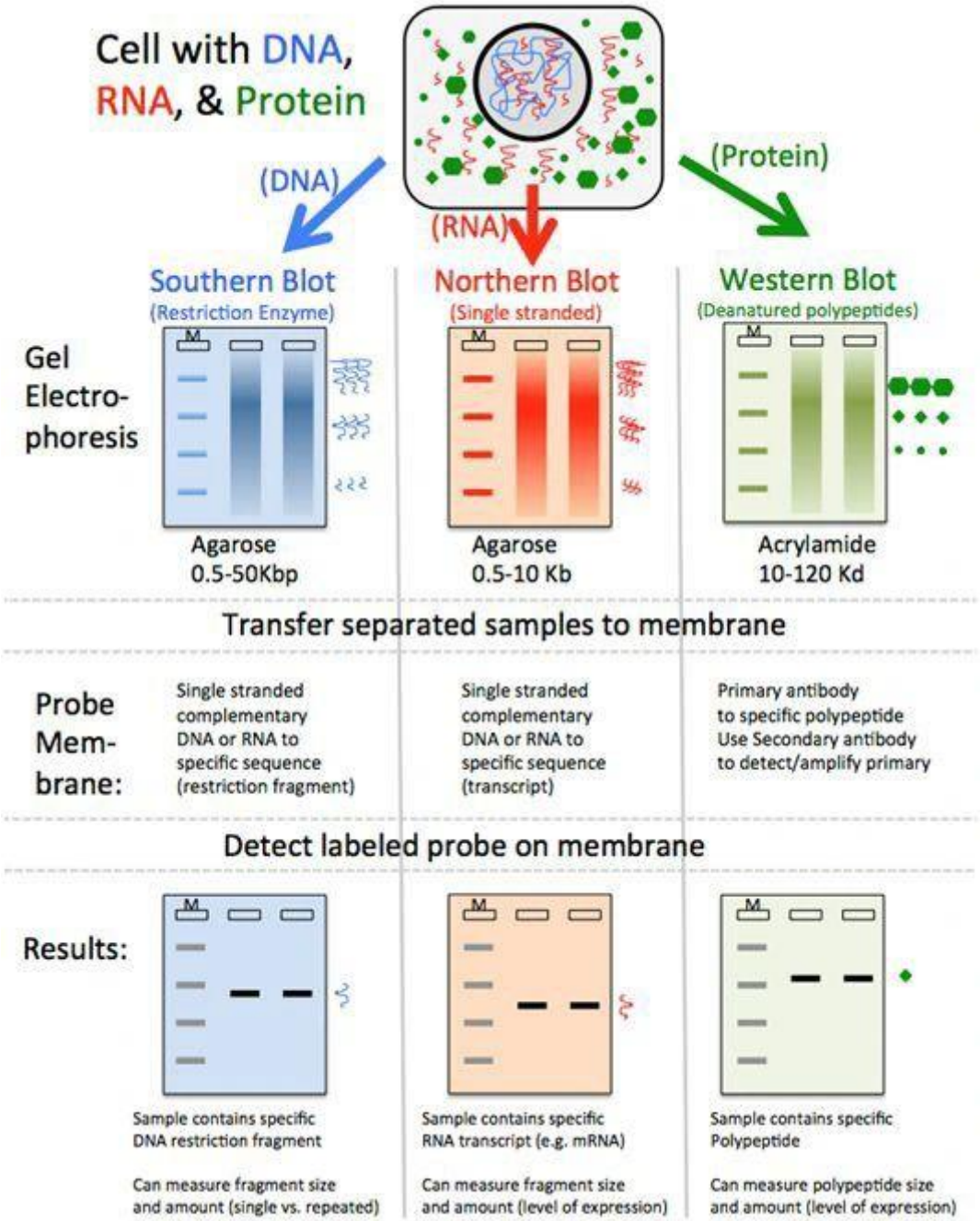


What are your interpretations?



What are your interpretations?

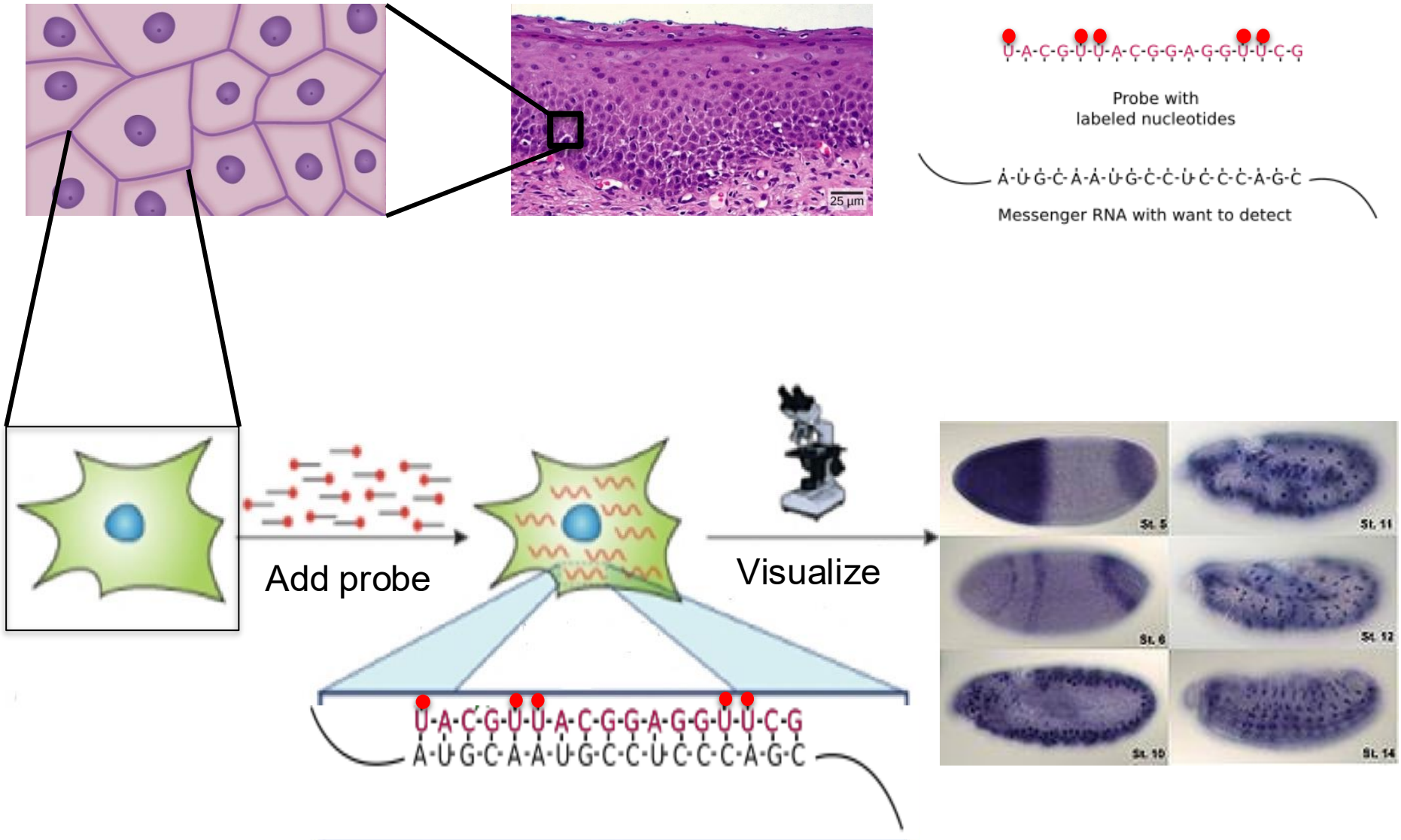



Cell with DNA, RNA, & Protein
(DNA)
(RNA)
(Protein)
Southern Blot
(Restriction Enzyme)
Northern Blot
(Single stranded)
Western Blot
(Deanatured polypeptides)
Gel Electro-phoresis
M
Agarose
0.5-50Kbp
Agarose
0.5-10 Kb
Acrylamide
10-120 Kd
Transfer separated samples to membrane
Probe Mem-brane:
Single stranded complementary DNA or RNA to specific sequence (restriction fragment)
Single stranded complementary DNA or RNA to specific sequence (transcript)
Primary antibody to specific polypeptide
Use Secondary antibody to detect/amplify primary
Detect labeled probe on membrane
Results:
Sample contains specific DNA restriction fragment
Can measure fragment size and amount (single vs. repeated)
Sample contains specific RNA transcript (e.g. mRNA)
Can measure fragment size and amount (level of expression)
Sample contains specific Polypeptide
Can measure polypeptide size and amount (level of expression)

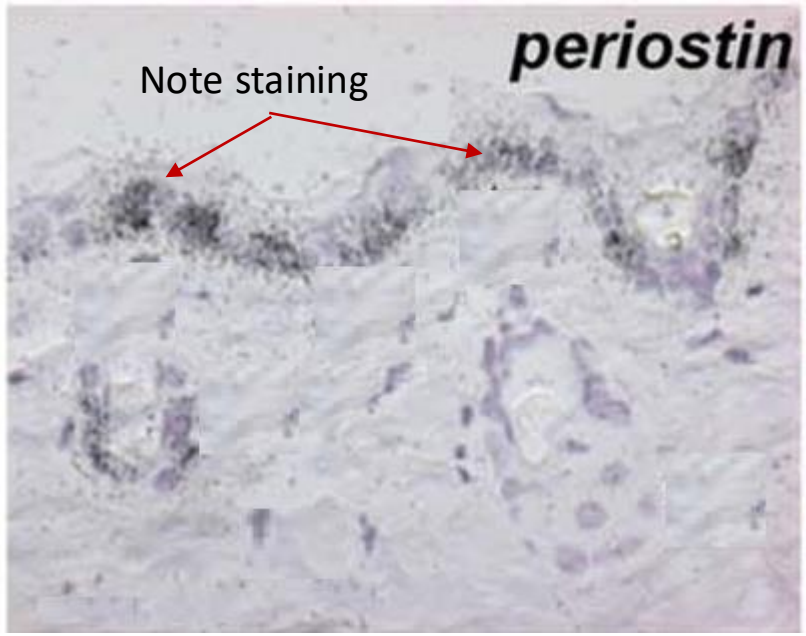
In situ hybridization

- 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)

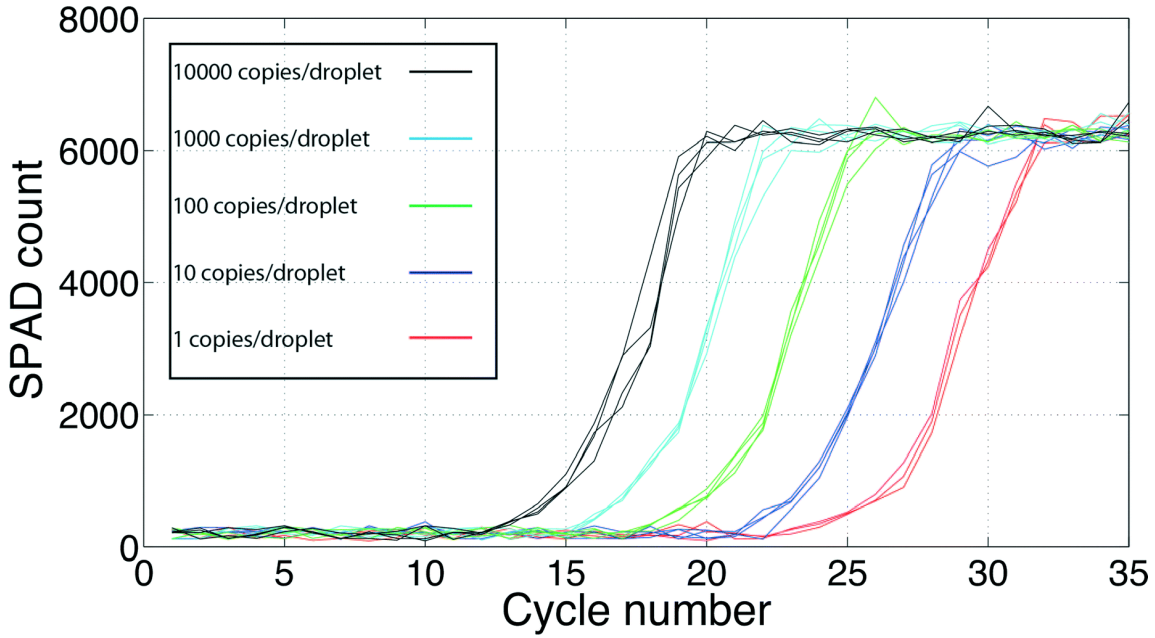
RNA and protein molecules do not coexist and are present in different places.

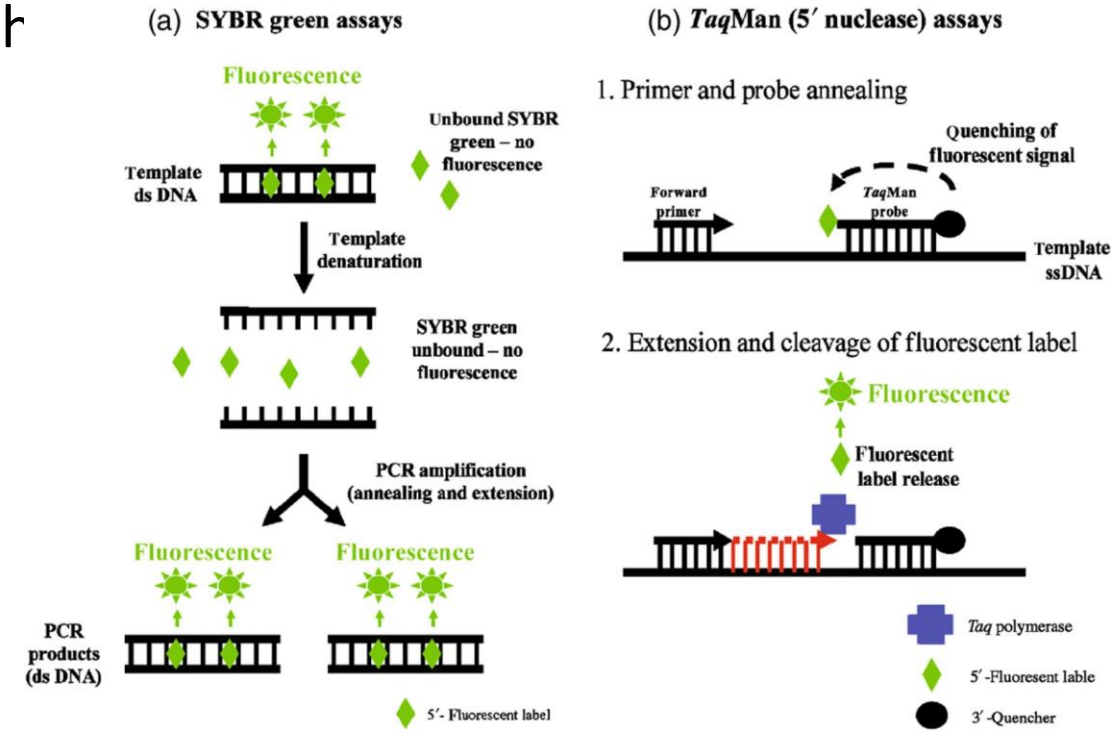
mRNA: inside cells along the basement membrane

Protein: outside cells in the basement membrane

Quantitative reverse transcriptase real-time RT-qPCR of mRNA

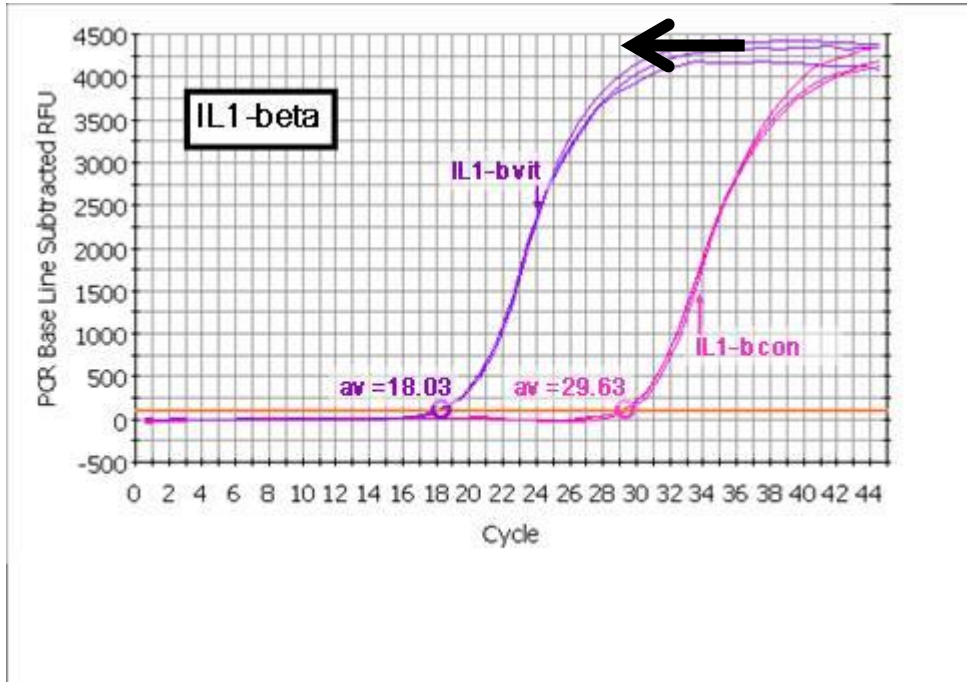
- Another way of relative quantitation of RNA expression is by converting RNA into cDNA followed by PCR in the presence of SYBR green.
- The higher the amount of RNA (cDNA), th detected.



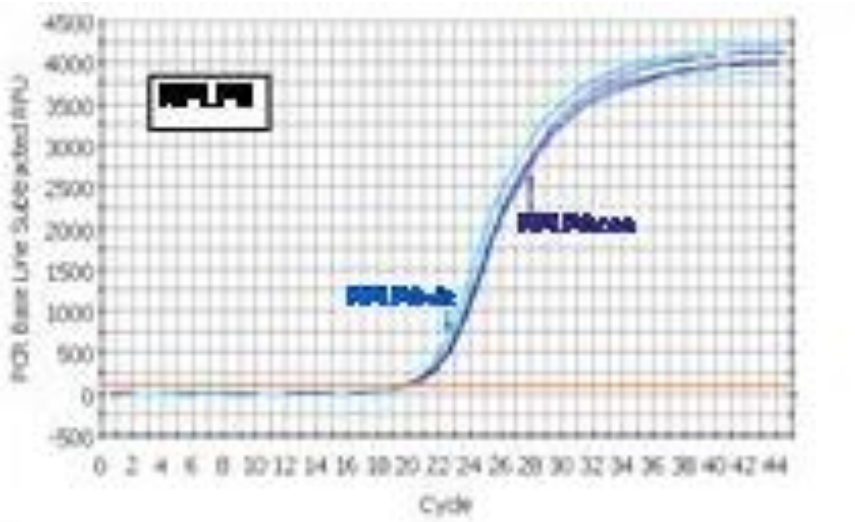


Example

A gene of interest

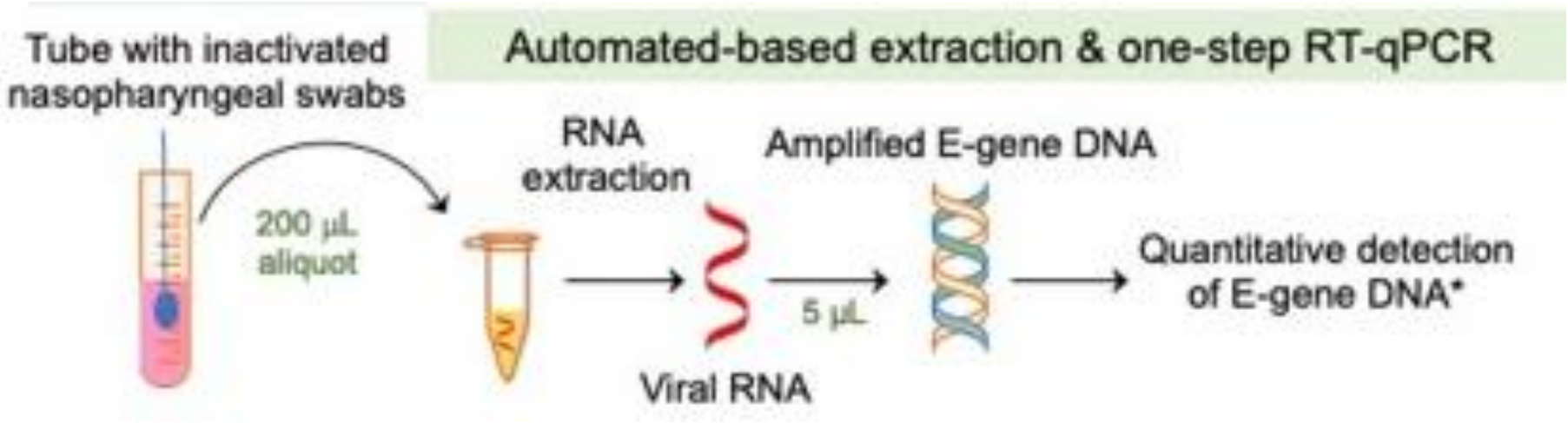


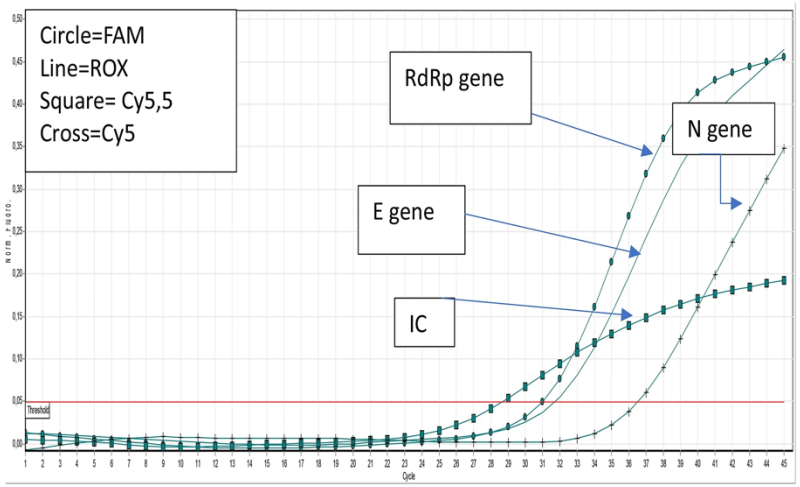
Housekeeping gene



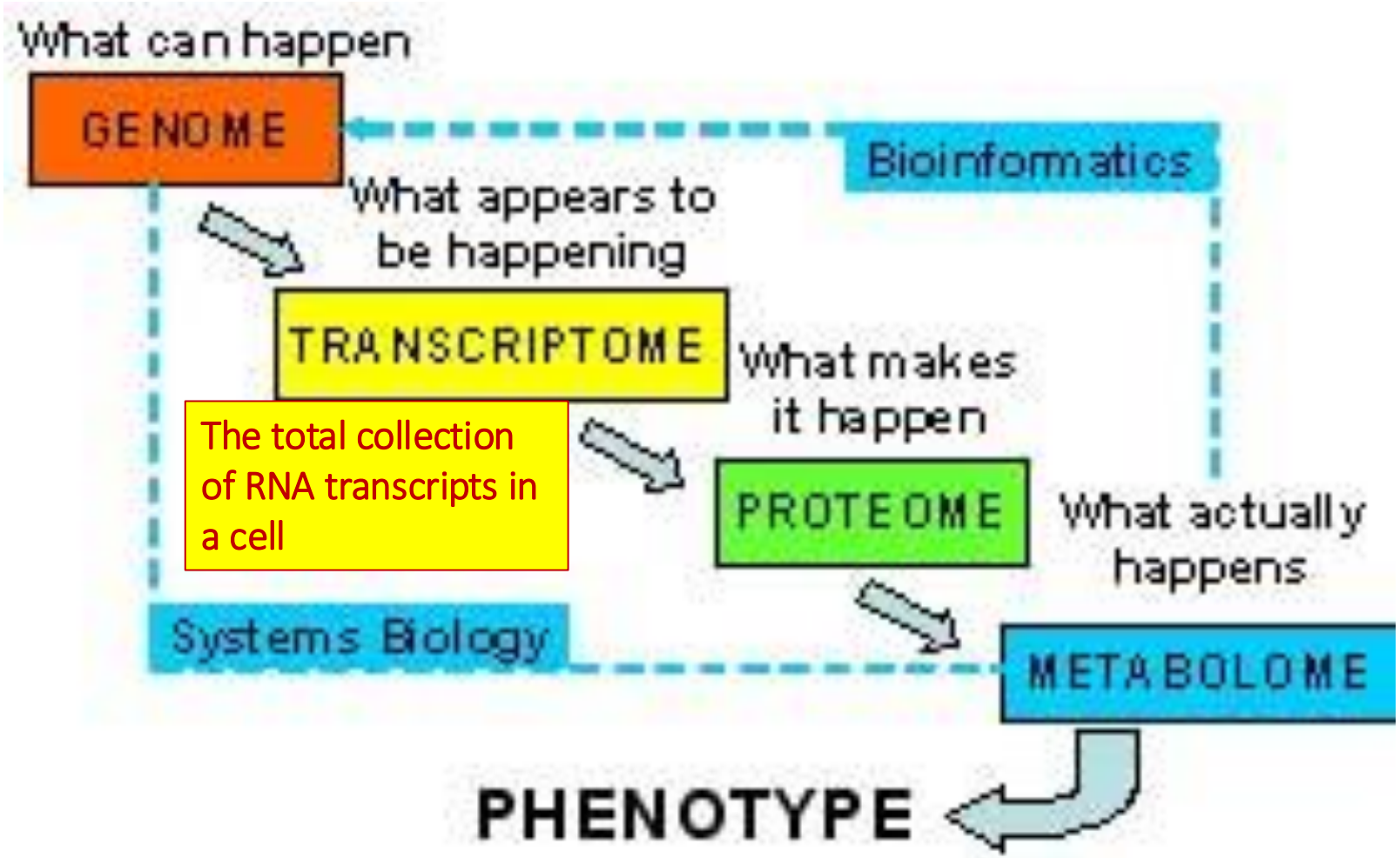
Unaltered expression

Detection of SARS-Co-2





The science of -omics



Studying the transcriptome

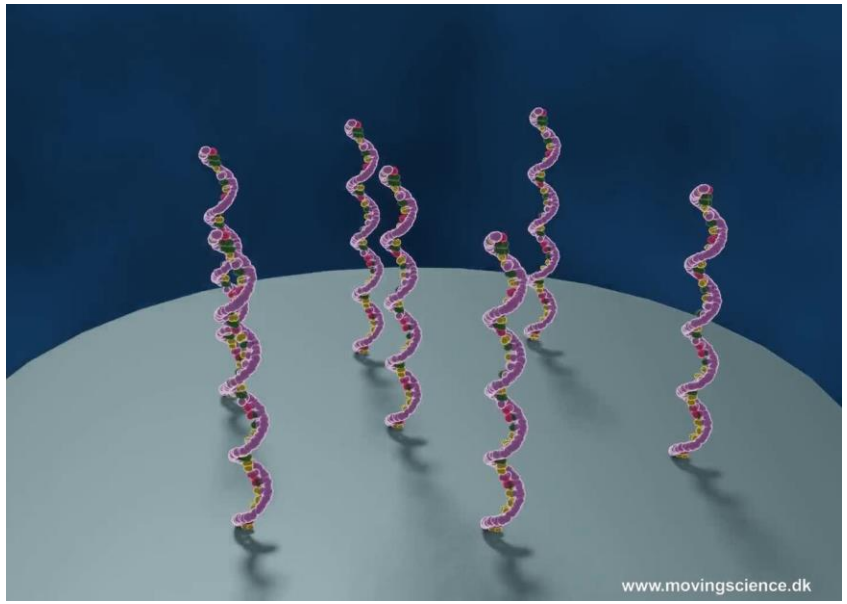
- One such method in studying transcriptomes is DNA microarrays, which allow the analysis of the RNA products of thousands of genes all at once.

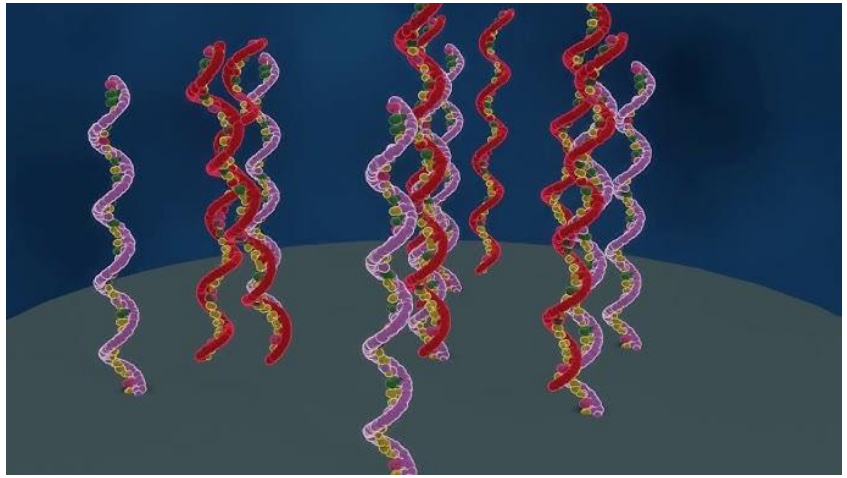
- By examining the expression of so many genes simultaneously, we can understand gene expression patterns in physiological and pathological states.

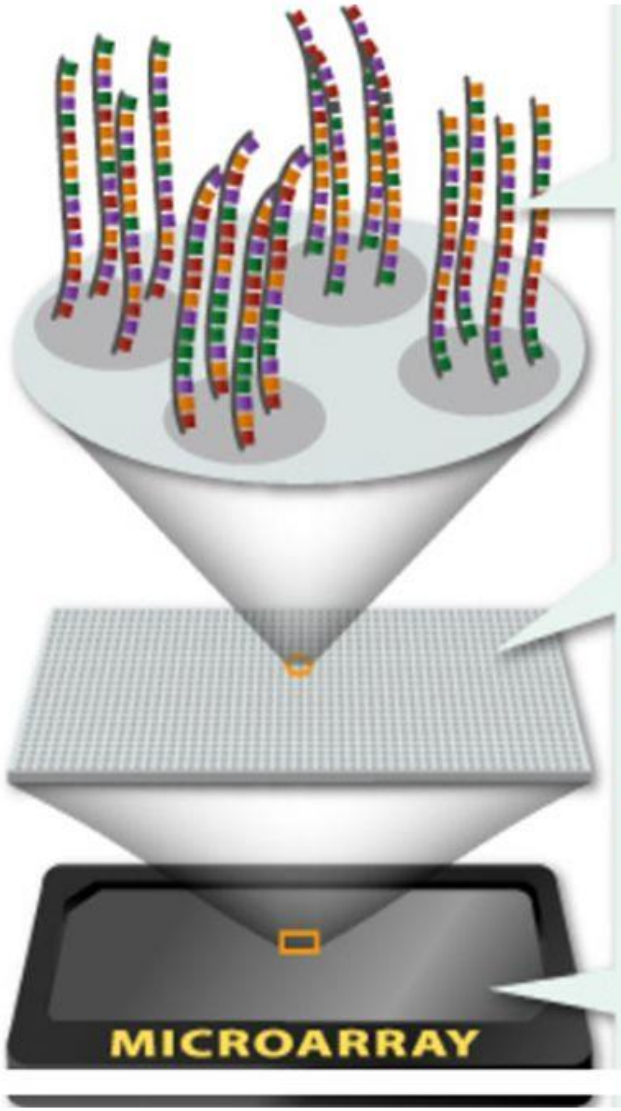
DNA microarrays

- DNA microarrays are 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 micorarray 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.

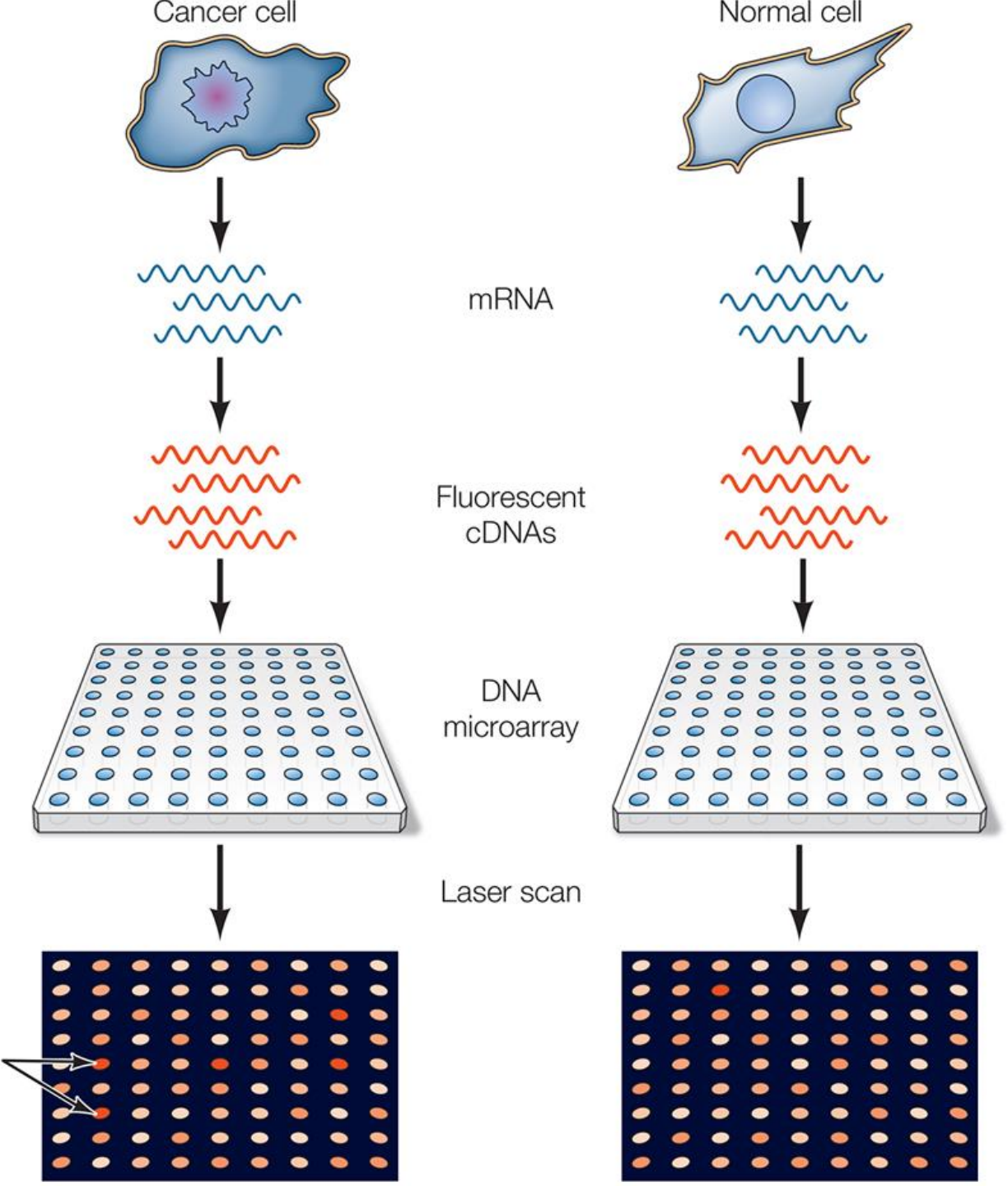
The DNA sequence on each spot is unique.

Each spot represents one gene.

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

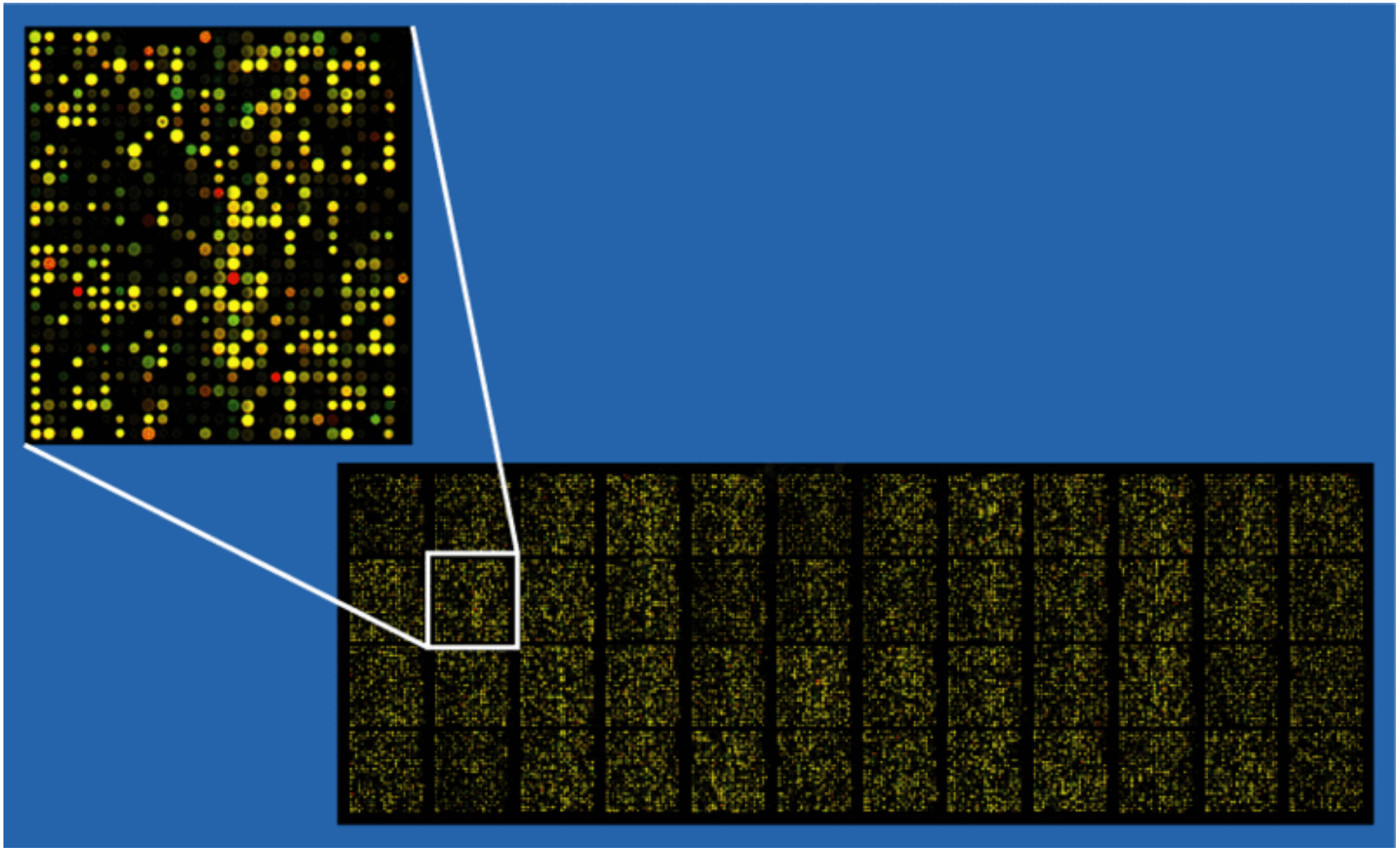
The precise location and sequence of each spot is recorded in a computer database.

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

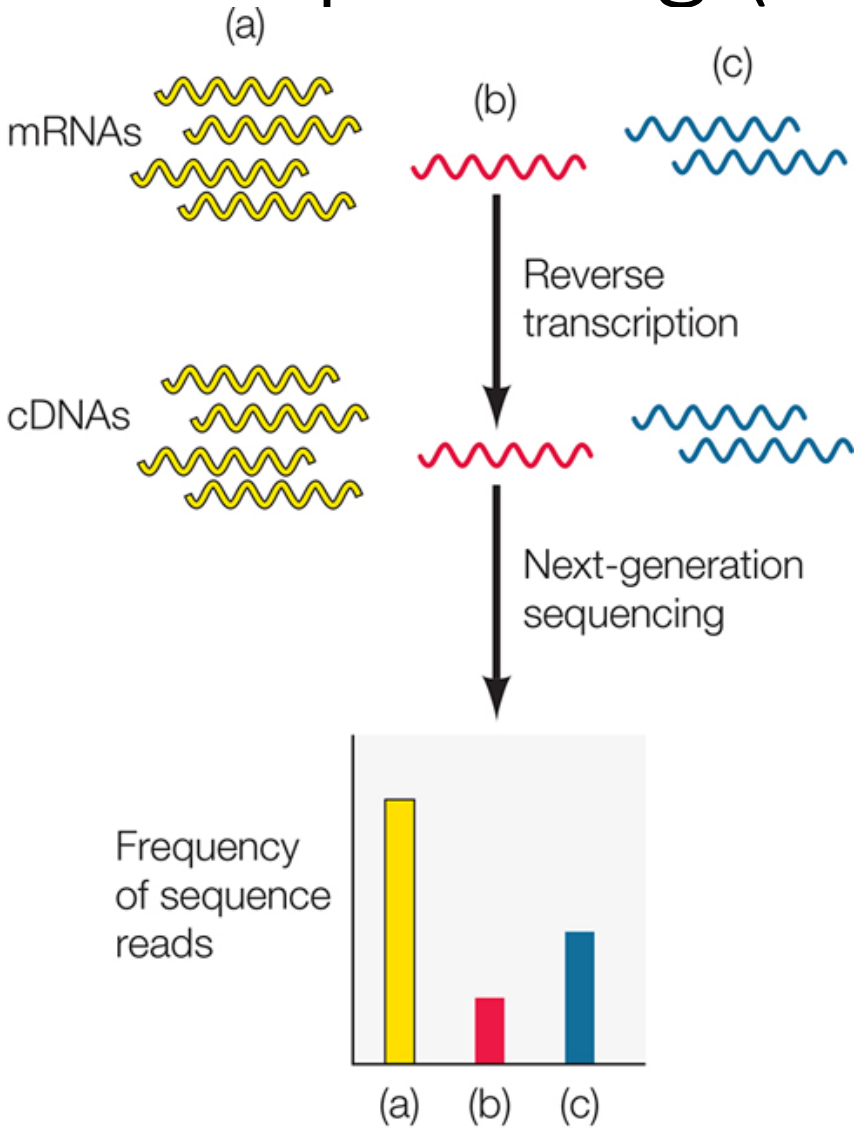


The procedure

- mRNAs are extracted from cancer cells and normal cells and converted to cDNAs, which are labeled with a fluorescent dye.
- The cDNAs are then hybridized to a DNA microarray containing spots of oligonucleotides corresponding to 20,000 or more distinct human genes.
- The relative level of expression of each gene is indicated by the intensity of fluorescence at each position on the microarray, and the levels of expression in cancer cells and normal cells can be compared.



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

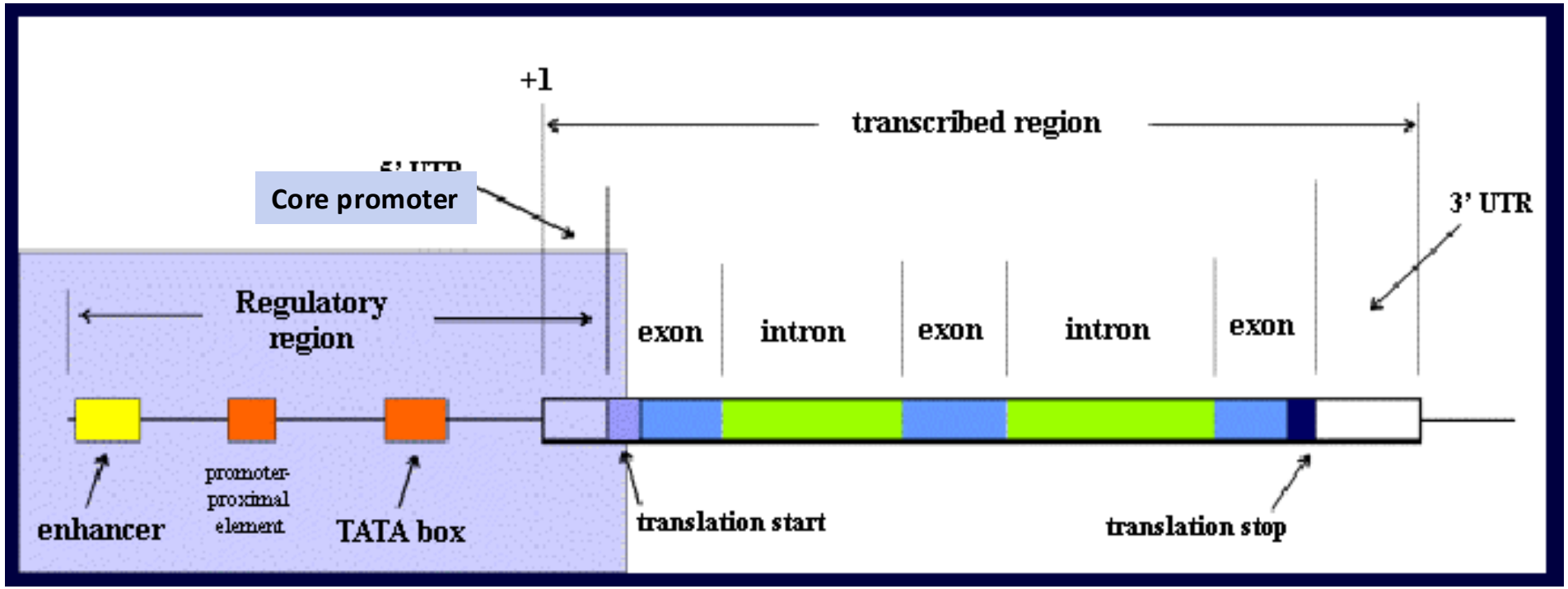
- RNA-seq can be used to
  - characterize novel transcripts
  - Identify splicing variants
  - profile the expression levels of all transcripts

- Microarrays are limited to detect transcripts corresponding to known genomic sequences.

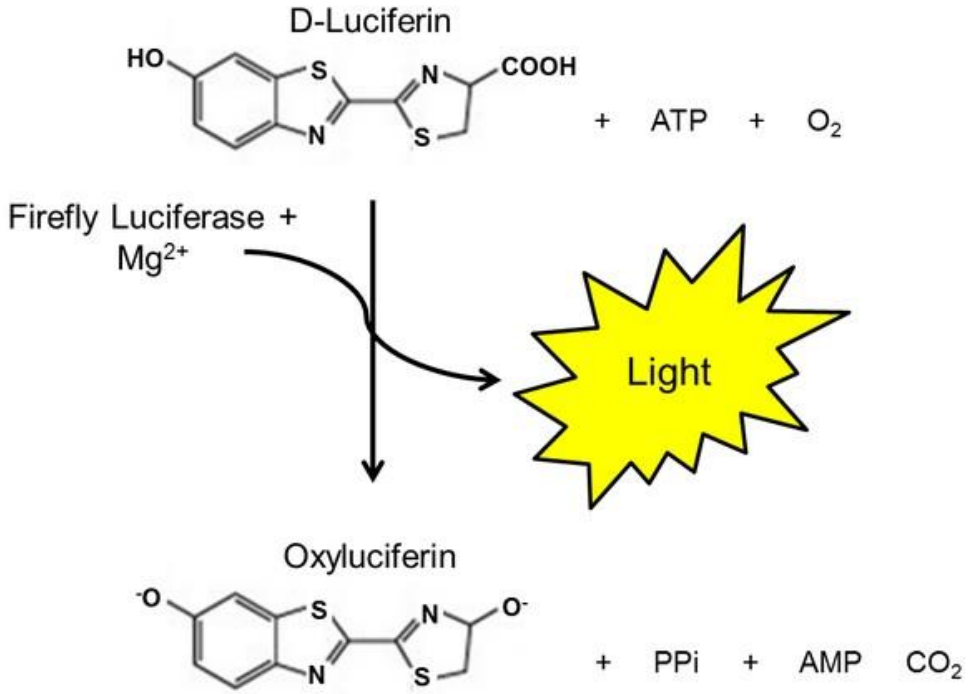
Analysis of transcriptional regulatory sequences

What are transcriptional regulatory sequences?

- Promoter (core promoter): A region of DNA upstream of a gene where relevant proteins (such as RNA polymerase and transcription factors) bind to initiate transcription of that gene.
- Promoter-proximal elements: Any regulatory sequence in eukaryotic DNA that is located close to (within 200 base pairs) a promoter and binds a specific protein thereby modulating transcription of the associated protein-coding gene.
- Enhancers or silencers: Regulatory DNA sequences that, when bound by specific proteins, regulate the transcription of an associated gene. They can be located near, within, after, and/or very far away from the gene, and, if lipped or relocated, are still functional.

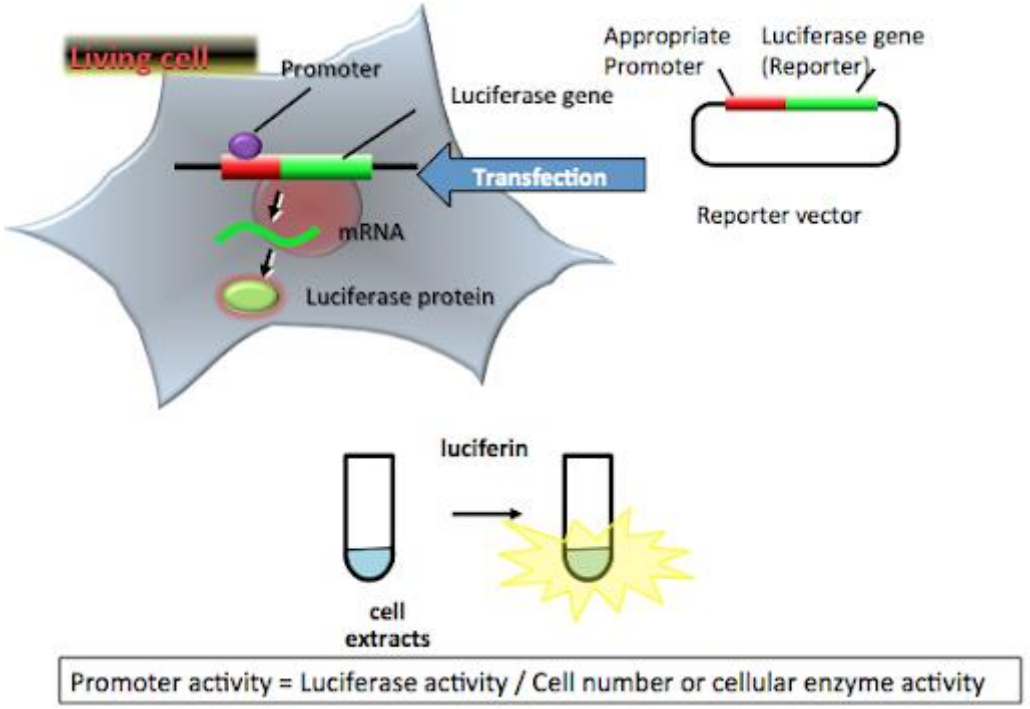


Firefly luciferase

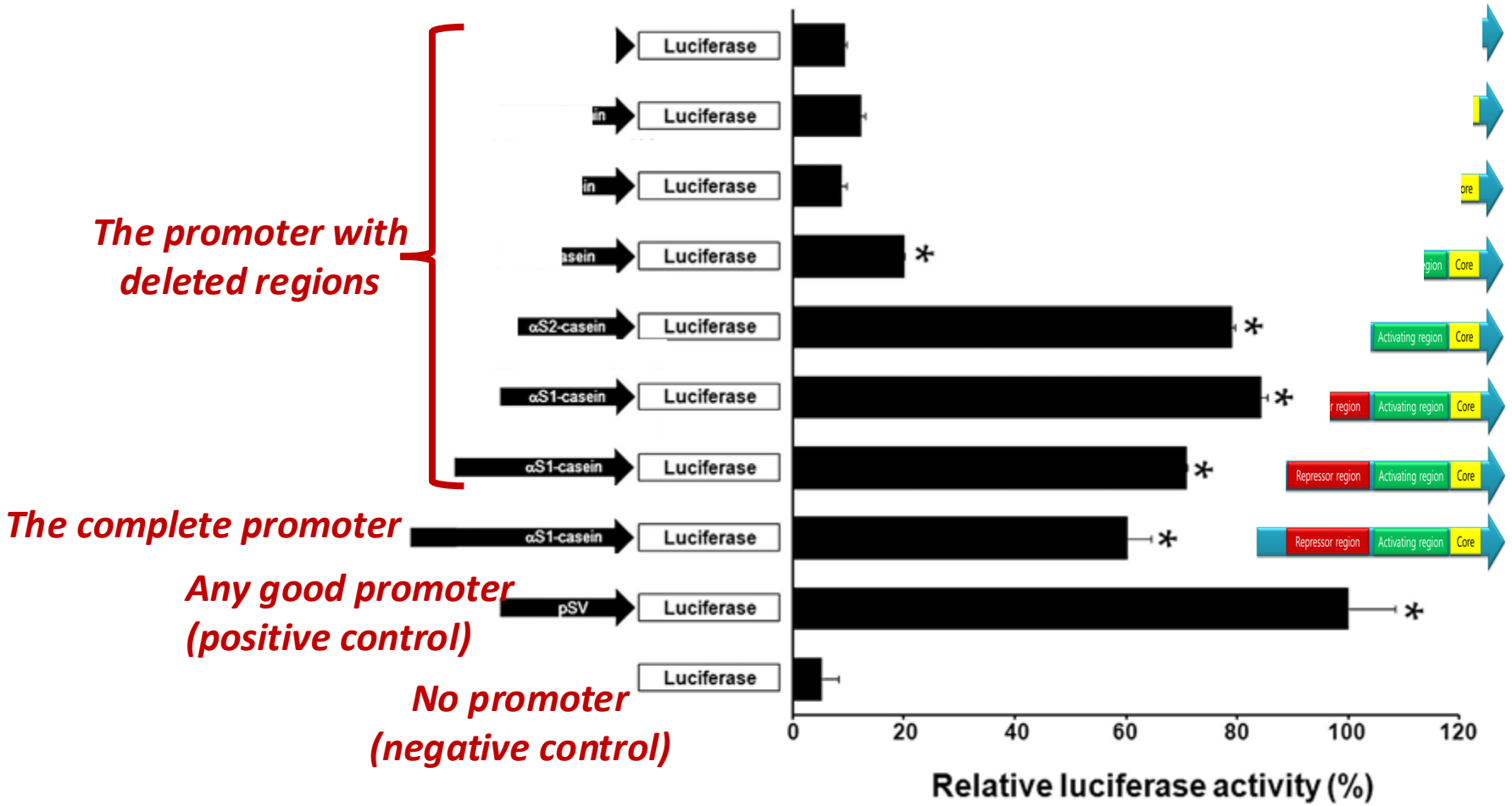


Luciferase reporter assay

- Purpose: study the activity of a gene at certain conditions or identify the function of certain regions of the promoter.
- **Only** the regulatory region (e.g. promoter, PPE, etc.) of the gene is placed upstream of a "reporter gene" such as the luciferase gene in a plasmid.
- The plasmid is transfected (inserted) into cells, and the expression level of luciferase (instead of the original gene itself) is measured.



Identify the functions of the different regions



Identify the regulatory protein-binding sites

- You have the promoter of a gene that can be regulated by multiple molecules.
  - Examples: estrogen, progesterone, and nutrients
- Prepare the promoter without the different regulatory regions and assess the luciferase activity in the presence of the different molecules.

- Think: what would happen if HRE is removed and the cells are placed under hypoxic condition knowing that hypoxia negatively regulates the gene expression?