بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 12

Analysis of gene expression & Analysis of transcriptional regulatory sequences

Written by : DST

NST

Reviewed by : NST members

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: المبرأ من النقائص والشريك، وكل ما لا يليق بالإلهية، الذي تُسبّحه وتقدّسه الخلائق وتنزهه عن كل سوء، لكمال أسمائه وصفاته وجمالها.

الورود: لم يرد في القرآن الكريم، وورد في السنة.

الشاهد: في أذكار الركوع والسجود، (سبوح قدوس رب الملائكة والروح).

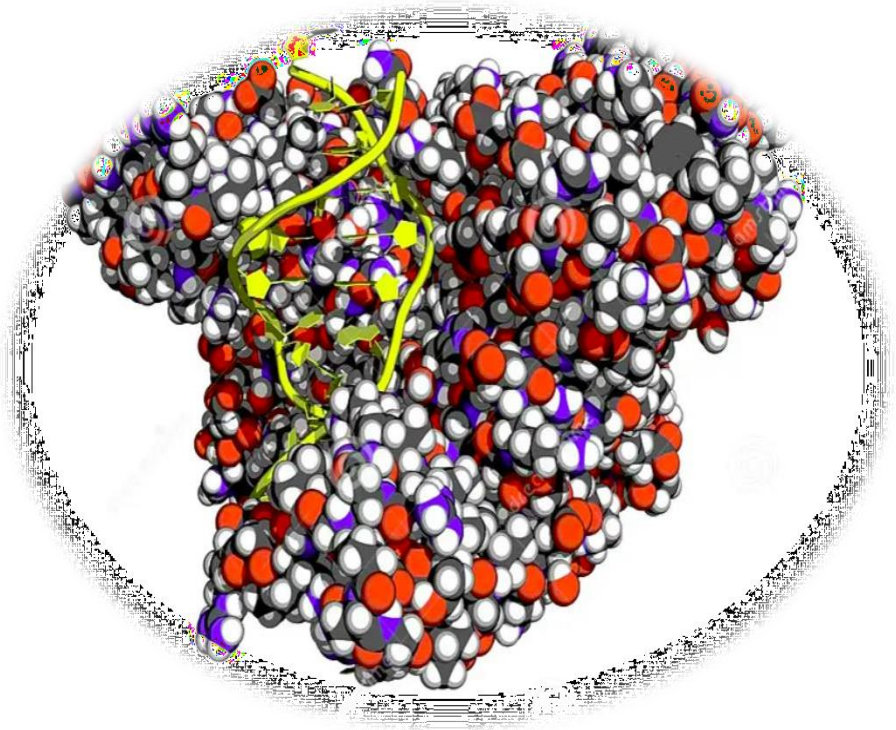
(أخرجه مسلم).

٢٥

اضغط هنا لشرحٍ أكثر تفصيلًا

Quiz on the previous lecture

Click on the pic



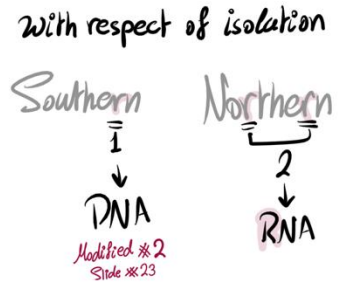
Analysis of gene expression

RNA level

This lecture discusses techniques for gene expression analysis, categorized into three levels of complexity:

1. *Basic methods:*
   - *Northern blotting.*
   - *in situ hybridization.*
2. Advanced methods:
   - real-time PCR.
   - DNA microarray.
3. Very advanced methods:
   - RNA-seq (sequencing)

Northern blotting

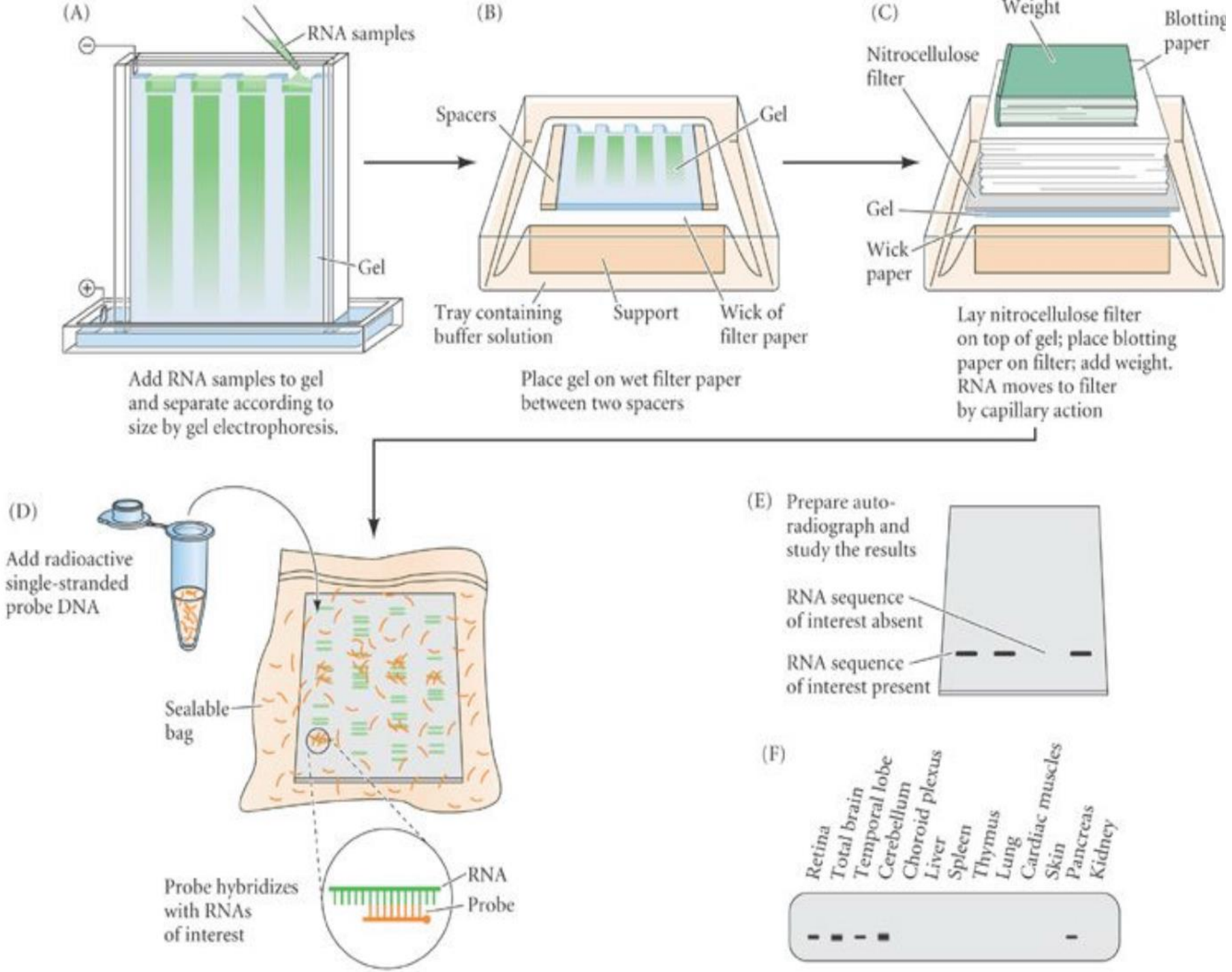


- This is done exactly like Southern blotting except that:
  - RNA from cells is isolated instead of DNA.
  - RNA molecules are fractionated based on size by gel electrophoresis.
  - The fractionated RNA molecules are transferred onto a membrane.
  - RNA molecules are targeted by a labeled DNA probe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

    (usually, DNA probes are used because they're more stable than RNA probes)

- What information can you deduce from it?

1. **Expression, if the gene expressed or not.** (if expressed, mRNA (transcript) would be detected)
2. **Size of the RNA of interest.**
3. How active the gene is/ **to what extent is the gene expressed.** (More band intensity, more expression)



Examine the figure carefully before proceeding to the explanation in the following slides.

Northern Blotting Technique

- It is similar to Southern blotting, but specifically used for detecting RNA.

Here, the term "RNA" specifically refers to mature mRNA.

1. First, **RNA** is obtained from cells, NOT DNA.
   - RNA molecules obtained are variable in size, depending on the size of the gene.
2. RNA molecules are then fractionated by gel electrophoresis.
   - RNA molecules are separated based on size, with smaller fragments migrating further and faster than larger ones.
3. After that, RNA molecules are transferred onto a membrane.
   - RNA molecules are already separated by size, serving as an exact replica of the gel.
4. Next, radioactively labeled complementary DNA probes bind to the target RNAs of interest (hybridizing to it).
5. A signal is emitted where the probe binds, identifying the target RNA.

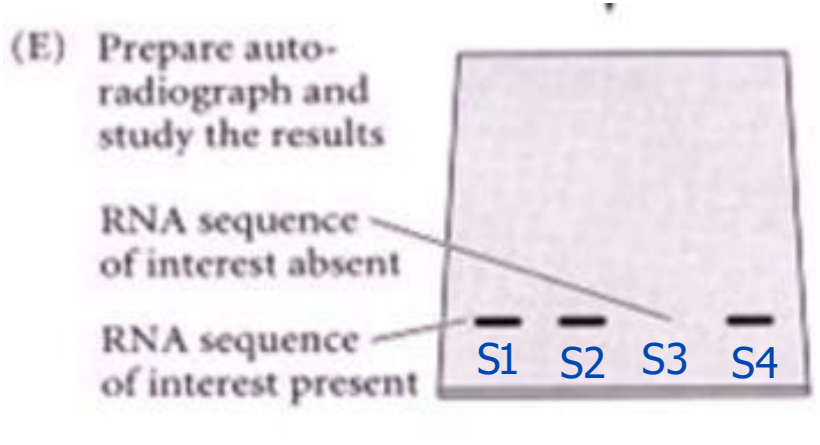
Northern Blotting- Results interpretation(1)

- In this example there are 4 samples, put in different wells.

❖ From the results, the following can be determined:

1. Samples 1, 2, and 4 **express** the gene, as bands are observed, whereas sample 3 does not express the gene, as no band is present.
2. RNA molecules are relatively small, as they are positioned closer to the anode than the cathode.

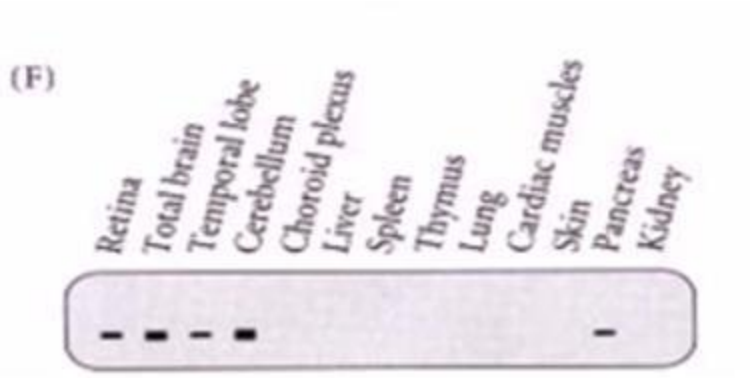
Cathode (-)



Anode (+)

Northern Blotting- Results interpretation(2)

Same gene, different tissues.



Here, the expression of the **same gene** but in **different tissues** is examined.
For this specific gene:

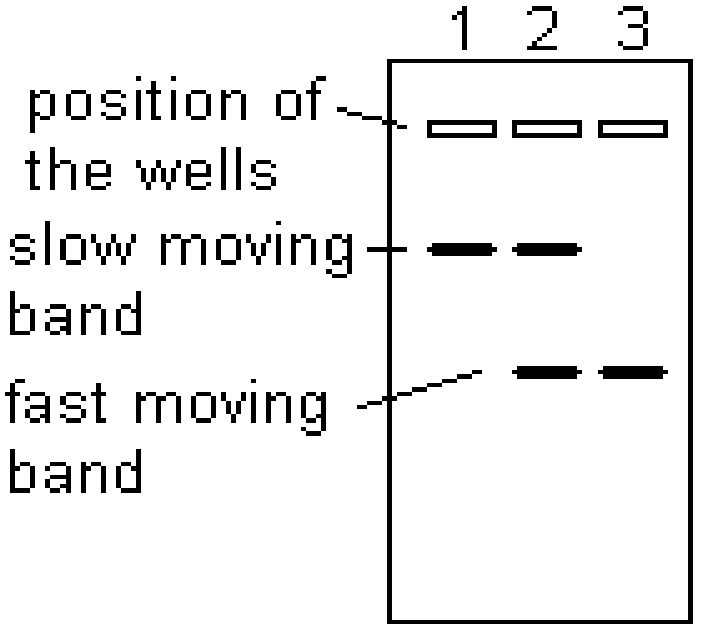
- The cerebellum (sample 4) expresses the gene at a much higher level than the temporal lobe

(sample 3), as indicated by the greater intensity of band 4 compared to band 3.

- In tissues such as the liver and spleen, where no RNA band is observed, there is no expression of this gene.
- So, northern blotting can determine if a specific gene is expressed in a particular tissue and to what extent.

What are your interpretations?

- Here, we have three samples, and after performing northern blotting, the following results were observed:
  1. Sample 1 expresses the gene and produces a relatively large mRNA.
  2. Sample 2 expresses the gene but produces 2 different mRNA molecules; a large mRNA and a smaller mRNA.
  3. Sample 3 expresses the gene but produces a small mRNA. But why?



- The generation of different RNA molecules of varying lengths from the same gene can be attributed to:
  1. RNA molecules can be produced from **different promoters**.
  2. RNA can be **alternatively spliced**.
  3. Variation in the **termination** of transcription.

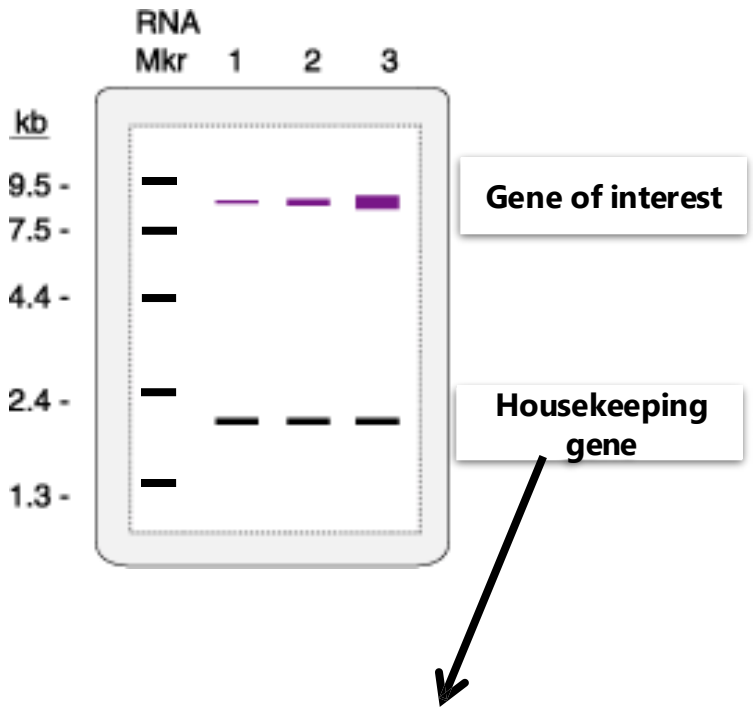
(Recall lecture 11)

Again : Before adding the probe we separate the RNA molecules based on size.
The probe binds to mRNA regardless its size, as long as it's complementary to it; so the probe does not affect how the mRNA migrate through the gel

What are your interpretations?

REMEMBER, RNA is single stranded so no base pairs → kb not kbp.

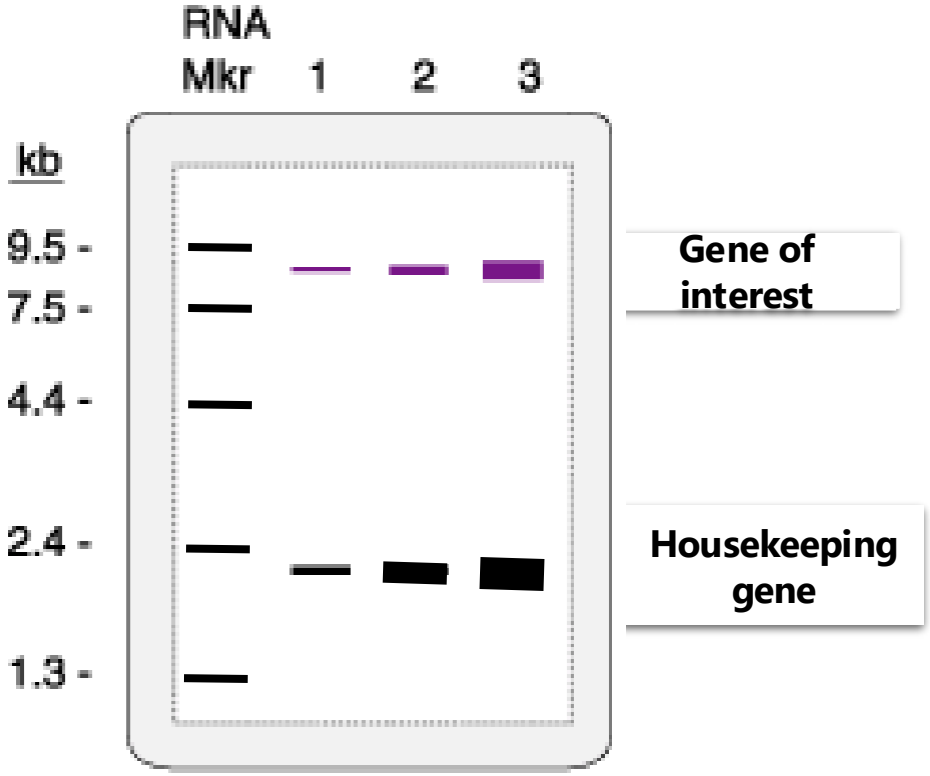
- Here comparative expression is done among 3 samples.
  - **Comparative expression:** comparing the level of a certain gene expression among different samples, meaning that samples can be placed under varying conditions to observe changes in gene expression levels.
- The gene of interest produces RNA molecules about 9 kb long.
- Sometimes different conditions (ex. volumes) lead to false results (see next slide), so, to ensure that the interpretations are error-free, the expression of house keeping genes is observed.
  - **House keeping genes:** genes whose expression isn't changed under different conditions (constant), for example: histone genes are always expressed and doesn't change, also actin, certain metabolic enzymes; all cells need them, so their expression isn't changed.
- Here real changes in intensity can be observed (3 most intense), because the expression of the housekeeping gene is equal among all 3 samples.



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

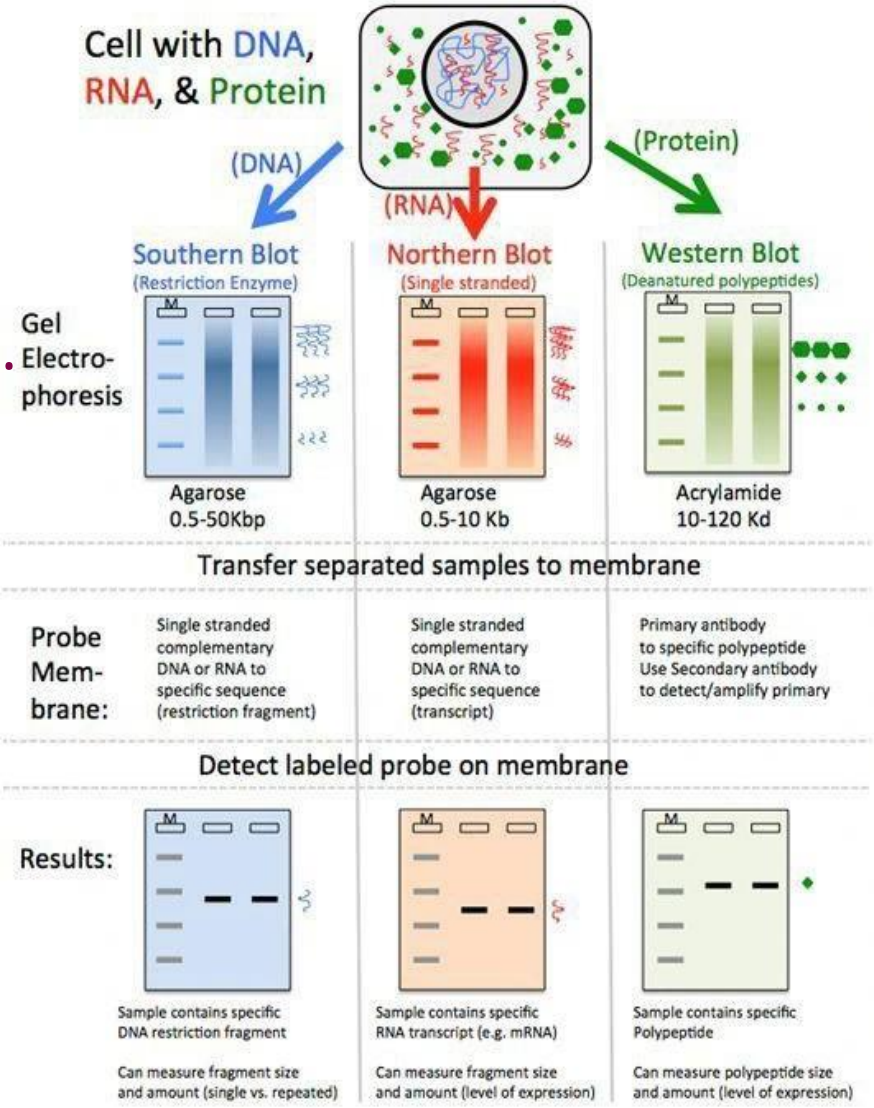
What are your interpretations?

- ❖ Here, we have three samples, but the expression of the housekeeping gene varies among them, indicating that the initial amounts of the samples are **not equal.**
- ✓ As a result, the observed increases in intensity are not real.



Summarizing Techniques - Macromolecules Analysis

1. Proteins.
   - Proteins are separated based on size, then targeted with **antibodies** is process known as **immunoblotting** or **western blotting**.
   - ❖ Mechanism.
     - SDS-PAGE is done, separating proteins based on size.
     - Proteins are transferred into a membrane.
     - Addition of specific antibodies.
     - Detection of proteins and their sizes (MW).
2. RNA.
   - **Northern blotting** can be used to analyze gene expression.
   - Examining the presence of RNA bands, specifically RNA fragments that are complementary to a particular probe.
   - ✓ We assess not only their **presence** but also their **size** and **intensity**, with the latter reflecting the level of gene expression.
3. DNA.
   - **Southern blotting** is a technique used to analyze the sizes of DNA fragments, typically generated by restriction endonucleases, for example.



SDS: sodium diacyl sulfate.
PAGE: polyacrylamide gel electrophoresis.

In place

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.

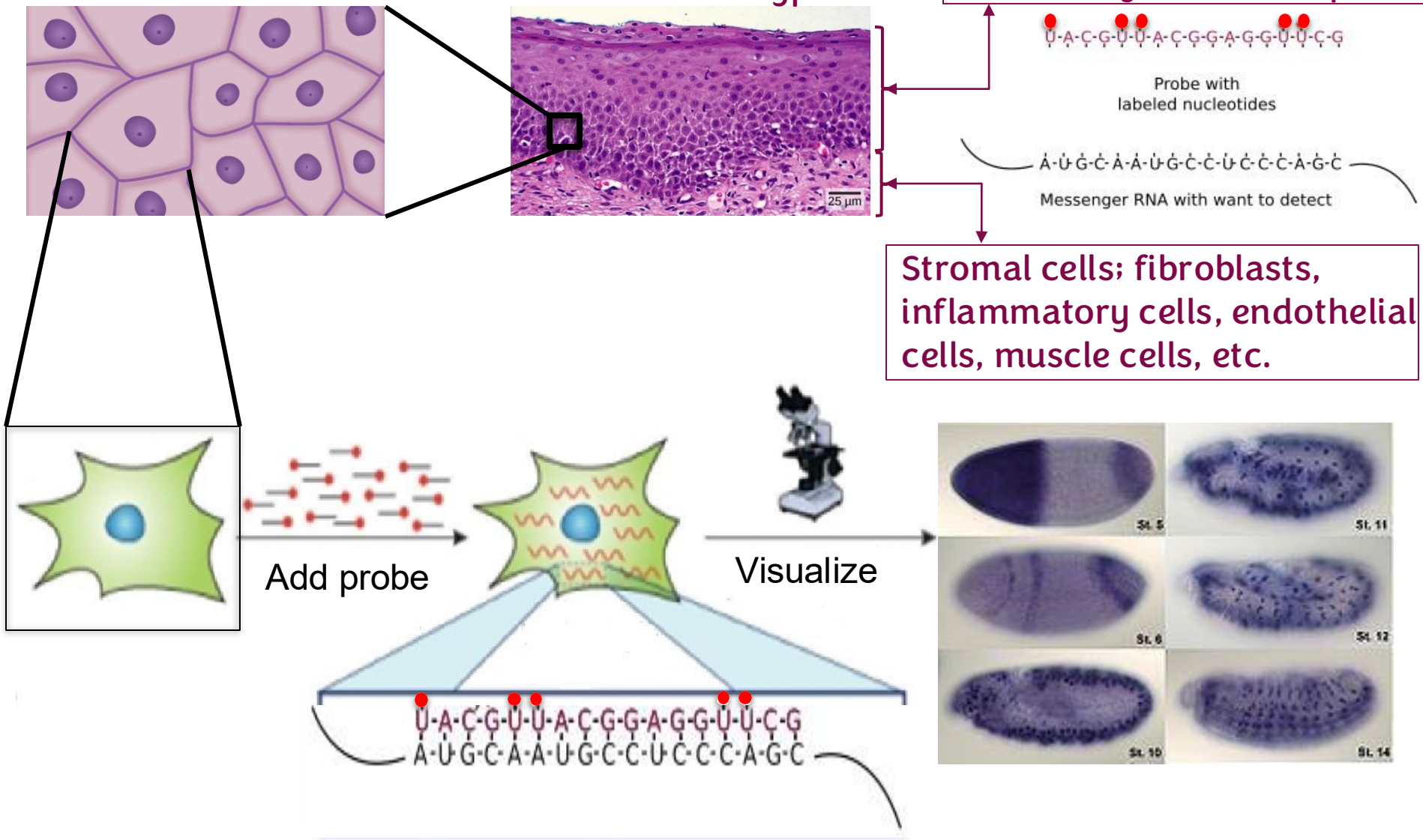
✓ Looking for exactly where the gene is expressed in a tissue section.

✓ A tissue section has multiple cell types (differentiated, less differentiated and stem cells, ex: epithelial cells, endothelial cells, fibroblasts, etc.), and this allows the identification of which specific cell is expressing a particular gene.

Procedure of in situ hybridization

Tissue section with different cell types.

Well differentiated cells, immature cells, ex: epithelial cells (keratinocytes for example).



Stromal cells; fibroblasts, inflammatory cells, endothelial cells, muscle cells, etc.

In Situ Hybridization - Procedure

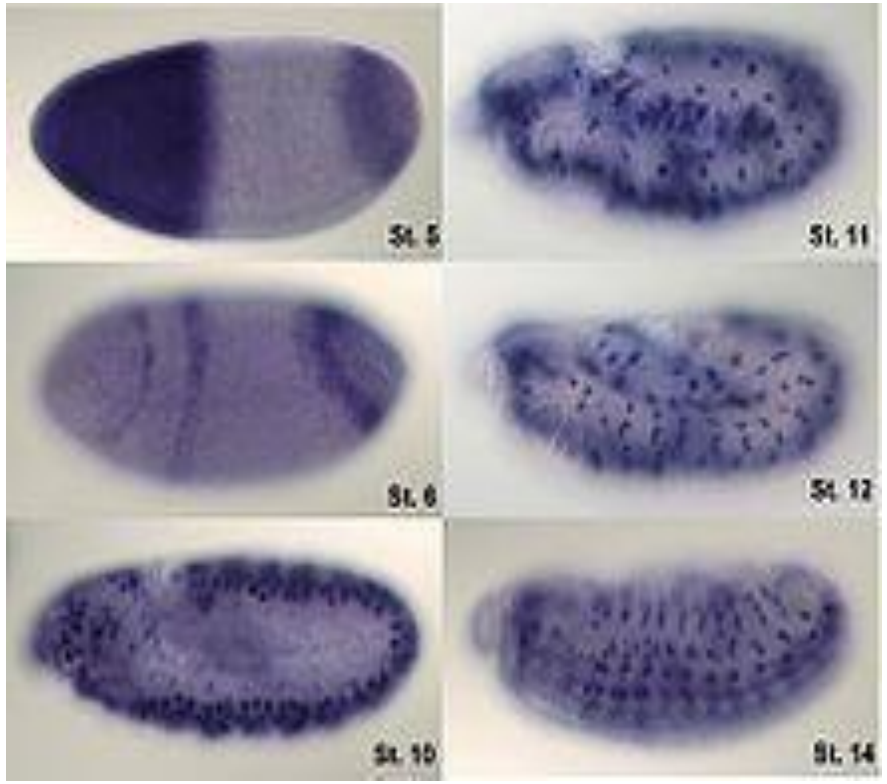
1. A tissue section is obtained.
2. A probe (DNA or RNA) is added to the tissue section.
3. Upon hybridization with an mRNA, the probe emits a signal which allowing the identification of the specific cell where the signal originates.
   - Zooming in on a particular cell, the probe binds to messenger RNA (mRNA) molecules in that cell.
4. The signal is visualized, confirming that a specific cell expresses the gene of interest.

In Situ Hybridization – Visualization

This is taken from a Drosophila embryo, the embryo of fruit flies.

- Various in situ hybridization techniques were performed using different probes targeting specific genes.

➢ These techniques reveal the distinct patterns of expression for certain proteins involved in processes like development and embryogenesis in fruit flies.



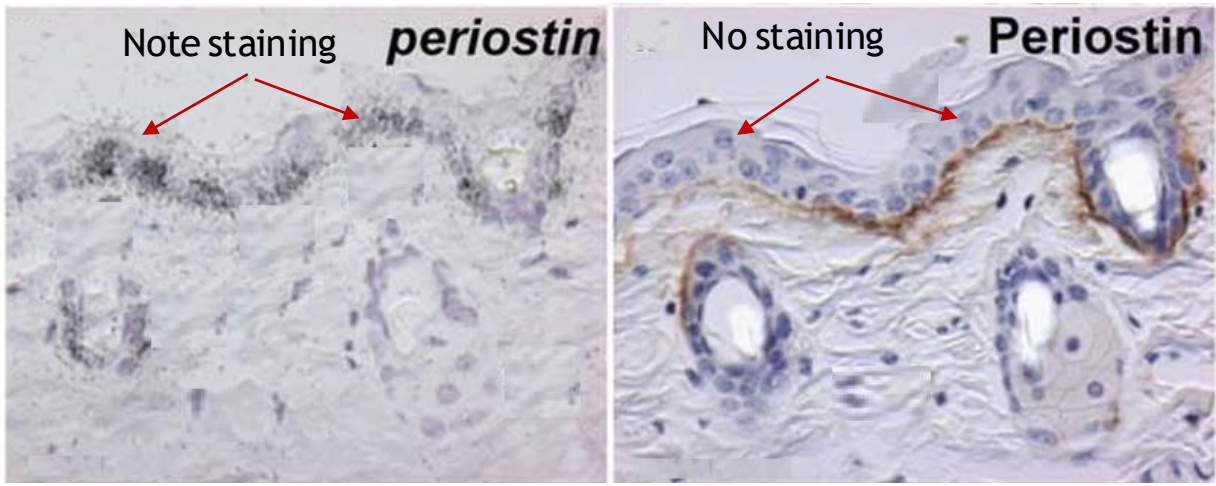
Why northern blotting and not immunohistochemistry?

- Sometimes, a gene may be expressed in certain cells, but the protein is located elsewhere.
- For example,
  - In in situ hybridization (ISH), the expression of a gene is observed at the RNA level in cells lining the basement membrane (this example ➔).
  - However, when using immunohistochemistry (IHC), the protein was found in the basement membrane.

✓ This means that the cells express the gene, and the protein is immediately exported to the basement membrane.

➢ **In situ hybridization helps identify the source of gene expression and provides insight into protein localization within tissue sections.**

ISH (RNA) **IHC (protein)**



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

mRNA: inside cells along the basement membrane

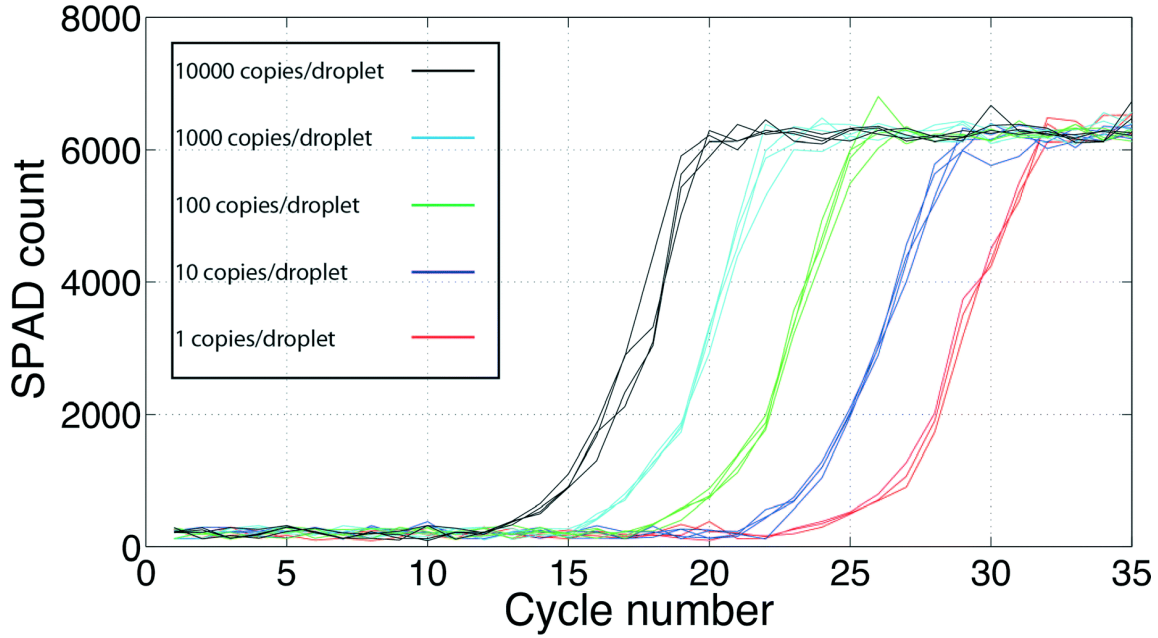
Protein: outside cells in the basement membrane

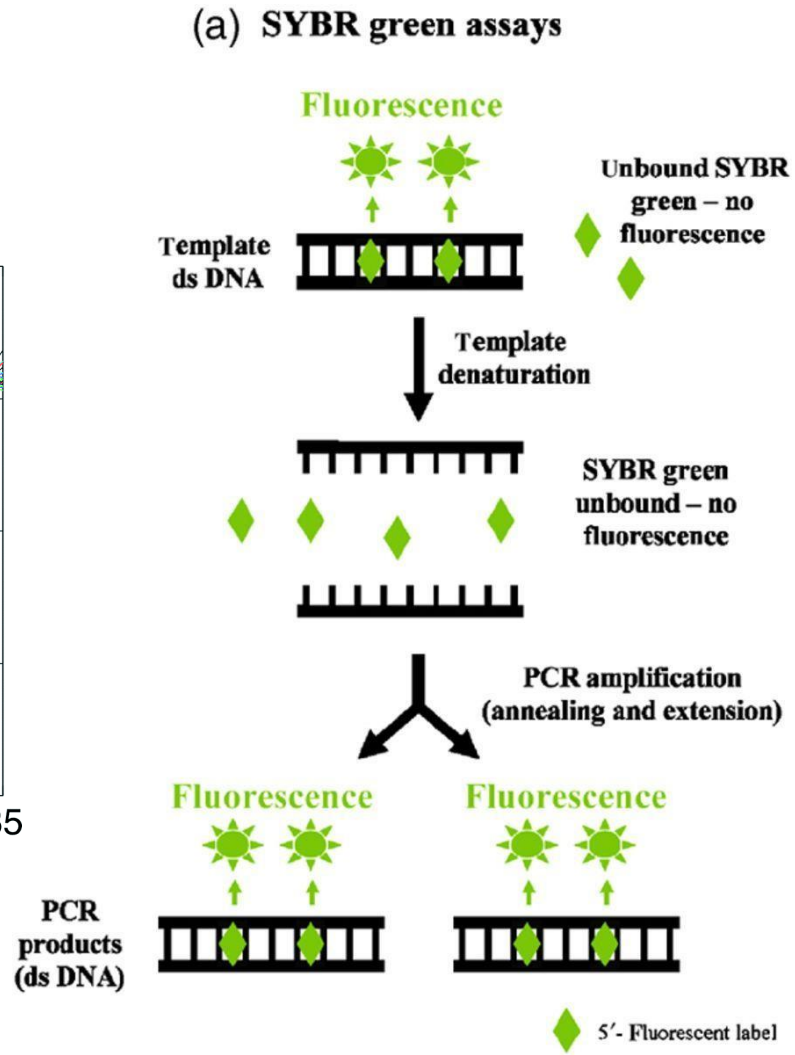
So, you can know that stained cells are the source of the protein, but not any other cell.

Quantitative reverse transcriptase real-time RT-qPCR of mRNA

RT ⇔ Reverse Transcriptase (RNA → cDNA)

- 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.



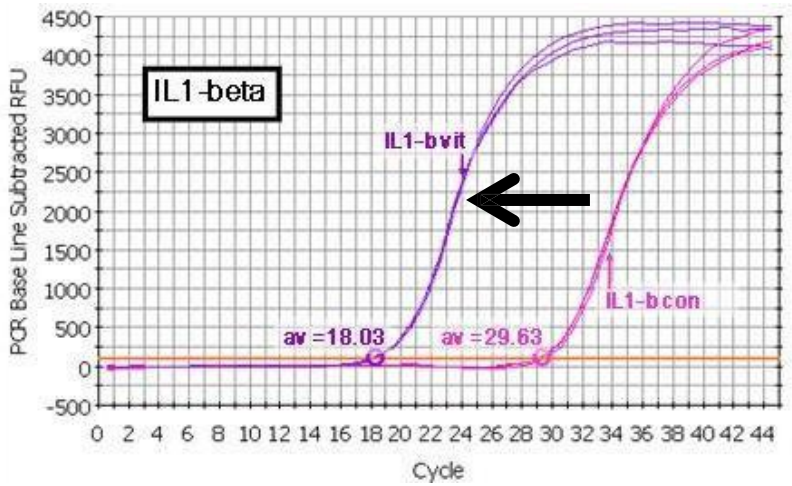


Apart from the reverse transcription here, this is completely equivalent to the technique discussed in *Lecture 6*.

Recall that the **higher** the initial load (RNA load here), the **lower** the threshold cycle (see x-axis in the graph above).

Example

A gene of interest

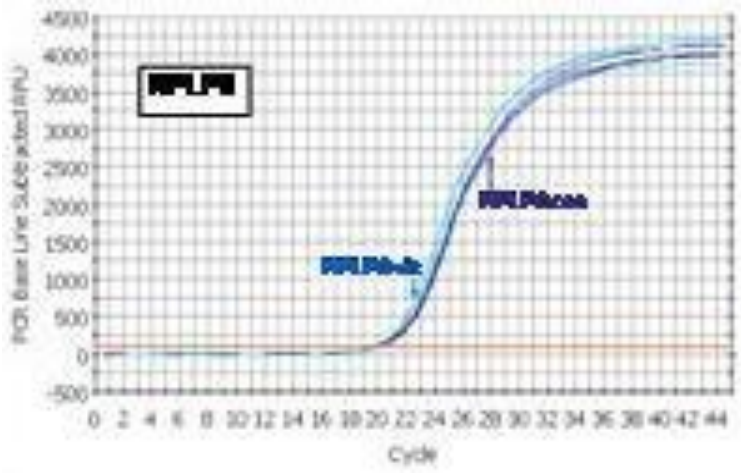


Notice the 2 curves in the graph above:

The right has higher threshold cycle → less RNA (cDNA)
The left has lower threshold cycle → more RNA (cDNA)

This indicates that this particular gene is transcribed to a greater extent in the sample shown on the left.

Housekeeping gene



Unaltered expression

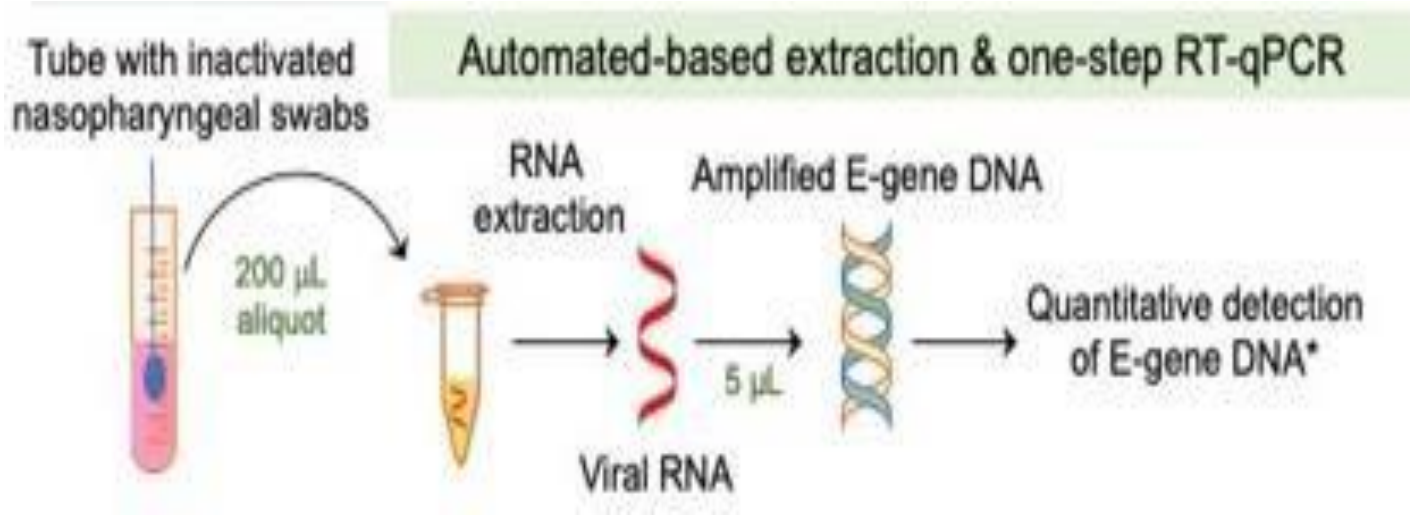
As discussed earlier in slide 10, we use a housekeeping gene to ensure that the experiment was well-conducted, and that both samples are initially equivalent.

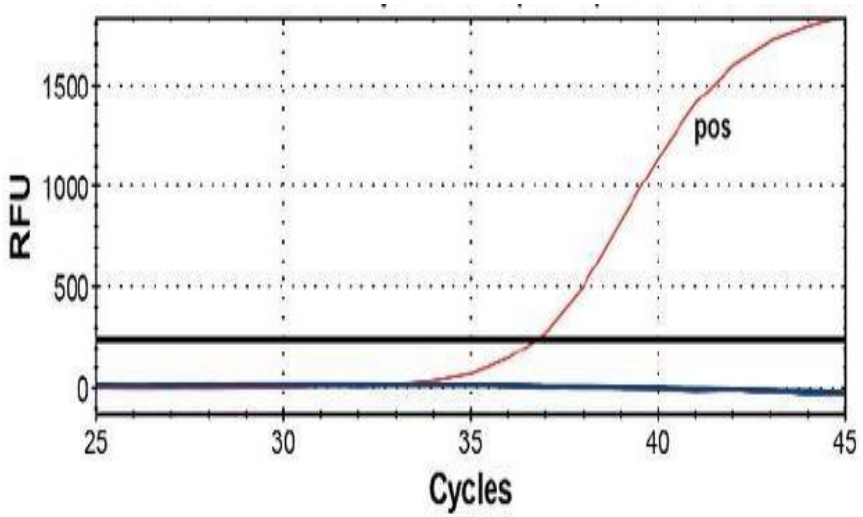
Detection of SARS-Co-2

كل التعب أجره عند الله

This RT-qPCR was famously used during the COVID-19 pandemic to assess the presence of coronavirus.

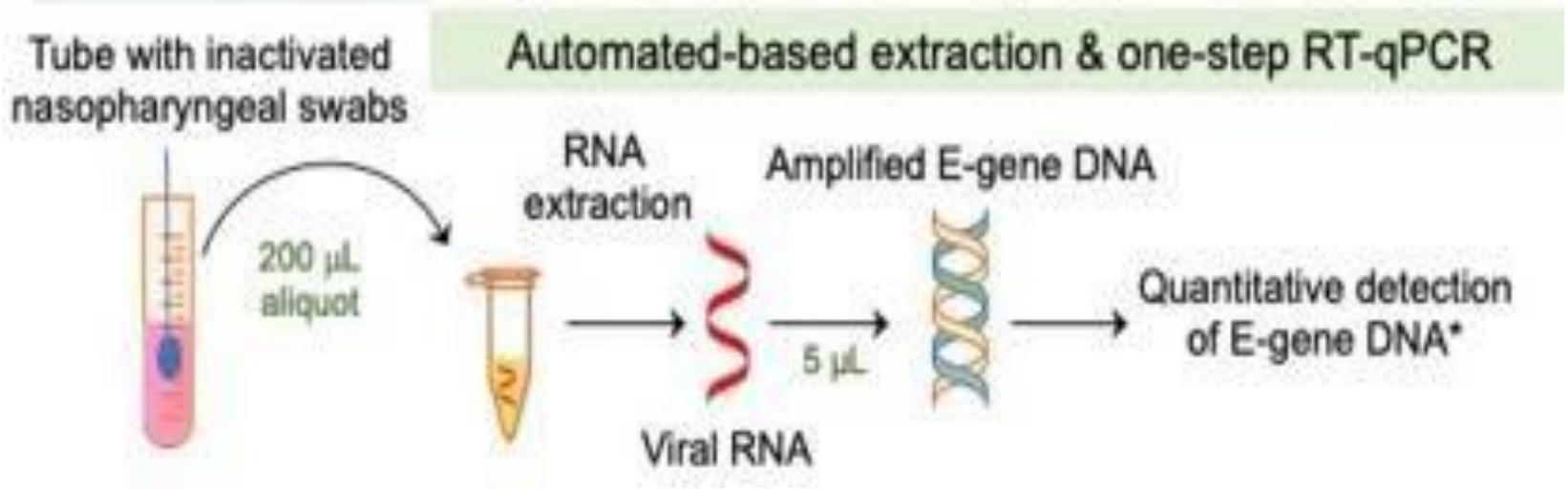
3 genes were monitored simultaneously (multiplexing).





The 3 genes are expressed → positive →Person has Coronavirus

Detection of SARS-Co-2

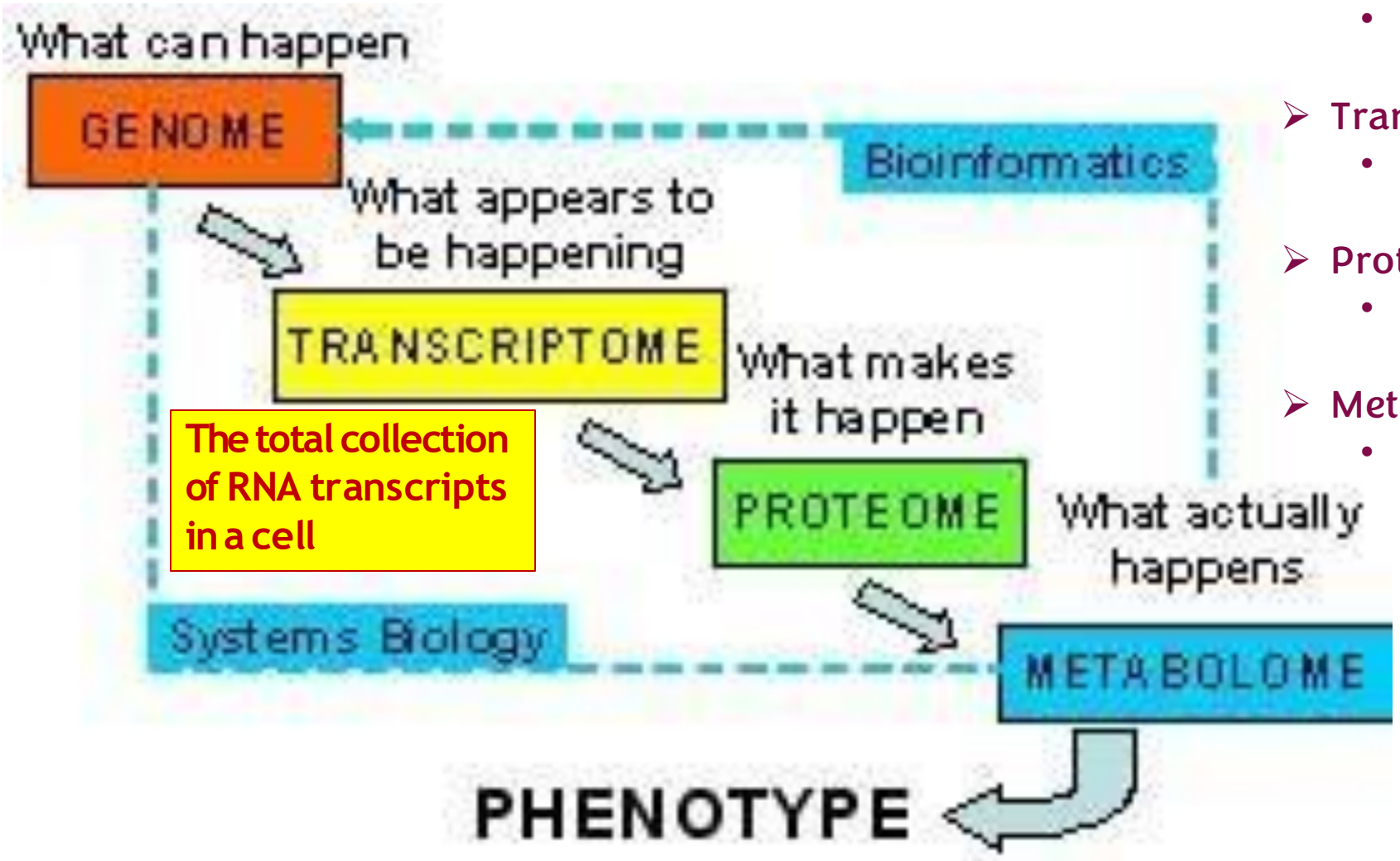


Detection of SARS-CoV-2 by RT-PCR

1. A nasopharyngeal swab is collected from a patient suspected of having COVID-19.
2. The viral RNA is extracted from the sample.
3. Because PCR amplifies DNA and not RNA, the viral RNA is first reverse-transcribed into complementary DNA (cDNA).
4. The cDNA is then amplified using PCR to detect the presence of the virus.
5. To confirm that the detected virus is SARS-CoV-2, three viral genes are amplified simultaneously.

✓ This technique is known as **multiplex PCR**, in which multiple DNA targets are amplified in the same reaction tube at the same time, increasing specificity and diagnostic accuracy.

The science of -omics



On a larger scale, similar to the genome which accounts for all the DNA in a sample, there exist other concepts:

- Genomics (the study of genomes)
  - DNA sequence
- Transcriptomic (// of transcriptomes)
  - RNA produced by transcription
- Proteomics (// of proteomes)
  - Proteins translated from mRNA
- Metabolomics (// of metabolomes)
  - Metabolites altered by enzymes

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.

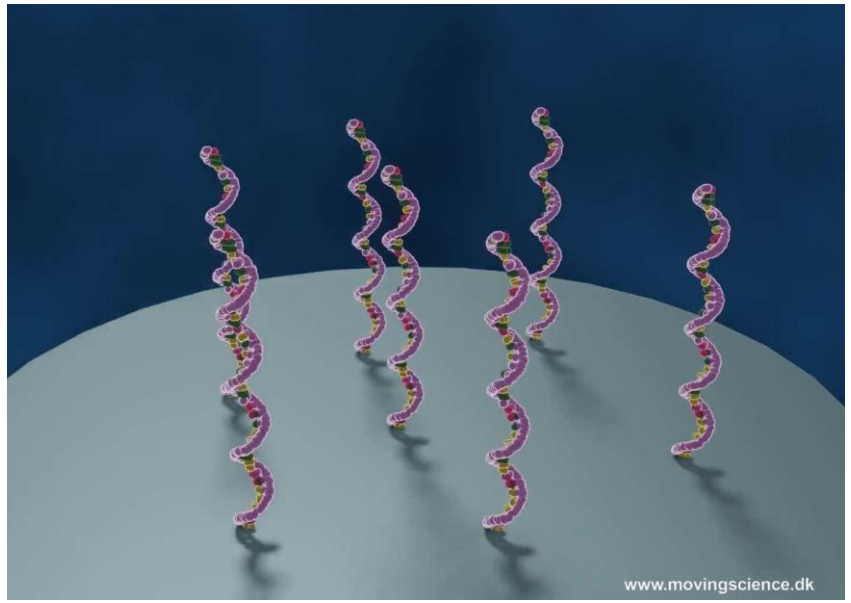
- By examining the expression of so many genes simultaneously, we can understand gene expression patterns in physiological and pathological states.

DNA microarrays

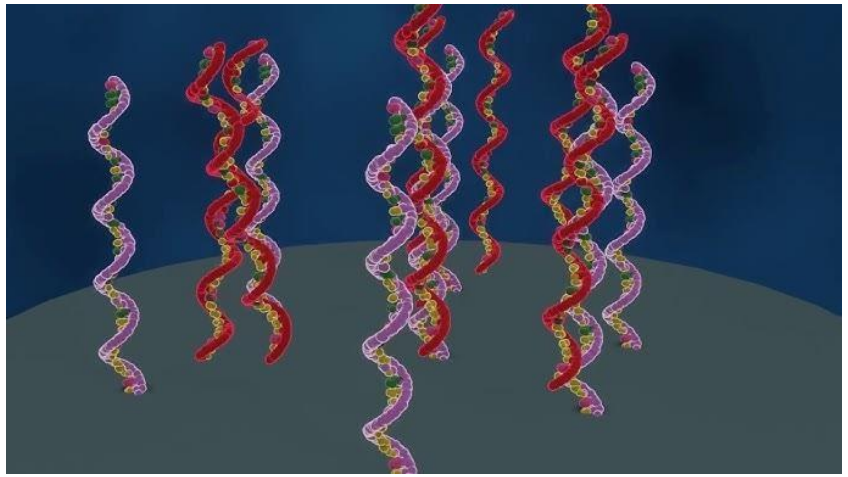
- DNA microarrays are solid surfaces (glass microscope slides or chips) spotted with up to tens of thousands of DNA fragments in an area the size of a fingernail.
- The exact sequence and position of every DNA fragment on the array is known.

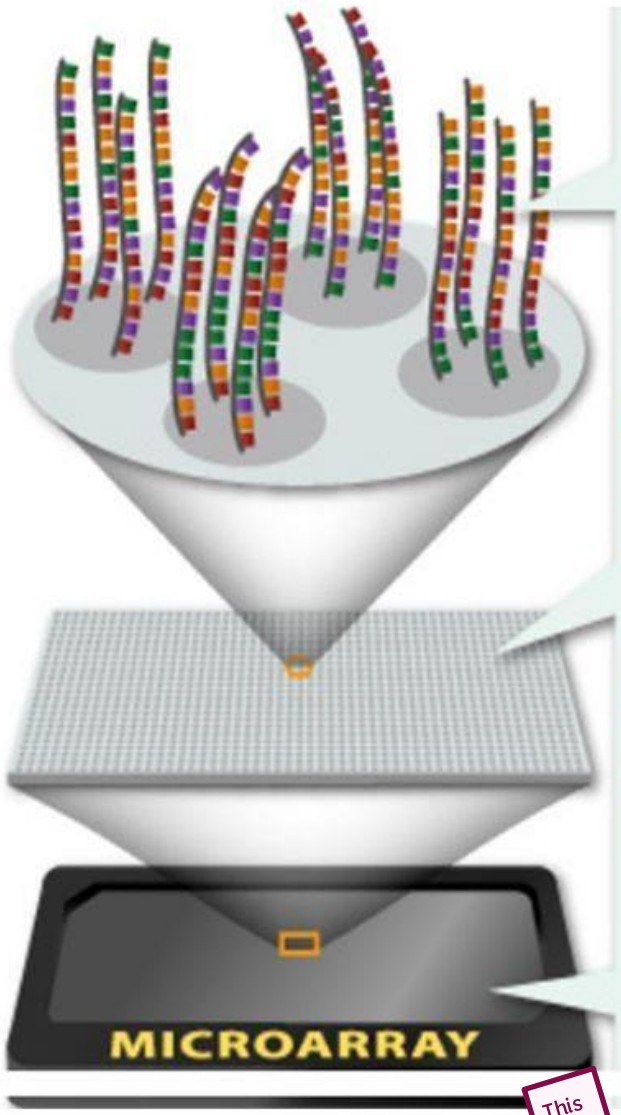
- http://learn.genetics.utah.edu/content/labs/microarray/
- http://www.sumanasinc.com/webcontent/animations/content/dnachips.html

Nonhybridized spot



Hybridized spot (partially)





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.

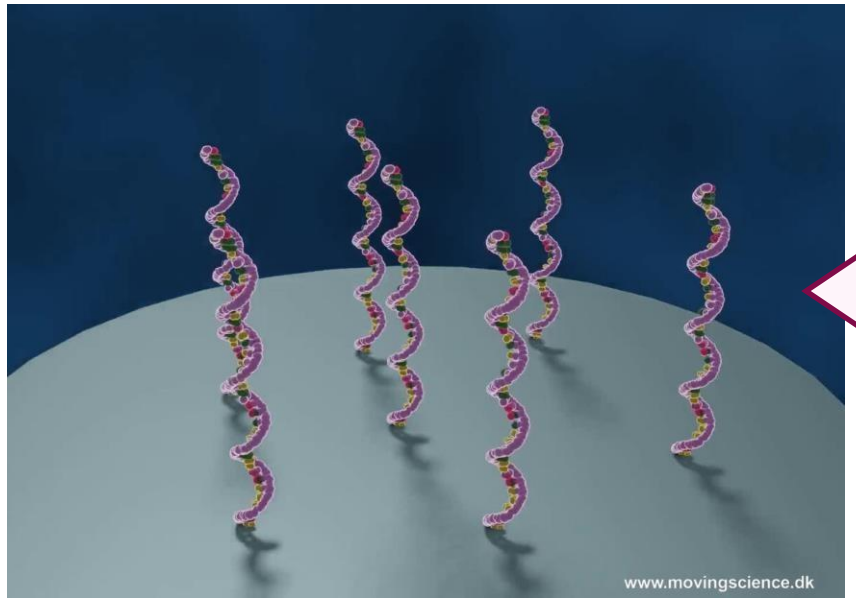
Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass).

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.

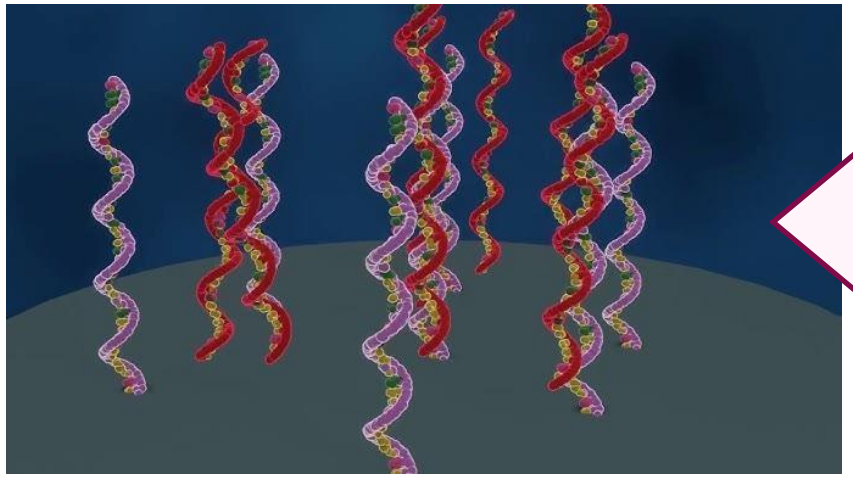
This whole thing is actually as small as that

Nonhybridized spot

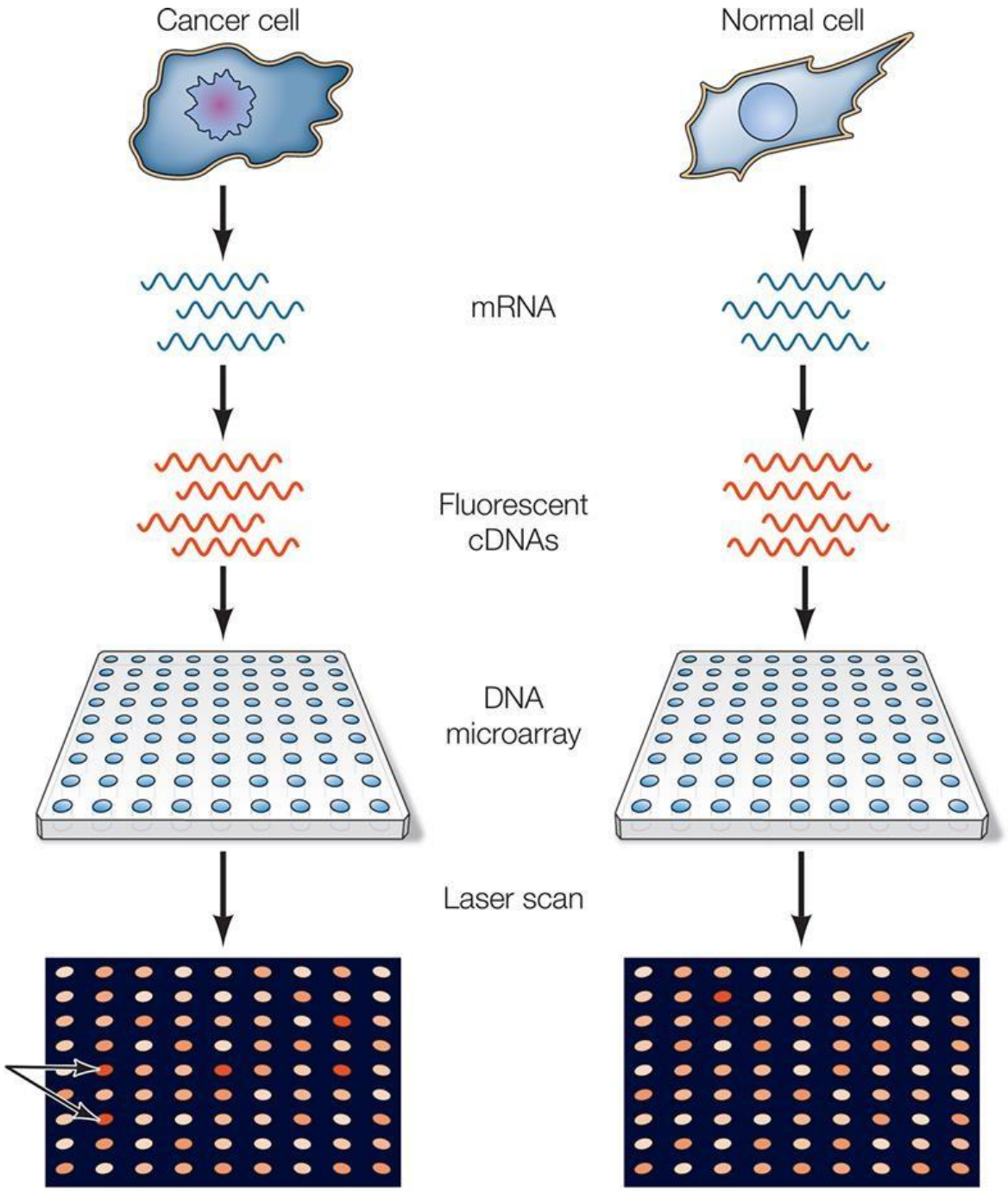


- A single-stranded nucleic acid probe is fixed to a solid surface at a specific spot.

Hybridized spot (partially)



- When DNA or RNA from the sample is added, it will bind only if it is complementary to the probe sequence.
- This binding occurs through nucleic acid hybridization (base pairing).
- After hybridization, a detectable signal is produced from that exact location on the solid surface.
- The presence of the signal indicates the presence of the target DNA or RNA sequence in the sample.



The procedure

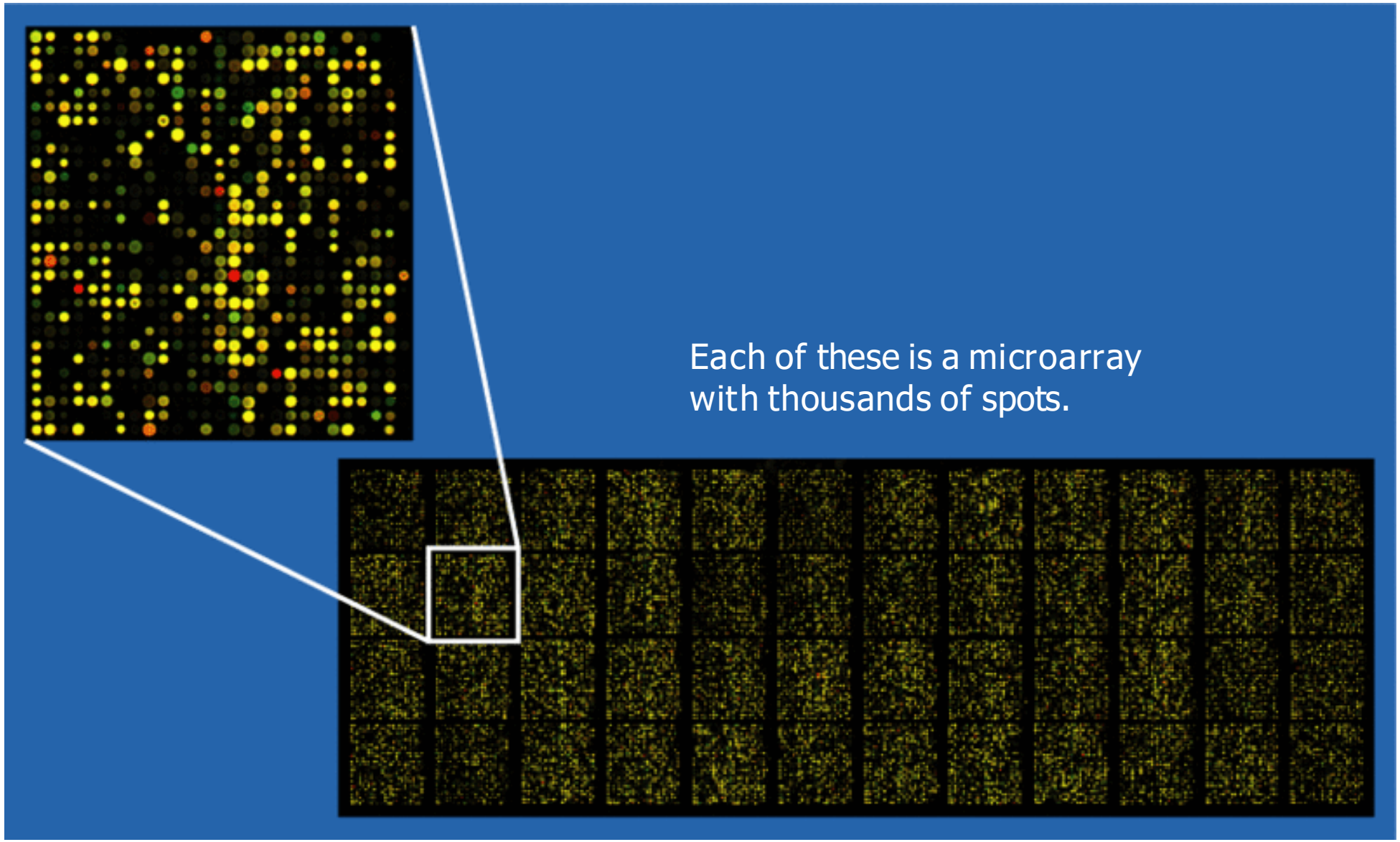
- mRNAs are extracted from cancer cells and normal cells and converted to cDNAs, which are labeled with a fluorescent dye.
- The cDNAs are then hybridized to a DNA microarray containing spots of oligonucleotides corresponding to 20,000 or more distinct human genes.
- The relative level of expression 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.

DNA microarrays – More explanation

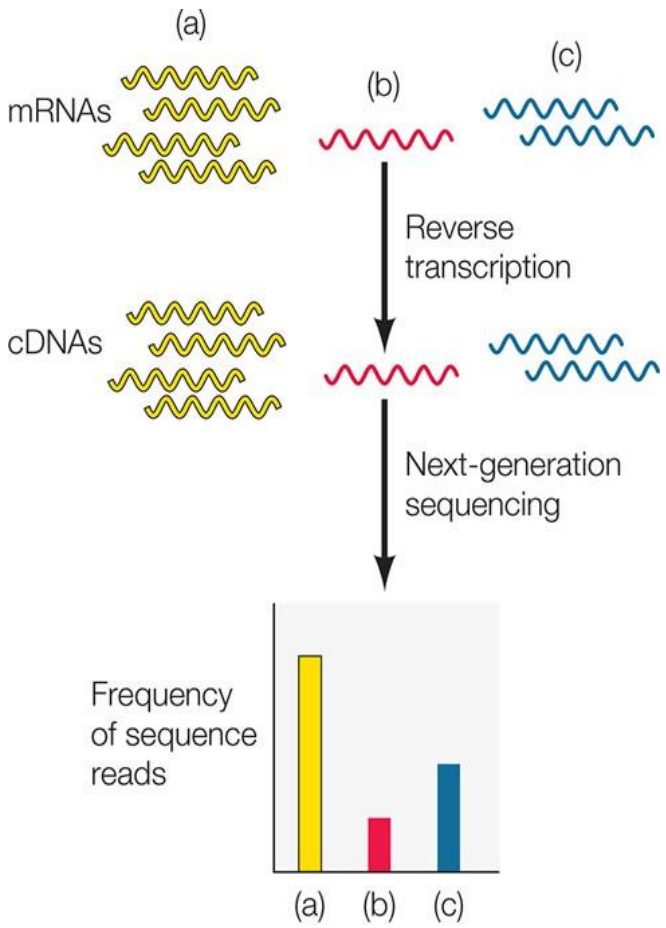
- The microarray is divided into thousands of spots.
- 20000 for protein-coding RNA + others for other RNA types.
- Each spot is composed of many copies of DNA probes attached to the underlying surface.
- DNA fragments at each spot are identical and are complementary to the cDNA of **exactly one gene.**
- After exposition to the cDNA sample, the microarray obtains a unique configuration related to the intensity of the signal from the fluorescent cDNA from the sample; the **amount of cDNA of a gene is proportional to the extent of expression of that gene in the sample, and thus the intensity of the signal at each spot reports the expression of the gene whose cDNA is complementary to the DNA fragments at the spot.**

• E.g., RED may indicate strong signal, PINK is weaker, and WHITE with no signal.

➢ Notice that each spot reflects the expression of one gene.



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.

Recall Lecture 7 where next-generation DNA sequencing was discussed.

The idea here is that reverse-transcripted RNA molecules cDNA are sequenced just like any DNA.

The more frequent a **cDNA** is sequenced, the more its **RNA** was initially made, and the more active the gene is.

Note: that we don't know the exact identity of that gene but we can know its sequence

RNA-seq vs. microarray

- RNA-seq can be used to
  - characterize novel transcripts the genes that we don't know about
  - Identify splicing variants it's important because we might not be able to do that with microarray
  - profile the expression levels of all transcripts

This technique, unlike microarrays, doesn't need a known complementary sequence to capture the DNA sought for.

Instead, it "sequences" the cDNA for all genes present; after that we can refer to databases to identify the sequences.

This is how it was known that about 75% of the genome is transcribable (not only protein-coding genes).

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

RNA-seq can overcome these limitations.

Analysis of transcriptional regulatory sequences

- **Let's now discuss how we can analyze transcriptional regulatory sequences.**

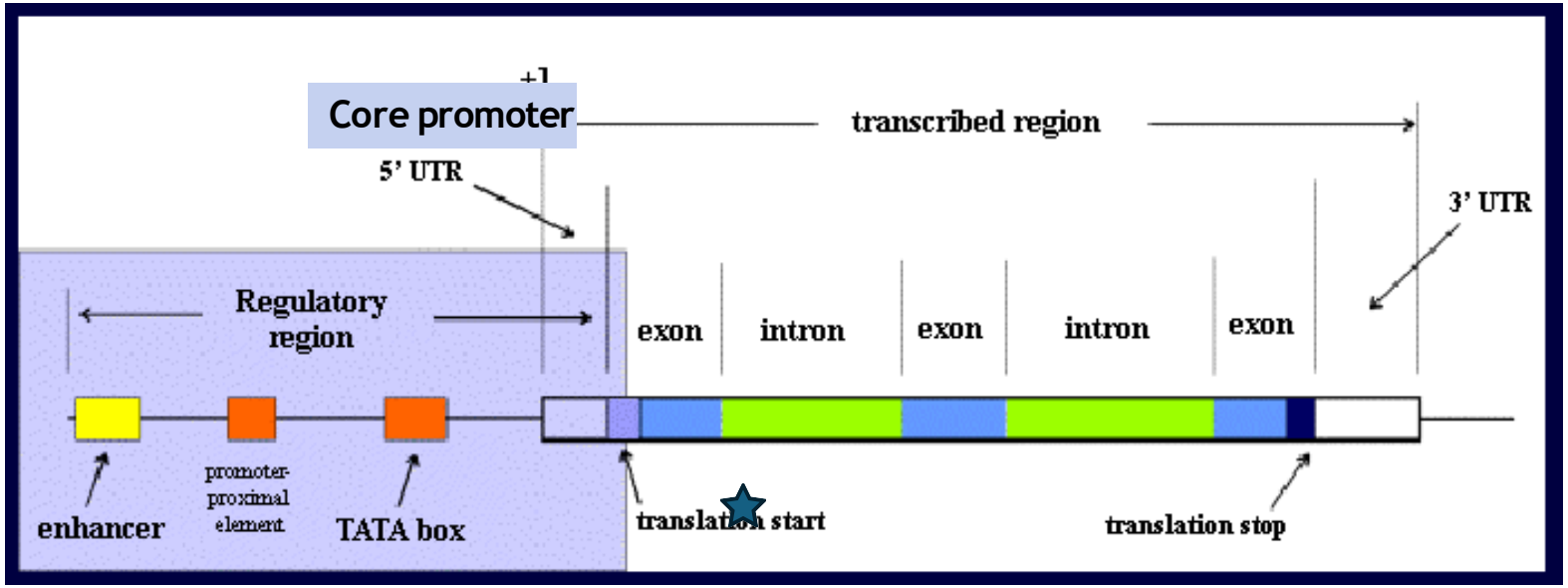
These include regulatory regions of DNA, such as promoters and enhancers, which serve as binding sites for specific regulatory proteins. For example, general transcription factors and specific transcription factors like the cAMP response element-binding protein (CREB) bind to promoter-proximal elements. Similarly, specific proteins, often called transcriptional activators or repressors, bind to enhancers. These proteins typically bind only to their specific enhancer sequences.

Now, suppose I want to study **how well** a gene is expressed in a specific cell type under certain conditions—for example, in the presence versus absence of a hormone or in light versus darkness. In this case, **I am not focusing on the gene itself but rather on how effective its promoter is under these conditions.**

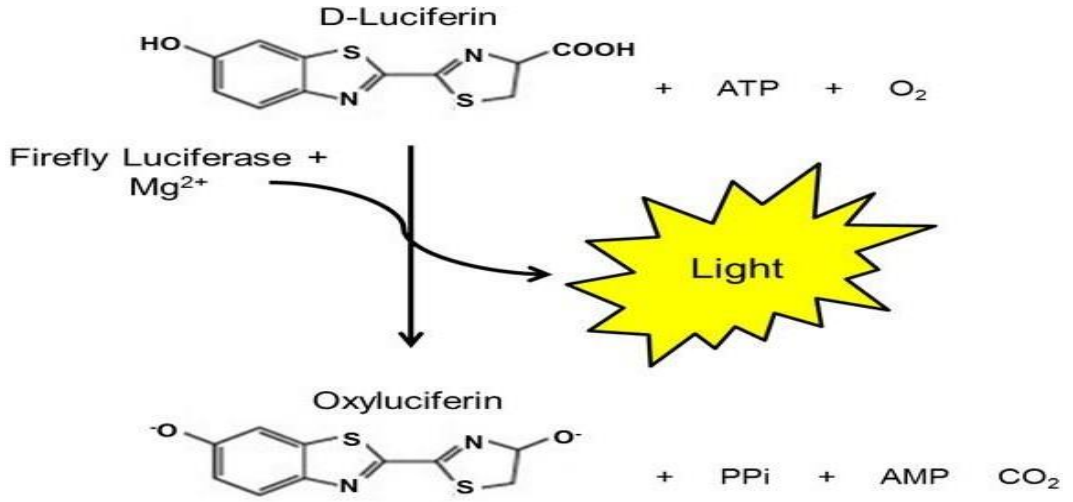
To study promoter activity, we can use DNA cloning to create recombinant DNA constructs and analyze promoter function under various conditions.

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

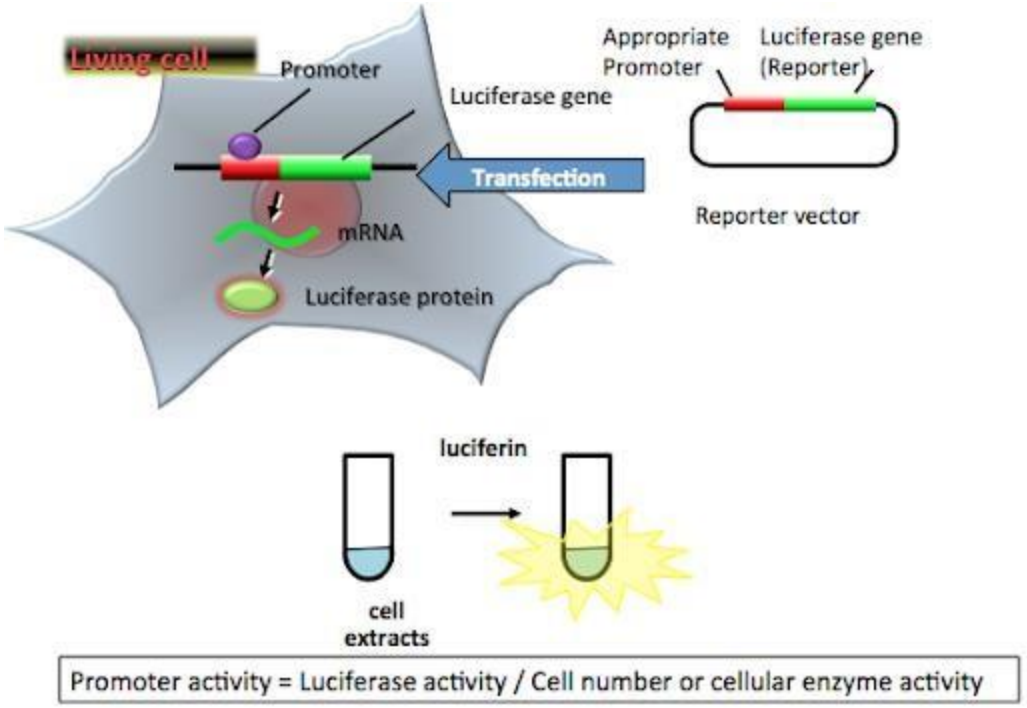


We utilize an enzyme found in fruit flies, which are insects known for their bioluminescent abilities. These flies have glowing abdomens that create a beautiful display at night. The enzyme responsible for this glow is called **luciferase**. It interacts with a substrate known as **luciferin**, which gets cleaved or converted by luciferase into a product called **oxyluciferin**. This is the molecule that produces the light. While the specifics of the reaction don't have to be our main concern, what we have to care about is that this enzyme cleaves luciferin to create a product that emits light.

So how can we take advantage of all these tools, molecular tools?

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.

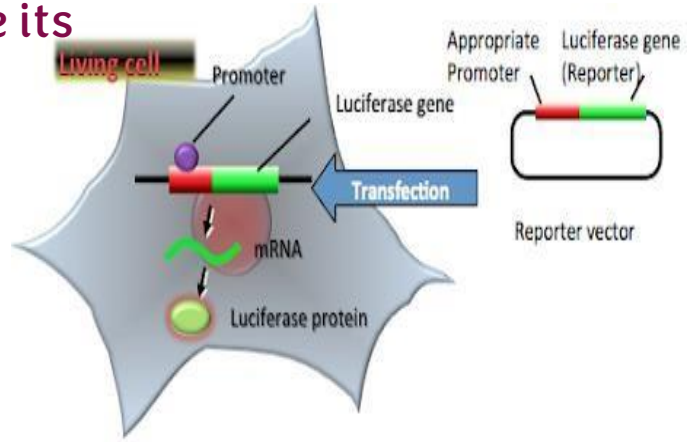


- This is how we study promoter activity:

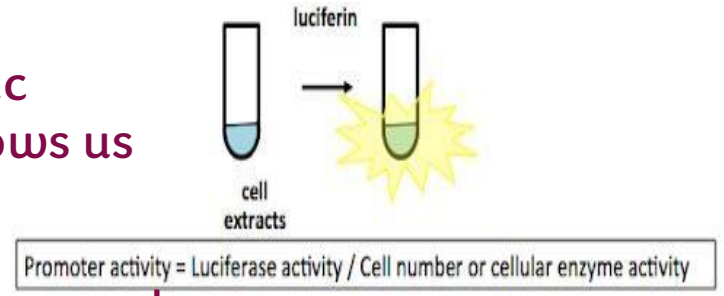
We create recombinant DNA using bacterial plasmids. For example, we use a plasmid containing the luciferase gene, originally derived from fireflies, and replace its native promoter with the promoter we want to study.

○ Let's say we place the luciferase gene under the control of the dopamine hydroxylase gene promoter. We create this recombinant DNA construct and then transfect it into a human cell, such as a nerve cell.

○ At this point, the nerve cell contains the recombinant DNA, including the luciferase gene regulated by the promoter of interest.



○ We can then expose these cells to different conditions—such as light versus darkness, the presence versus absence of a hormone, or the presence of specific neurotransmitters—and measure the expression of the luciferase gene. This allows us to study how the promoter responds to these conditions.



We then add luciferin, a substrate for the luciferase enzyme. If luciferase is expressed, it catalyses the oxidation of luciferin, producing a light-emitting product.

- The intensity of the light signal correlates with the amount of luciferase in the cell, which reflects the activity of the promoter under study.
- The stronger the light signal, the higher the promoter activity. We expose these cells to different conditions—such as the presence versus absence of a hormone, neurotransmitter, or stress—and measure the amount of light produced.
- This provides a quantitative measure of how the promoter is regulated under various conditions.

Another approach is to study the regulatory sequences within a specific DNA region.

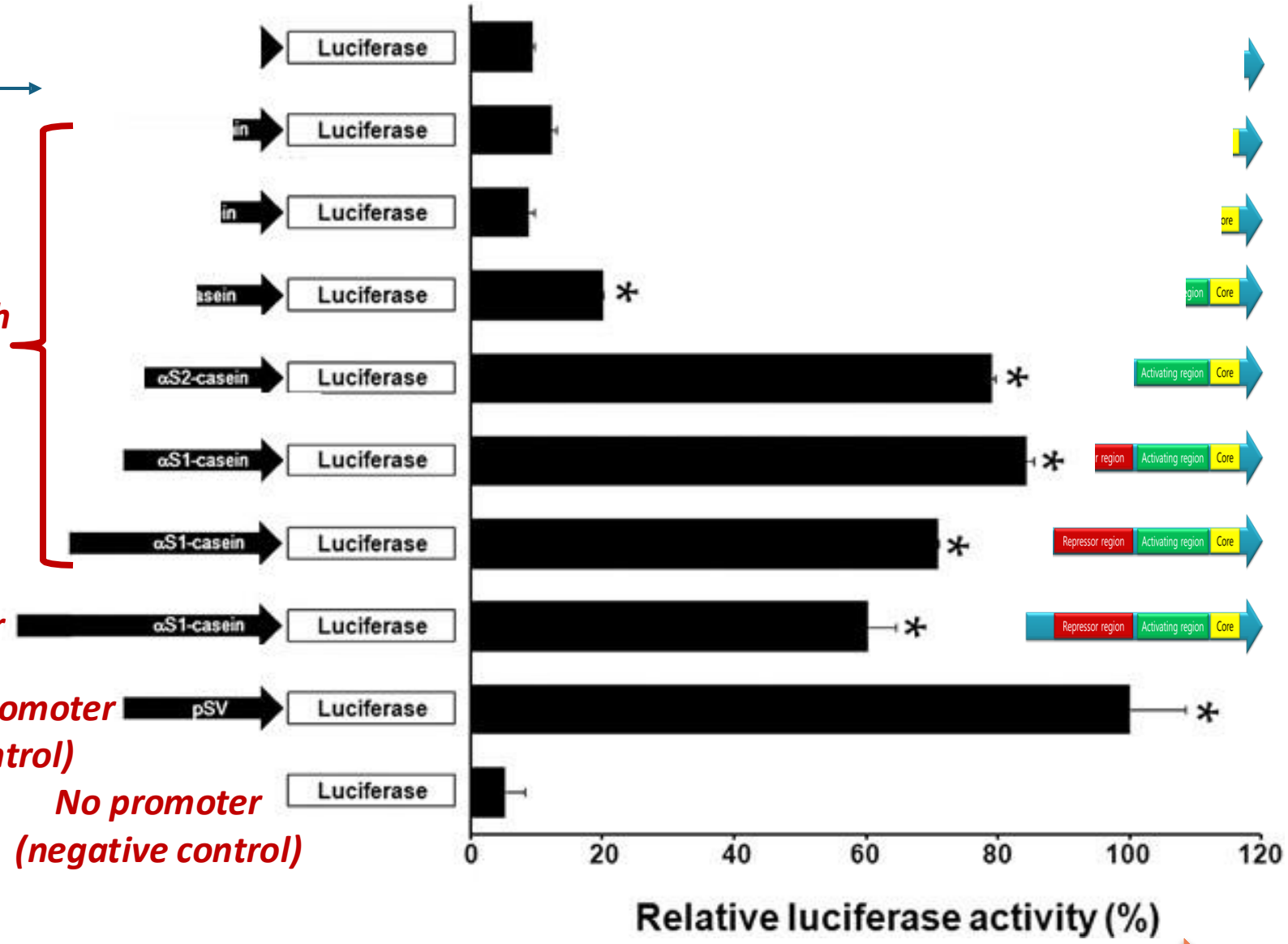
- For example, we may examine the core promoter, proximal promoter elements, and enhancers to determine their exact positions and functions.

We aim to identify the precise nucleotide sequences responsible for transcriptional regulation within these regions.

-To accomplish this, we use methods such as DNA footprinting to detect protein-binding sites, chromatin immunoprecipitation (ChIP) to map protein-DNA interactions, or reporter gene assays to test the functional activity of specific sequences.

Further details ↓

Example →



The promoter with deleted regions

The complete promoter

Any good promoter (positive control)

No promoter (negative control)

Repressor region | Activating region | Core

Explanation ↓

- We have the luciferase gene placed under the control of a promoter of interest. The promoter may contain regulatory sequences, including a repressor region, an activating region, and a core promoter sequence. The purpose of this experiment is to determine the location and function of these sequences.

- To study these regions, we clone the promoter DNA fragment into a plasmid containing the luciferase gene, creating recombinant DNA. This plasmid is then transfected into different cell types (e.g., nerve, skin, or stomach cells). Luciferase expression is measured based on light produced when luciferin is converted by luciferase.

- We start with the full promoter region, which includes all regulatory sequences, and systematically delete portions to observe how expression changes. Removing the **repressor** region should **increase** expression, indicating that this region **inhibits** transcription (luciferase expression).
Conversely, deleting the **activating** region should **decrease** expression, showing that it enhances transcription.

- If the **core** promoter is removed, transcription should **nearly stop**, confirming its essential role in initiating transcription.

Controls:

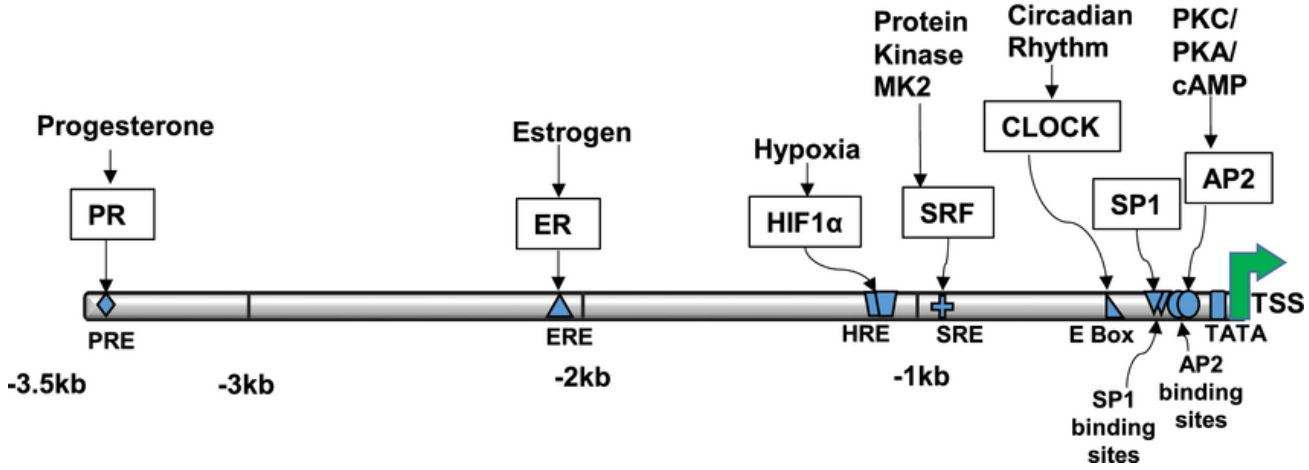
Negative Control: The luciferase gene without a promoter shows minimal expression due to possible background “leakage.”

Positive Control: The luciferase gene with a known strong promoter produces maximum light output, ensuring the experimental setup works properly.

- This method is called the luciferase reporter gene assay(experiment that measures something) , where luciferase acts as a “reporter” to indicate gene expression levels. The assay helps evaluate how promoters behave under different conditions (e.g., hormones, stress, or nutrients) by measuring light output.

The more luciferase we have the more light we have.

Identify the regulatory protein-binding sites



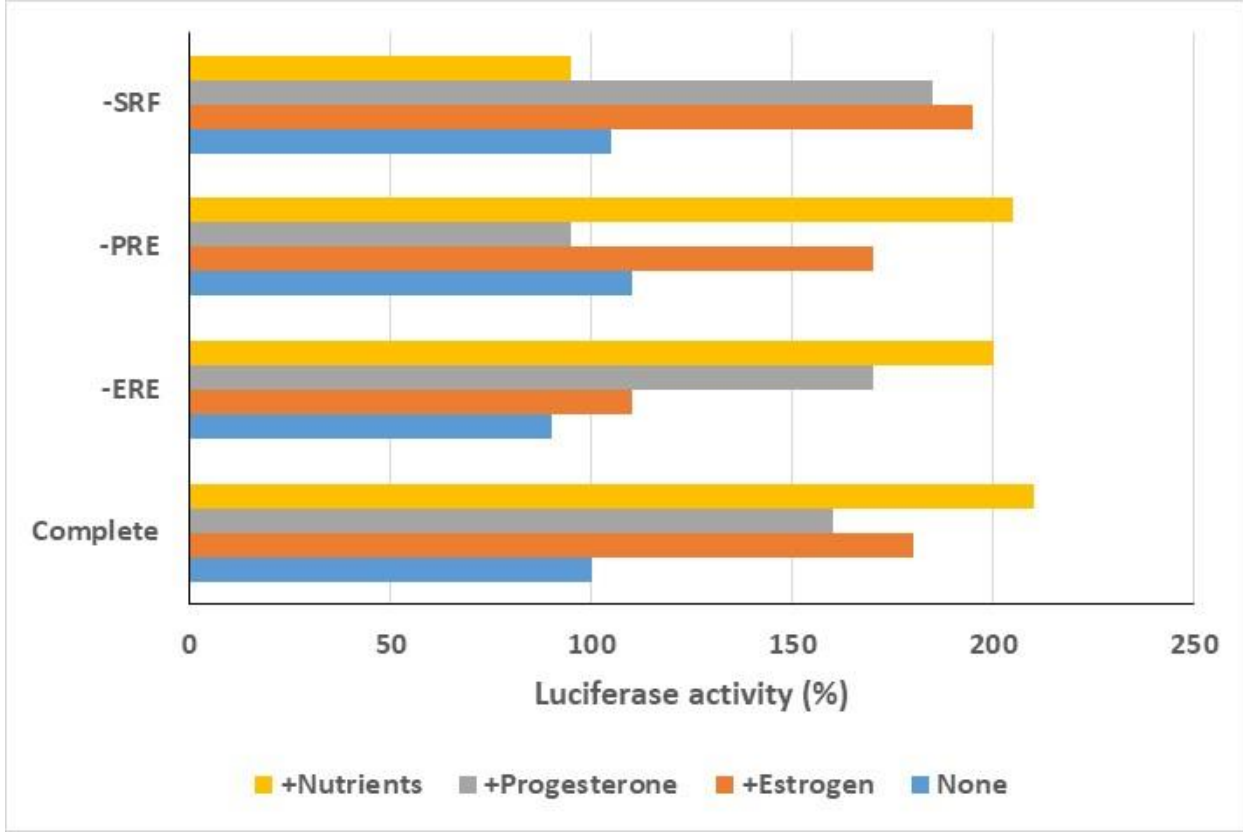
You have the promoter of a gene that can be regulated by multiple molecules.

- 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?

Next slide to understand



Luciferase Reporter Gene Assay and Identification of Response Elements

The **luciferase gene** is placed under the control of a specific **promoter region**. This promoter–luciferase construct is cloned into a **plasmid**, which is then introduced into cells by **transfection**. Consequently, luciferase gene expression becomes regulated by different experimental conditions such as **estrogen, progesterone, or nutrients**.

Experimental Conditions and Basal Expression

Several experimental conditions are established to assess gene regulation:

1.Basal Expression (Control Condition)

The plasmid is transfected into cells without adding any hormones or stimulatory factors.

The measured luciferase activity in this condition is considered **100% luciferase activity** and is referred to as **basal expression**, which represents the **minimal level of gene expression** in the absence of activators or inhibitors.

This condition serves as the **reference control (ruler)** against which all other conditions are compared.

Hormone- and Nutrient-Induced Stimulation

2.Estrogen-Stimulated Condition

When cells are treated with **estrogen**, gene expression is stimulated. Estrogen binds to its specific **estrogen receptor**, and the estrogen-receptor complex translocates into the nucleus, where it binds to the **estrogen response element (ERE)** located in the promoter region.
This binding leads to **enhanced transcription**, resulting in an **increase in luciferase enzyme activity**.

3.Progesterone-Stimulated Condition

In the presence of **progesterone**, gene expression is also stimulated. Progesterone binds to its specific **progesterone receptor**, and the hormone-receptor complex binds to the **progesterone response element (PRE)** in the promoter region.
This interaction stimulates transcription and increases **luciferase activity**.

4.Nutrient-Stimulated Condition

When cells are exposed to **nutrients**, gene expression is stimulated through signaling pathways such as the **MAP kinase signaling pathway**. This leads to activation of the **serum response factor (SRF)**, which binds to the **serum response element (SRE)** in the promoter region.
As a result, transcription of the luciferase gene is enhanced, and luciferase activity increases.

Functional Analysis of Response Elements

To confirm the specific role of each response element, deletion experiments are performed:

- **Removal of the Estrogen Response Element (ERE):**

When the ERE is removed from the promoter and the plasmid is transfected into cells, basal luciferase expression becomes **lower than 100%**.

Upon addition of estrogen, luciferase activity shows **minimal or no increase**, indicating that the ERE is essential for estrogen-mediated gene activation.

In contrast, addition of progesterone still results in increased luciferase activity, demonstrating that progesterone does not act through the ERE. Nutrient-induced stimulation is also observed, confirming that these effects are mediated by different elements.

- **Removal of the Progesterone Response Element (PRE):**

Deletion of the PRE abolishes the response to progesterone. As predicted, gene expression does not change in the presence of progesterone because the binding site for its receptor is absent, while responses to estrogen or nutrients may still occur.

- **Removal of the Serum Response Element (SRE):**

When the SRE is removed, nutrient-induced stimulation is lost, and the addition of nutrients no longer increases luciferase activity. This confirms that the SRE is responsible for nutrient-dependent regulation of gene expression.

Minor increases or decreases in luciferase activity are considered **experimental variation** and are not biologically significant. Only **large and reproducible differences** are interpreted as meaningful regulatory effects.

Conclusion

Using this approach, the specific **transcriptional regulatory protein binding sites** (ERE, PRE, and SRE) within the promoter region can be identified and functionally validated. Each response element is responsible for mediating gene expression in response to its corresponding stimulus.

رسالة من الفريق العلمي:

< من البُنية الجزيئيّة إلى البُنية الكونيّة : والخالقُ واحد >

تبيّن حديثًا للعلماء أنّ الكون ليس فراغًا ساكنًا، بل بناءٌ مُحكم ذو بنية هندسيّة دقيقة؛ نسيجٌ كونيّ يتمدّد ويتشكّل وفق قوانين محسوبة، حتى صار من الشائع في الأدبيّات العلميّة وصفه بـ البنية الكونيّة (Cosmic Structure)

فقد أظهر الرصد الفلكي المتقدّم أنّ المجرّات لا تتوزّع عشوائيًّا بل تنتظم في جسور كونيّة (Cosmic Filaments) وجدران كونيّة (Cosmic Walls) تفصل بينها فراغات هائلة، في صورة تشبه البناء المتماسك، لا الفضاء الخالي.

أكّدت الدراسات الحديثة باستخدام منظارَي هابل وجايا
(Hubble Space Telescope & Gaia Space Observatory)
معًا أنّ الكونَ في توسُّعٍ سريع ومُستمرّ، وأنّ معدّل توسُّع الكون يقترب من ثابت هابل : $H_0 \approx 73.5\ \mathrm{km/s/Mpc}$

فسبقت الآية الكريمة كلّ هذه الاكتشافات الحديثة إلى وصف السماء بلفظ (البناء):
{والسّماءَ بنَيْناها}، وأنّها في حالة توسُّع: {وإنّا لَمُوسِعون}
• **لُغويًّا** : (موسِع) اسم فاعل، يدلُّ على الأزمنة الثلاثة؛ الماضي والحاضر والمُستَقبل؛ والمعنى أوسعناها في الماضي ونوسِعُها في الحاضر وسنوسِعُها في المستقبل..

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			