Sheet no.12

Molecular biology

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Lecture Topic: Measurement of Transcriptional Activity

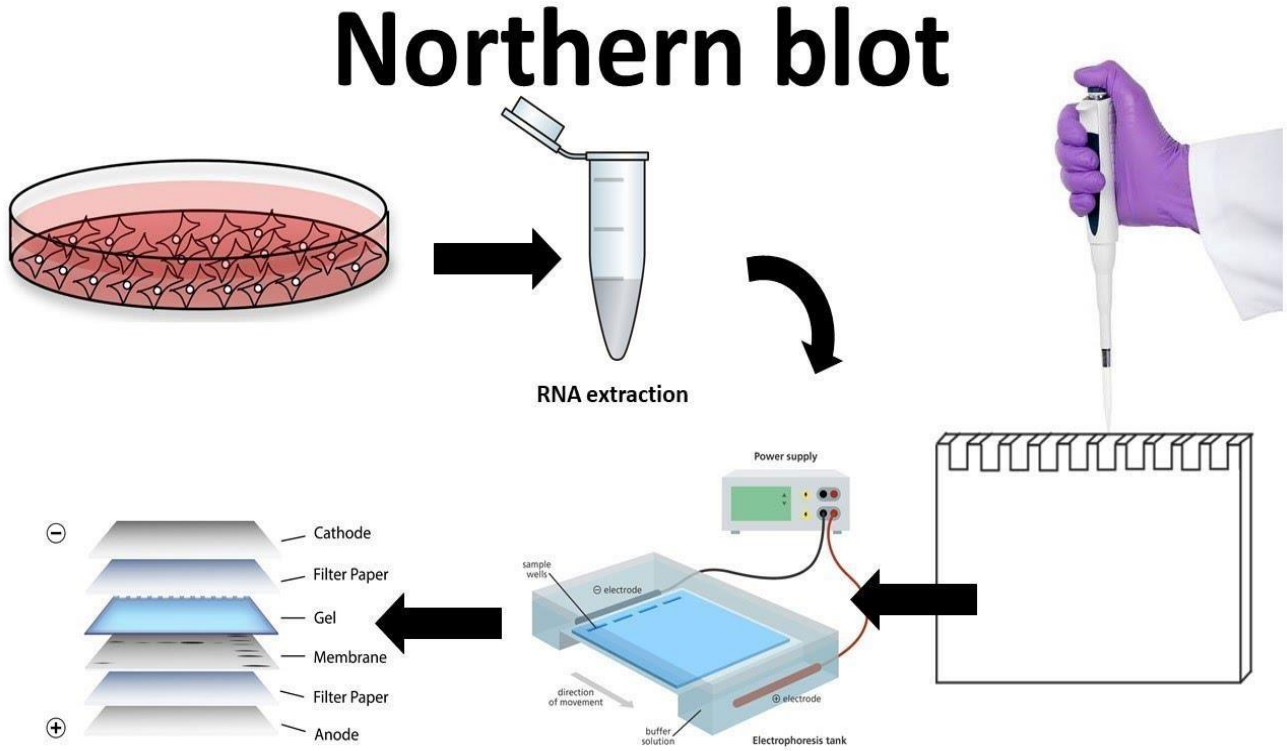
- In this lecture, we will learn about different techniques used to
- measure the **amount of RNA present** in our samples and identifying **sites of gene expression**.

  Recall the following from previous Lectures:

  - the amount of RNA indicates the degree of transcription (Large amounts of RNA mean high transcriptional activity and vice- versa)
  - Although we might have around 22000 protein-coding genes within our Human Genome, Not all of these genes are active (In regards to gene expression) . Around **5000-6000** genes are only active for any type of cell .
  - We have a variety of RNA molecules due to
    1- Formation of Isoforms via Alternative slicing
    2- Large number of genes as we mentioned previously.

- This is important as it allows us to know and understand many things about our sample, for instance:
  - We can measure and compare the transcriptional activity in 2 cells; one is healthy, while the other is affected by a disease. We can understand then the effect of the disease on the transcriptional activity of the cell (if it affects it at all) and this will help us in finding the treatment for various diseases.

- The big question is: How can we measure RNA levels and site of expression ? The answer lies in these two basic methods :
  - **Northern blotting**
  - **In situ hybridization**



Northern Blotting

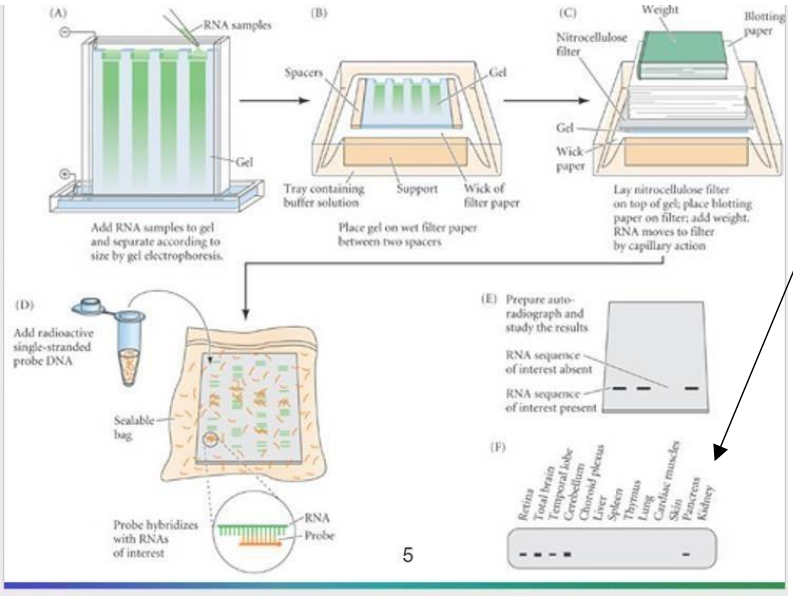
- Remember **southern blotting** that we have studied previously.
- Southern blotting gets its name from the scientist Edward **Southern**. After developing this technique, another scientist who lived in **Northern** U.S later developed the Northern blotting technique, hence the name **Northern Blotting** .
- There is also something called Western blotting used for proteins . (Not related to our lecture at all)
- RNA molecules range in length from 20 Bases to 10000 Bases. Due to this variability in RNA-lengths, when we form fractionation, we will get less distinct bands that are more or less smeared in comparison to DNA bands .

- Now, what is the procedure of this technique?

It is done **almost** exactly like Southern plotting except that :

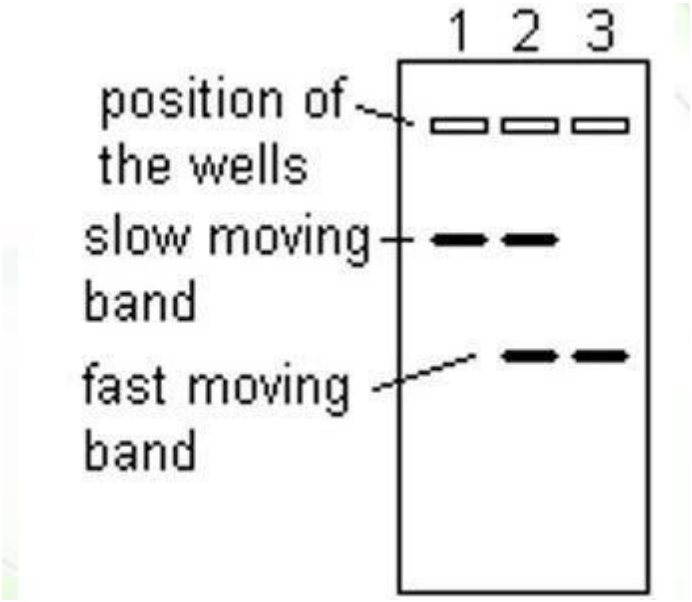
1- RNA from cells is isolated instead of DNA.
2- RNA molecules are fractionated based on size by **gel electrophoresis**. (using agarose gel as usual)
3- The fractionated RNA molecules are transferred onto a membrane (nitrocellulose fiber) .
4- RNA molecules are targeted by a labeled **DNA probe** with a sequence that is complementary to these specific RNA molecules.
    a- We can have Both RNA and DNA probes. **In northern blotting** , we use DNA probes that are complementary for a given RNA Molecule .Whereas in **Southern blotting** , we use RNA probes.
    b- Recall that these probes are labelled by a radioactive substance or a fluorescent substance In order to emit a detectable signal in Autoradiography.

- What information can you deduce from northern blotting?
    - Whether the gene for this specific RNA is expressed or not
        - That is: whether the probe emits signals or not.
    - The size of the targeted RNA molecule
        - Gel electrophoresis differentiates RNA strands of different sizes.
    - The amount of gene expression of a specific gene in different tissues.
        - Remember that all somatic cells of an individual's body have exactly the same genome. However, it is the level of transcription that is different.



- Look at the illustration of the **Northern blotting**. It is similar to southern blotting.
- Notice that the different tissues indicate different levels of transcription (different probe signaling) of the same gene. Some tissues do not transcribe it at all because they just do not need it!

- **Housekeeping genes**: These are the genes that are transcribed in all cells in all tissues, simply because the cell and the tissue as a whole just can't survive without them. So, the probe that detect them will always emit signals whenever it is applied.
    - **In other words**: They are genes with <u>constant expression</u>.
        - Examples include:
            - Histone genes: All cells have DNA that must be packed.
            - Myosin genes, actin genes, etc.
- Look at the following results of northern blotting of different samples:

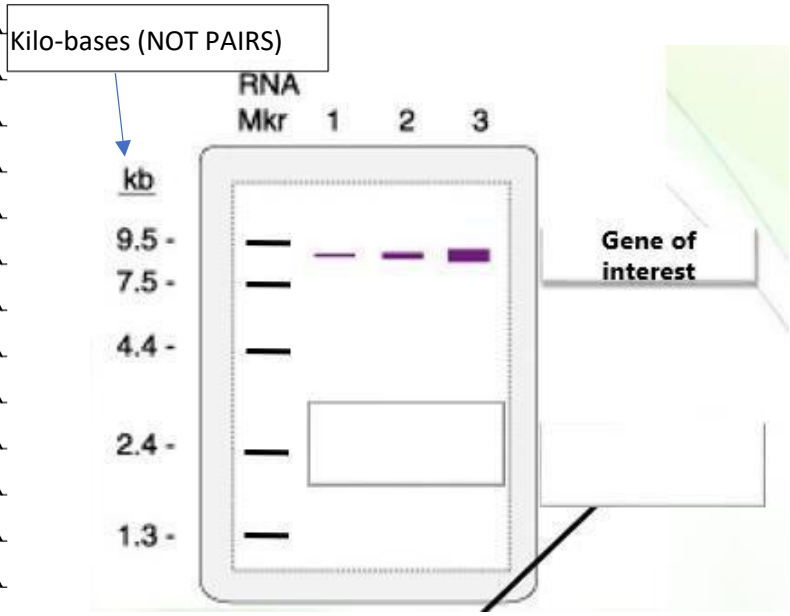


- What are your interpretations of the results?
    - Different interpretations are possible, as we have no information about what these samples are, or where they come from. The possible interpretations are:

1. All these samples are from the same gene, but they have different transcripts (mRNA) due to **<u>alternative splicing</u>**.
2. The probe is not specific and can bind to RNAs with related (not identical) sequences. Samples 1 and 3 each transcripts its own gene, but sample 2 has transcriptional activity for both genes and the probe can bind to both RNAs as it is not specific. To make things clearer:

- Sample 1 →Gene A is transcribed.
- Sample 2 →Genes A and B are transcribed.
- Sample 3 →Gene B is transcribed.
- The probe binds to both RNA molecules because their sequences are similar.
- Note: RNAs with similar sequences synthesize proteins that belong to the same family. For example, 2 RNA molecules that have similar (NOT IDENTICAL) sequences, one can synthesize H2A protein and the other can synthesize H2B protein.
- That was another possible interpretation of the results, think of more

- Let's see another example of test results:



- Ask yourself these questions:

1. Is the gene expressed?
a. Yes, in all 3 samples.
2. What is the size of the detected RNA?
a. About 9000 bases in all 3 samples.
3. What is the extent (activity level) of transcription?

- If you want to answer the third question, you will most likely say that sample 3 (since it's the thickest band) has the highest transcriptional activity while sample 1 has the lowest transcriptional activity because the thickness of sample 3 indicates that it has more RNA (transcript) than the others.
- This answer is not entirely true. The previous result could be of three samples that have been filled in the wells **in unequal proportions** (That is , having different masses) and therefore the thickness of sample 3 is simply because we have added too much of that sample .
- To further explain , Assume that the researcher have used 1 gram of sample (1) , 10 grams of sample (2) and 100 grams of sample (3) . In such a case, The Sample 3 band is going to be thickest simply because we used a larger mass and not necessarily that sample 3 has a high transcriptional activity .

How can we solve this problem?

- we use **housekeeping genes**. Remember what these genes are present All cells and therefore if our samples have Housekeeping genes-transcribed bands that are the same in thickness, then we are dealing with samples that are equal in amount and our previous results are valid . Because samples of the same weight have the same transcriptional activity of these housekeeping enzymes. On the other hand, samples with different amounts will show different thicknesses of the Housekeeping-genes bands.

- Here , Since each sample has a different amount of RNAs transcribed from the housekeeping genes, and housekeeping genes have constant expression (**equallevels of transcriptional activity in all cells**), The results regarding the transcriptional activity of the gene of interest are not valid .

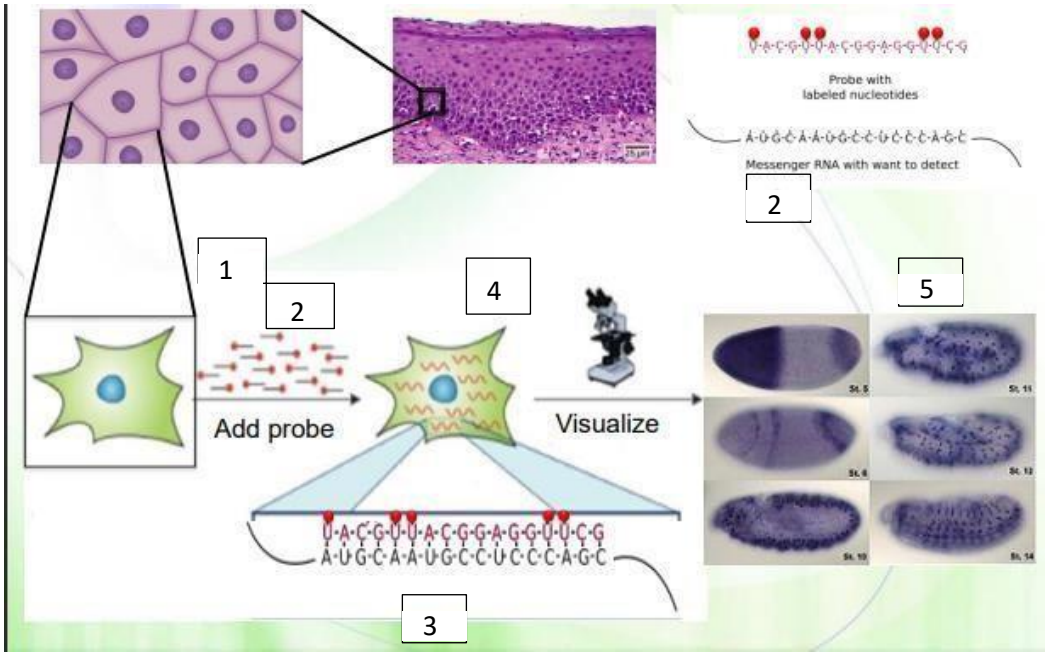
- Now lets examine this result. Here, since all the band of the housekeeping gene are of the same thickness (i.e of the same transcriptional activity) , all samples are equal in amount and therefore, the results regarding the transcriptional activity of the gene of interest are valid with Sample 3 having the highest transcriptional activity for that RNA molecule .

In Situ Hybridization

- "In situ hybridization" method reveals the distribution of specific RNA molecules in cells in tissues.
    - The term "**in situ**" means "**in place**".

- This means that RNA is labeled by a probe in its location wherever it exists.
- RNA is not extracted; it is labeled as it is in the tissue.

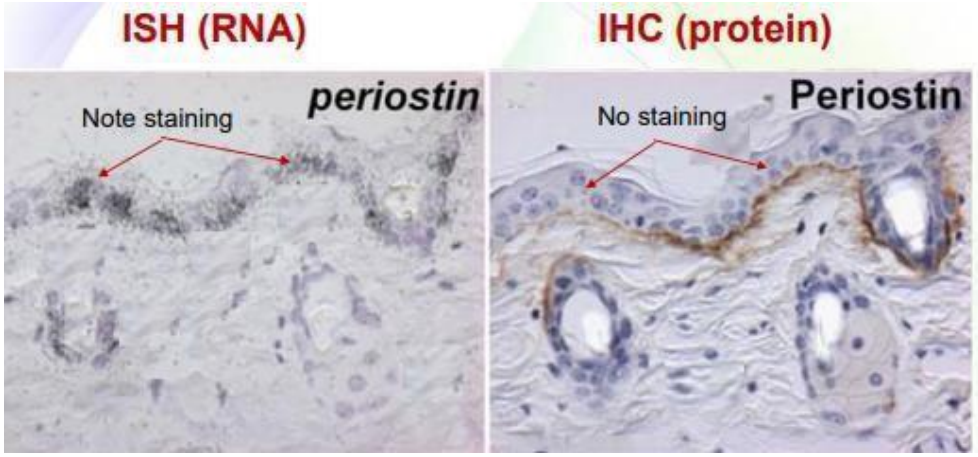
- RNA molecules can hybridize when the tissue is incubated with a complementary DNA or RNA probe.
  - RNA can bind to both DNA and RNA probes if the strands are complementary. (Not necessarily 100% complementary; depends on the external factors that affect hybridization)
- In this method you are looking for 2 things:
  1. Patterns of differential gene expression (The level of transcriptional activity)
  2. The location of specific RNAs in the tissue
- This picture explains the whole procedure of this method:



- **Steps of in situ hybridization**:

1. A histological section of one cell is taken and prepared.
2. The probe with its labeled nucleotides is added to the cell.
3. The probe will look for and find the RNA molecule we want to detect.
4. The probe emits its signal when it binds to the RNA molecule in its location.
5. These are results of different experiments in the same method. They are visualized using a microscope. The dark areas indicate the locations of the probes. Notice that each RNA molecule exists in a specific part (location) of the cell. Different RNA molecules have different locations in the cell based on their function. The Bold areas indicate more signaling . In other words, high transcriptional activity of that specific RNA molecule .

- So, let's ask a final question: RNA is translated into protein and therefore detection of the RNA should the detection of the protein. Is that valid? **it is not**. 🔸
- Why? Because RNA and protein molecules do not coexist and are present in different places.
- Now that we know that in situ hybridization won't help out, how can we detect protein location in the cell?
    - We can incorporate a technique known as Immunohistochemistry (IHC) to detect the location of proteins by labeling them with anti-bodies .
- **To sum up:**
    - RNA is visualized by in situ hybridization technique which uses probes as labels.
    - Protein is visualized by immunohistochemistry technique which uses antibodies as labels.



- These 2 images are of identical histological sections.
    - One goes through in situ hybridization to visualize RNA.
    - The other goes through immunohistochemistry to visualize protein.
- We learn that the mRNA visualized by ISH (in the left image) marks the locations of the cells responsible for the synthesis of the proteins visualized by IHC (in the right image)

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