Genetics
& Cell biology

◉ Sheet ○ Slides

Number

13

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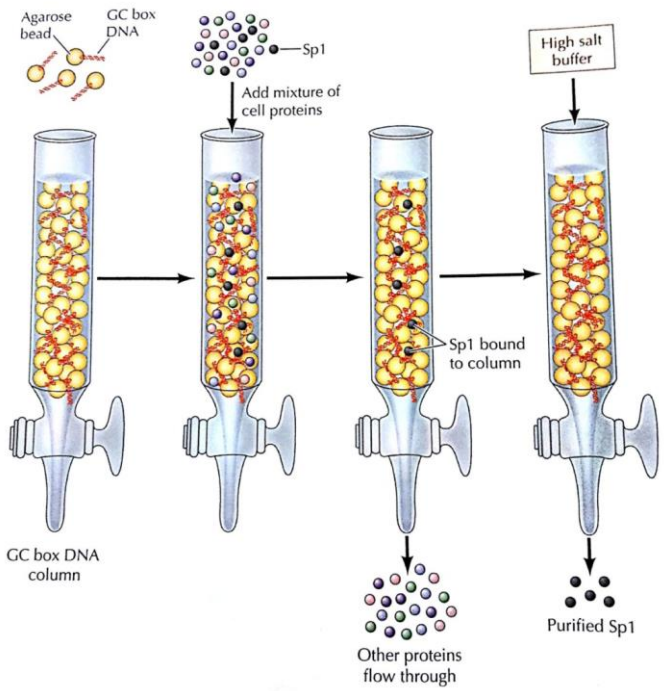
All the cells in our body have the same exact DNA sequence, yet they are completely different in their differentiation, function, structure, etc. What does make them different?? It's **gene expression**. All the cells have the 22 thousand genes but not all of these genes are expressed in every single cell; one cell expresses 6 thousand genes, the other expresses 10 thousand and so on. In addition to that, the **levels of regulation** (ex: RNA processing, protein processing and modification, transport, localization, phosphorylation, glycosylation, etc.) are variable. We have 22 thousand genes but the different products that can be produced or generated from these genes are millions of different proteins.

Another thing that makes cells different is the expression of **transcription factors** that regulate certain genes. For example, in endocrine cells the same receptor can be present in 2 different cells but the response is different. The ligand binds to the same receptor on both cells yet the response is different. In one cell it induces proliferation, but on the other, it induces apoptosis because the secondary messengers, the enzymes, and the transcription factors are different between cells.

In this lecture we will talk about several techniques that are used to investigate mRNA profiling, the genes that are expressed, and RNA expression and function in different cells (The patterns of mRNA can differentiate cell types from each other).

Affinity chromatography

We took before that affinity chromatography is the most specific type of chromatography techniques because it depends on a specific interaction between two different molecules. We can put a protein molecule on beads (stationary phase) in a column in which we can pass a lot of protein molecules, only one or two of these molecules will bind to that specific protein (added earlier) on the beads. The bound protein can then be eluted, isolated, and purified. We can put other molecules on the beads such as a sugar molecule or a DNA molecule (a DNA fragment).



A DNA fragment (promoter or enhancer region) can be put on the beads and then we pass proteins through the column. Specific proteins will bind to this fragment. So by using this technique we can purify these proteins.

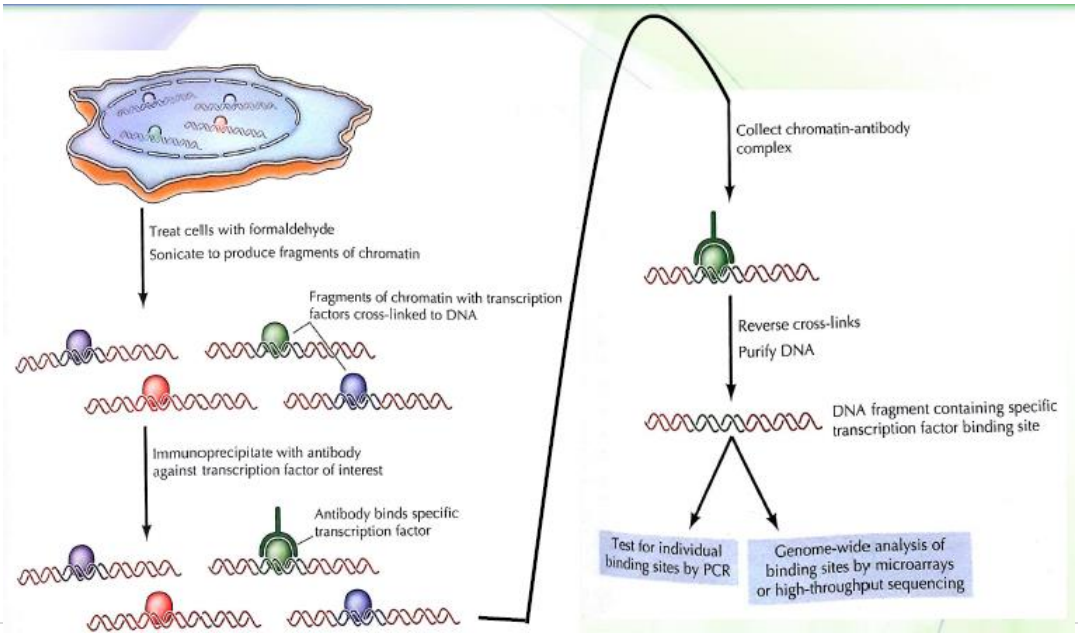
We can get the nuclear fraction using cell fractionation. We use centrifugation at different speeds in which we first get the nucleus, mitochondria, lysosomes, ribosomes and large proteins, and lastly the fluid (liquid part) by itself. We only take the nuclear fragment and pass it through affinity chromatography to look at the *nuclear proteins* only.

00:00-12:40

Chromatin immunoprecipitation

- ✓ Precipitation means deposition
- ✓ Immuno → we are using antibodies. Antibodies are really specific for certain proteins.
- ✓ Chromatin = DNA + proteins

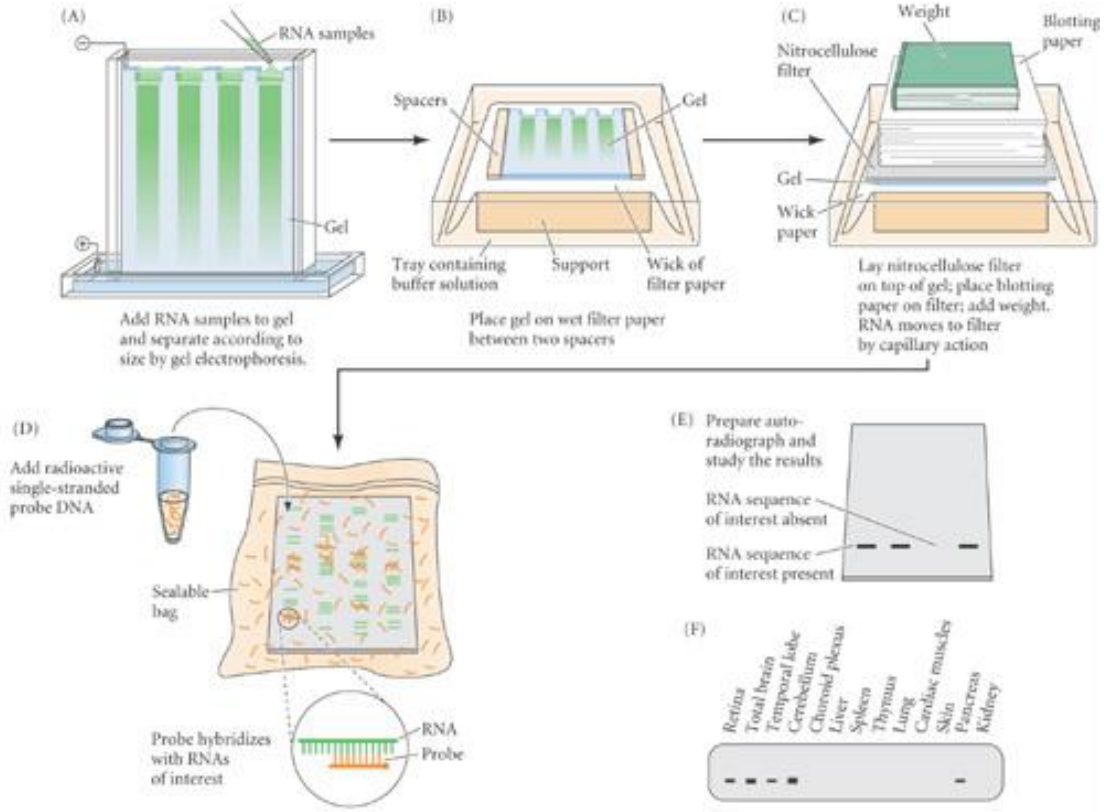
We take the cell and purify its DNA with its components. Then we crosslink the DNA with proteins so they become one unit using formaldehyde. After that, we add an antibody for a specific transcription factor and simply separate this protein with its crosslinked DNA away from other DNA molecules and proteins. After getting this protein and its DNA we can separate them and sequence the DNA we got. Since we know the protein we are targeting, we want to know the DNA sequence that binds to it. While in the previous technique we knew the DNA sequence and we wanted to know the protein that binds to that DNA sequence. We can Sequence the purified DNA fragment or identify by DNA microarray (which we talk about later in this lecture).



Northern blotting

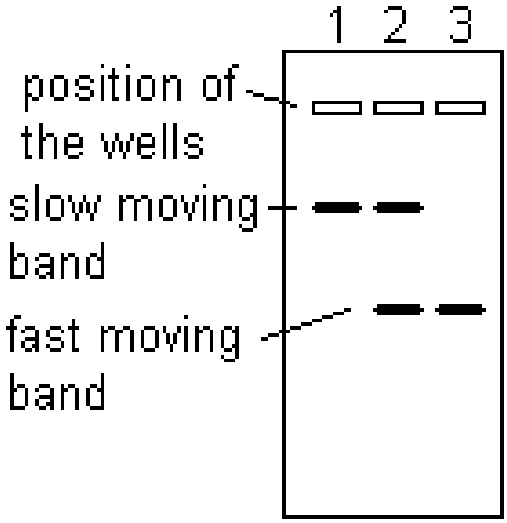
We talked about southern blotting before. **Northern blotting** is done exactly like Southern blotting except that RNA from cells is isolated instead of DNA. Western blotting is for proteins.

We isolate the RNA and fractionate it based on sizes by gel electrophoresis. Since we have a lot of RNA molecules of different sizes, they will appear as a smear on the gel (not as bands). Then we transfer them on a piece of paper (membrane). We add a labeled DNA probe whose sequence is complementary to a specific RNA sequence. Eventually, we will get specific RNA fragments (if they were present).



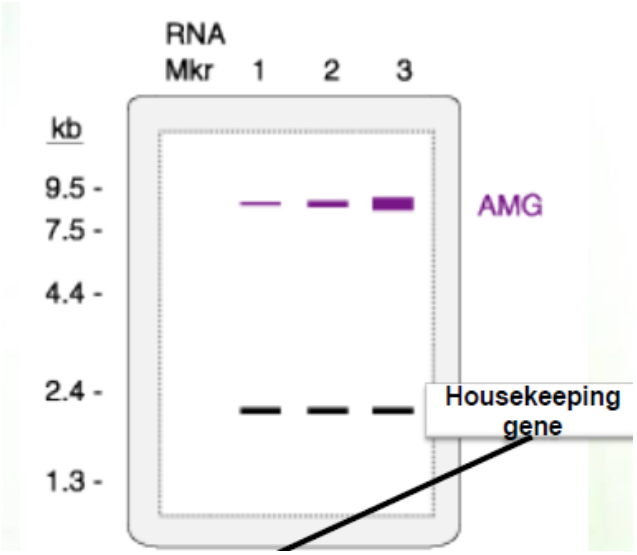
We can get 2 pieces of information from this technique:

- ✓ **If the RNA is expressed or not in a certain sample (cell) and its length (size)**. From the image we can see that sample 1 has an RNA fragment that is 5k bases long for example. Sample2 has an RNA fragment that is 5k bases long and one that is 3k bases. Sample3 has the 3k base RNA molecule but not the 5k base molecule.



So we can know if the gene is active or not and the length of its product. Genes are huge; they contain exons and introns. But in northern blot we are not looking at the primary RNA (with the introns), we are looking at the *mature RNA* (exons only).

- ✓ **The level of expression (density)**:
  Sample3 has a more intense fragment than sample1. This means there is more expression of this gene in sample3 than sample1 (the gene is more active in sample3).



*But what if this came from putting more amount of sample3 than of 1 in the gel (putting 5µL of sample1 and 20µL of sample3)?

- To make sure that doesn't happen, we use a **housekeeping gene**. This gene is active all the time (with constant expression) and is not affected by anything like actin and tubulin. In the figure we can see that the expression of the housekeeping gene is the same in all samples. This means that the increase of expression of RNA in sample3 is real (not from different amount of samples). If the housekeeping gene was more in sample3, I conclude that the amount I put was different.

In this technique, we are studying a certain RNA molecule (a certain gene) present in different tissues. For example, we see that a certain gene is expressed in the retina but not in the thyroid. Another example is the RNA product of a certain gene in the heart is 5k bases long, but in skin cells the same RNA is 3k bases long.
How can that be? The gene is the same but the processing is different; certain exons are needed in one tissue but not in the other.

In situ hybridization

"In situ" means "in place". It answers the question "**Where** the gene is expressed specifically in a certain tissue section?"

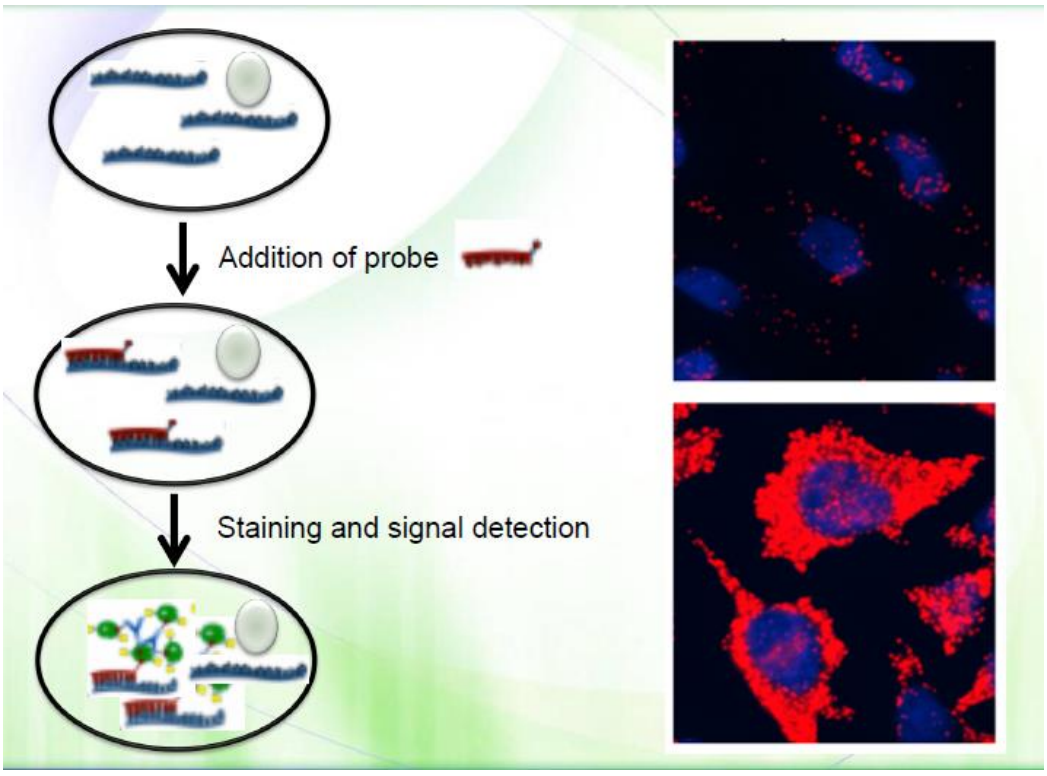
We are studying the presence of an RNA molecule in a specific cell.

If we took a tissue section and isolated all the RNA in this section. Then, we applied northern blotting and noticed that a certain gene is expressed a lot in this tissue.

*But since this tissue section has a lot of different cell types, how do we know in which cells is this gene expressed? Is it in the epithelial cells, inflammatory cell, or fibroblasts? - We use in situ hybridization.

To prove that the gene is expressed in epithelial cells for example, I take the tissue section and put a probe (DNA or RNA probe). The probe will bind specifically to the RNA in epithelial cells but not in fibroblasts because fibroblasts don't express this gene. The probe is labeled and it gives a signal when it binds the RNA and fluoresces.

In cancer cells for example we have different types of cells. There is the invasive, hypoxic, necrotic cells, etc. We can use this technique to see if there is a certain gene that is expressed in the invasive cells but not in hypoxic cells and the other way around.



There are some pictures in slides no12-14 showing RNA expression in drosophila during development. We use this technique (in which every probe gives a certain color) to identify the pattern of RNA expression in the periphery or in segments (vertical segments ➔ yellow florescence). Thus, this technique tells us about expression of RNA during differentiation, and the symmetry of expression: left and right, top and bottom, head and legs, …etc.

DNA library

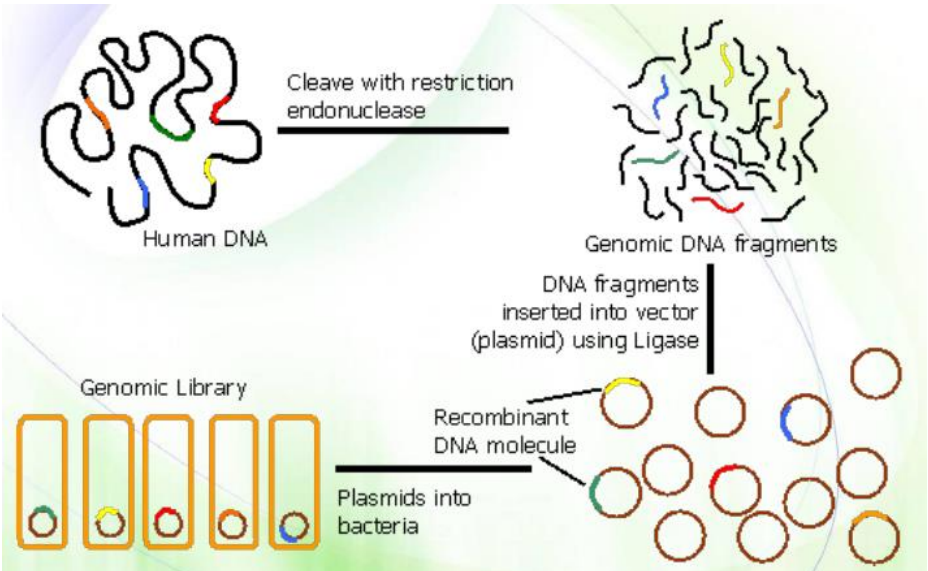
It is just like a real library but it's for DNA. We have a freezer in which there are tubes in boxes. Each box in a certain location has a certain DNA fragment.

There are 2 **types of basic DNA libraries:**

1. **Genomic DNA library:**

   In a genomic DNA library, I take the whole genome and fragment it using a restriction endonuclease to smaller fragments. Then, we insert these DNA fragments in a plasmid (which I cut with the same endonuclease); every plasmid will take a different DNA fragment. We put these plasmids in bacteria; each bacterium has a certain plasmid with a specific DNA fragment. We take bacterial colonies and analyze them to know which bacteria have which DNA fragment and put them in a freezer. When I want to study any DNA sequence, I take the bacteria with the sequence that I am interested in.

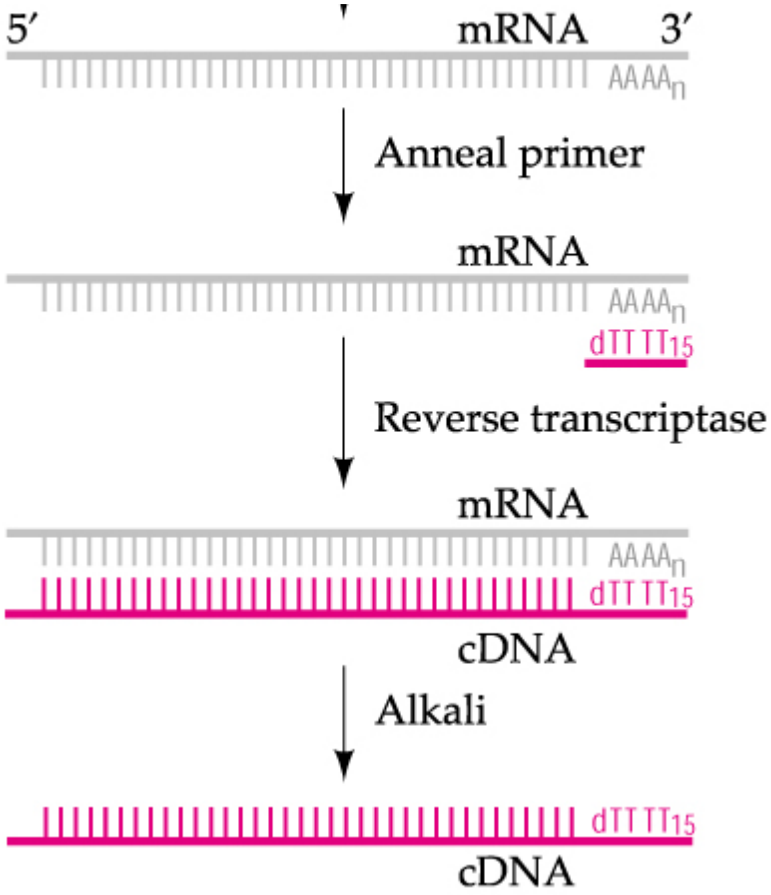
   This library contains the whole genome including introns, promoters, coding and noncoding regions, exons, telomeres, centromeres, etc.



2. **cDNA library:** more specific. c = complementary

We take the mature mRNA molecules (only exons without introns, promoters, or enhancers…). We convert mRNA to DNA by using **reverse transcriptase** enzyme (a retroviral enzyme); we call this DNA a cDNA (complementary to mRNA). cDNA can then be converted to double stranded DNA. We put the double stranded DNA in plasmids which in turn will be put in bacteria.

This library is a library of expressed genes specifically.



*How can I isolate mRNA form other types of RNA?

-mRNA has the poly-A tail that is not present in any other type of RNA. So we use a *poly-T primer*.

*If I made a genomic DNA library for skin cells and another for colonic cells, would they be identical?

-YES. The genome is the same in skin and colonic cells for the same organism.

On the other hand, if I made a cDNA library for skin cells, colonic cells and neurons, they will NOT be identical because the starting material is mRNA (the expressed genes only).

Genomic vs. cDNA libraries

♦ Genomic clones represent a random sample of all of the DNA sequences in an organism. By contrast, cDNA clones contain only those regions of the genome that have been transcribed into mRNA.

♦ Because the cells of different tissues produce distinct sets of mRNA molecules, a distinct cDNA library is obtained for each type of cell used to prepare the library.

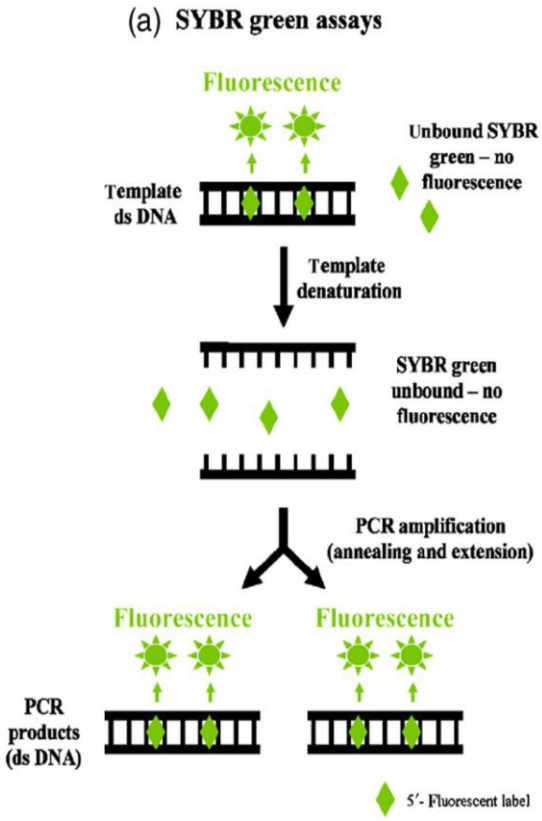
21:30-32:30

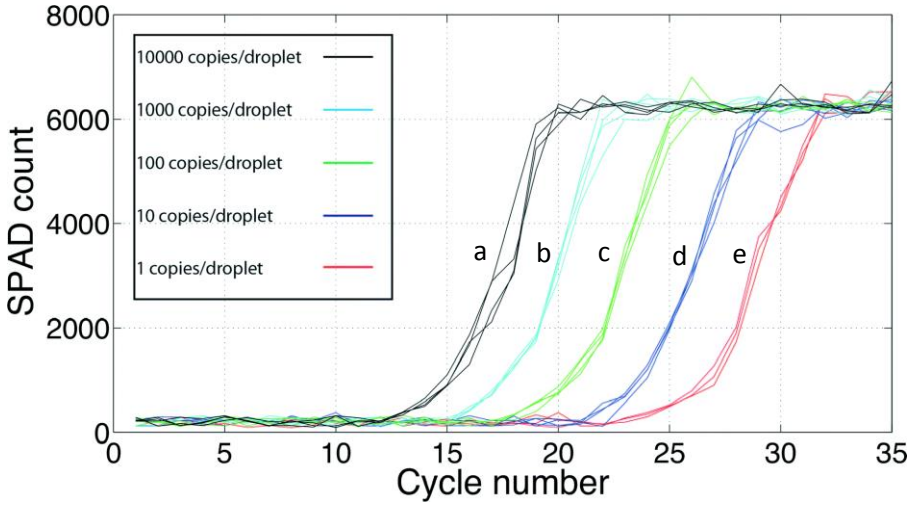
Quantitative real-time PCR of mRNA

We talked about PCR before. I can use it to quantify gene expression. We take mRNA and convert it to double stranded cDNA, and then we can do PCR in the presence of SYBR green.

If I have 2 different cells (neuron vs epithelial cell) and both cells express a certain gene but the *level* of expression is different. Using quantitative real-time PCR I can know if there is differential gene expression between the 2 cells.

The cell with higher expression of the gene will have more mRNA and thus more cDNA so the signal (SYBR green) will be detected sooner. According to that, sample (a) has the highest gene expression and (e) has the lowest.

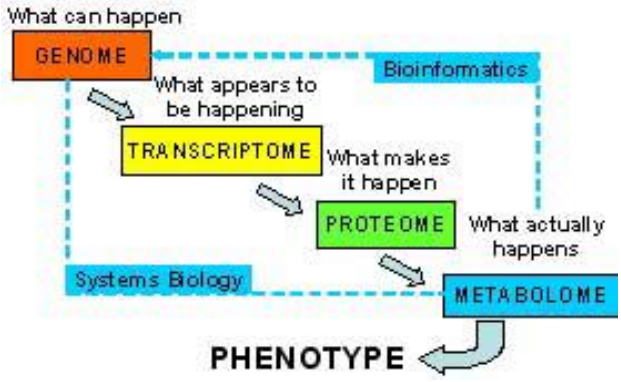




Here we also use a housekeeping gene to make sure I started with the same amounts. The figure below shows that the housekeeping gene for both samples appeared together, this means I started with equal amounts in each sample. The gene of interest appeared in the sample to the right first (less number of cycles), so gene expression is more.

The science of –omics

1. **Genomics:** studying a lot of genes at the same time.
2. **Transcriptomics:** studying RNA transcripts (gene expression).
3. **Proteomics:** studying proteins.
4. **Metabolomics:** studying metabolites.



Then there was phosphoproteomics, glycomics, etc. We are concerned in transcriptomics.

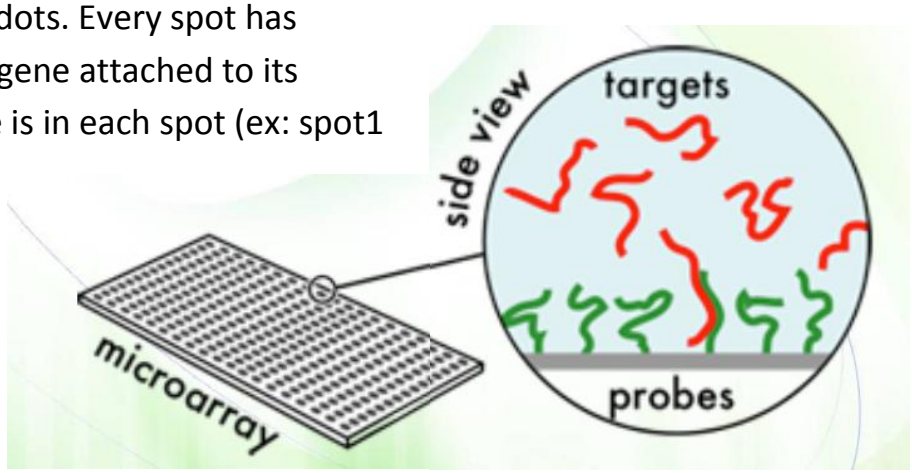
One such method in studying transcriptomics is **DNA microarrays**.

DNA microarrays

Array = arrangement

Micro → we are using a small chip (the size of a fingernail) but it can contain as much as 20 thousand different probes for 20 thousand different genes.

This chip has an array of spots/dots. Every spot has unlabeled probes for a specific gene attached to its surface, and I know what probe is in each spot (ex: spot1 has probes for actin gene, spot2 has probes for tubulin gene, spot3 has probes for e-cadherin….).

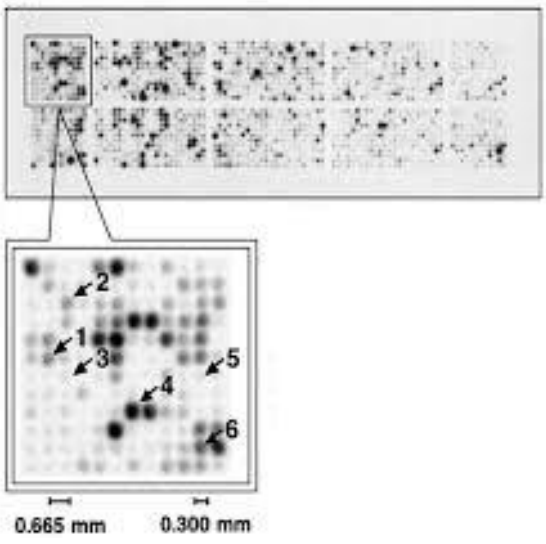


By examining the expression of so many genes simultaneously, we can understand gene expression patterns in physiological and pathological states.

To study gene expression, I take mRNA in the cell and convert it to cDNA. Then I label the cDNA with a radioactive probe so that the DNA fragments give me a certain signal, and put cDNA on the array. If a gene is expressed in this cell, its mRNA and thus cDNA will be present and it will bind/hybridaize with its probe[1] on the array. When the cDNA binds to the probe, the spot will give a signal. So whenever I see a signal this means the cDNA (and mRNA) for this probe is present and the gene is expressed. If the gene is not expressed I will not see a signal.

32:30-42:15

I took mRNA from one sample and used microarray technique. I can see an empty spot so that gene is not expressed. I can also see a faint spot, this means that the gene is expressed but it is not highly active. A dark spot means that the gene is highly expressed.



*Can I determine the cell type according to the signal I get?

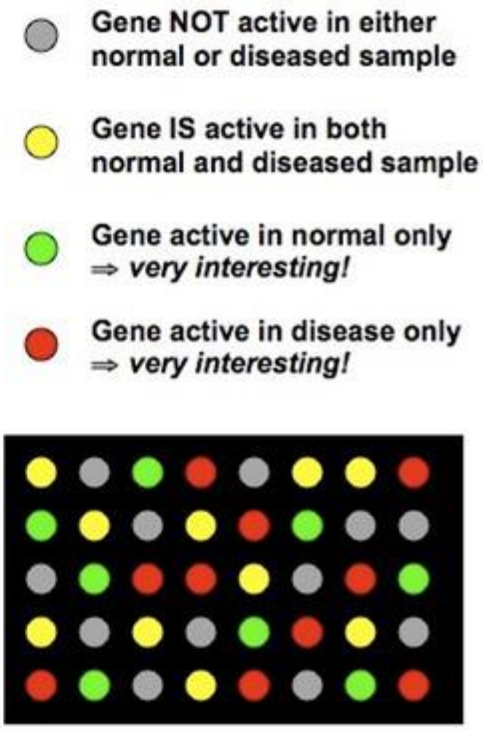
-Yes, because I know that a certain gene is expressed only in this cell for example.

The probes found in the spots are unlabeled[1]

Comparative expression: to compare expression of genes in two different samples. I take 2 different cell types (normal vs cancer cell or diseased vs healthy). For example, I treat a certain cell with a drug that caused changes in gene expression.Then I take the treated and the non-treated cell, and convert mRNA from both cells to cDNA. I label cDNA with a *fluorescent dye* that gives me a signal with different colors for the different cells (ex: green for the untreated and red for the treated cell) and add them to the array; cDNA will bind to its probe.

- If a gene is not expressed in both cells, the spot won't give me any signal (black spot).
- If the 2 cells have equal expression of a gene (the drug didn't affect its expression), the DNA with the red label and the DNA with the green label will bind to the same spot with equal amounts/intensity and the computer will show this as a yellow signal.

  *Remember: each spot contains many probes for the same gene
- If the drug caused a change in gene expression:
  - If it supressed gene expression in the treated cell, the green signal will be more than the red one and the spot will appear green.
  - If it induced gene expression in the treated cell, the red signal will be more than the green one and the spot will appear red.



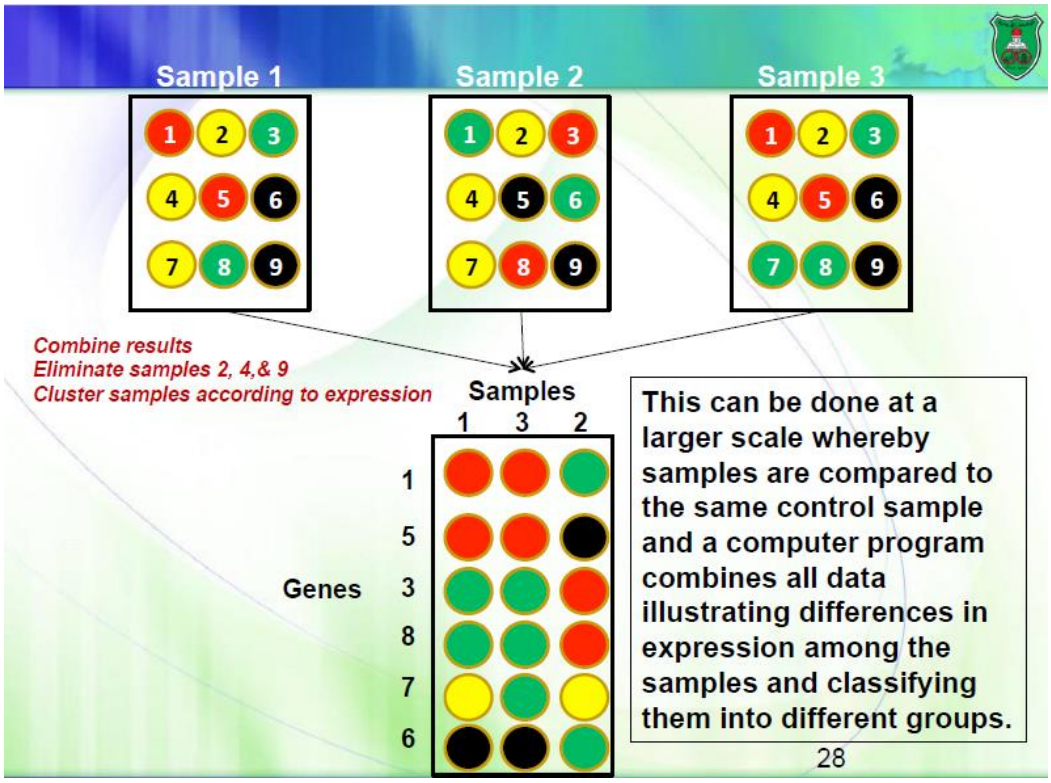
Black spot → no expression
Yellow → equal expression
Green → suppression for example
Red → induction for example

Example:

I did DNA microarray for 3 samples (from three tumors or cells treated with different drugs) and I want to compare them with the control. In sample1 for example gene1 is induced (up regulation), gene2 is equally expressed, gene3 is supressed and so on. The same is applied for the rest of the samples. A computer program combines the samples and comapre them to the same control sample and then combines all data illustrating differences in expression among the samples and classifying them into different groups.

Samples 1 & 3 are *similar* to each other while sample2 is different. Look at gene1 for example, it it induced in samples 1 & 3 but supressed in sample2 an so on. Samples 1 & 3 have the same pattern of gene expression but they are not identical; they differ in gene7.

Note: the computer eliminated genes 2 and 4 because they are equally expressed in all samples. Also, it eliminated gene9 because it is not expressed in any sample.

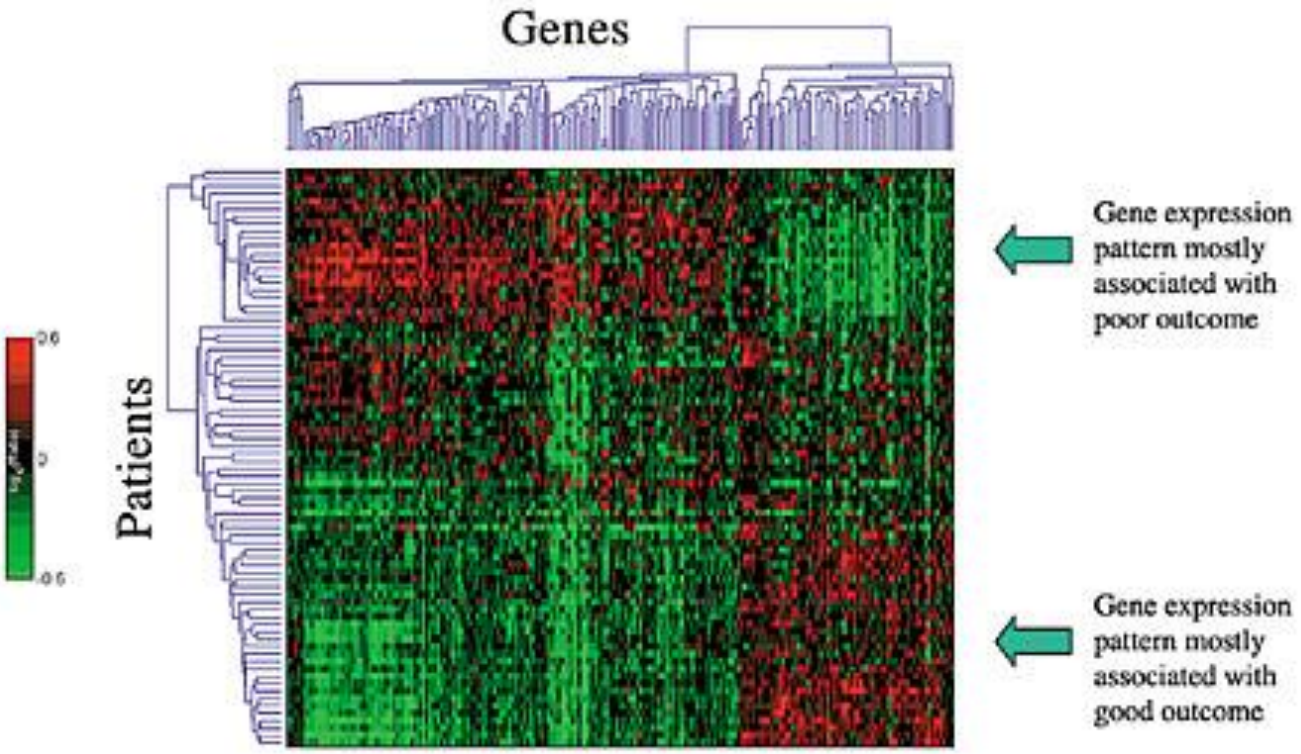


DNA microarrays and breast cancer

Samples were taken from breast cancer *patients*, and the computer program arranged them and put similar samples next to each other.

These genes could differentiate patients; patients with poor outcome and others with good outcome. We can also differentiate patients according to treatment response, type of the cancer, or the outcome depending on gene expression.

Now, the gene expression profile of the tumor in any patient with breast cancer can be determined, and based on this we can predict if the patient will have a good or poor outcome and we can know which treatment to give.



42:15-52:30

The End