Gene Expression

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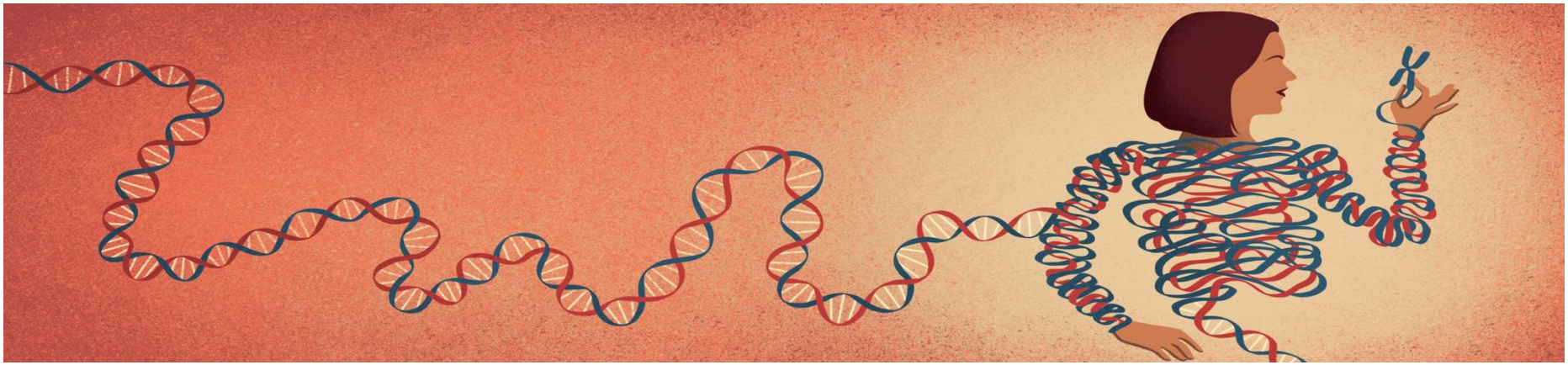
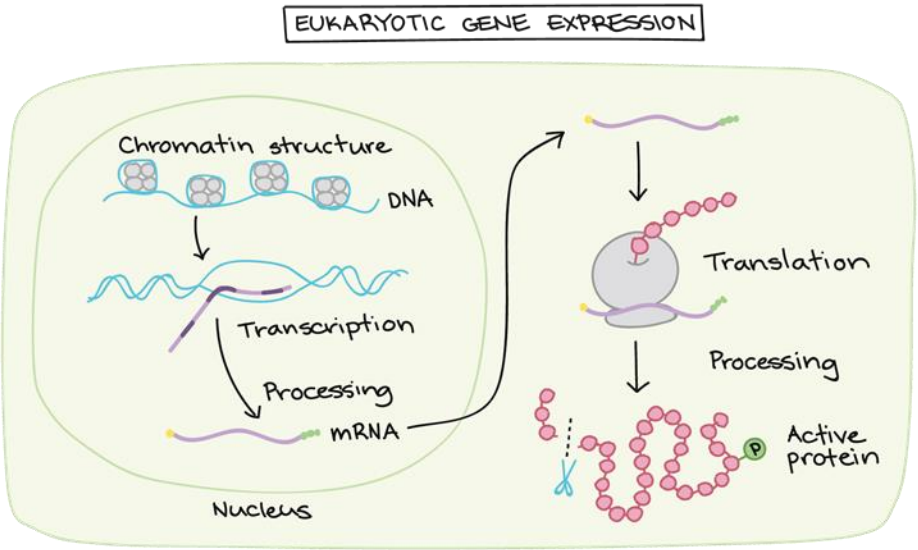
Dept. Pharmacognosy

Advance Pharmacognosy



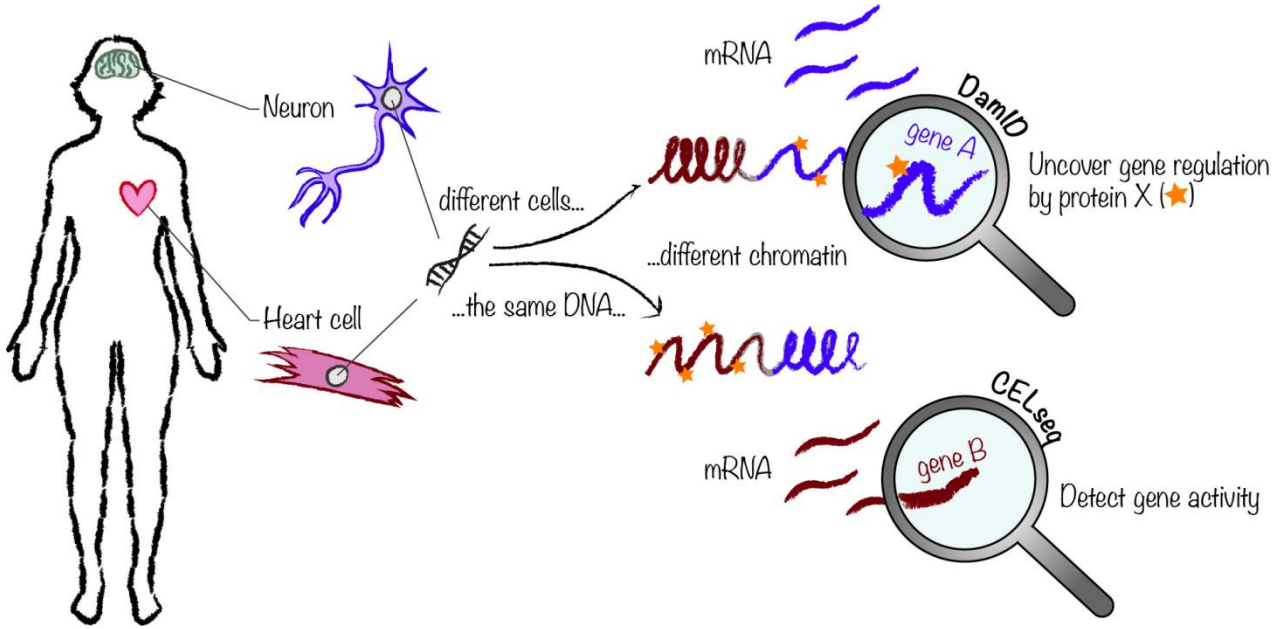
What Is Gene Expression?

Using a gene to make a protein is called gene expression. It includes the synthesis of the protein by the processes of transcription of DNA and translation of mRNA. It may also include further processing of the protein after synthesis.

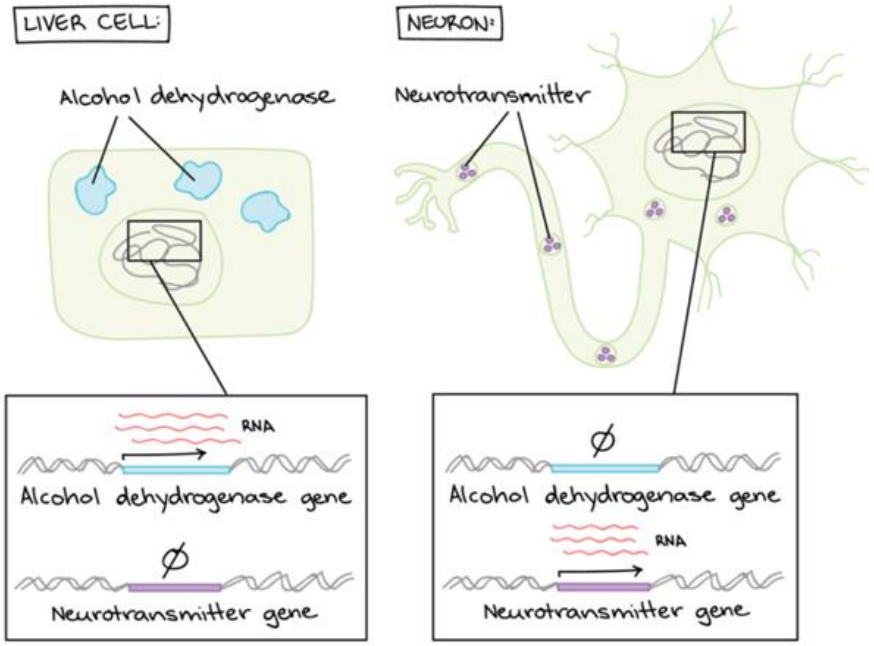


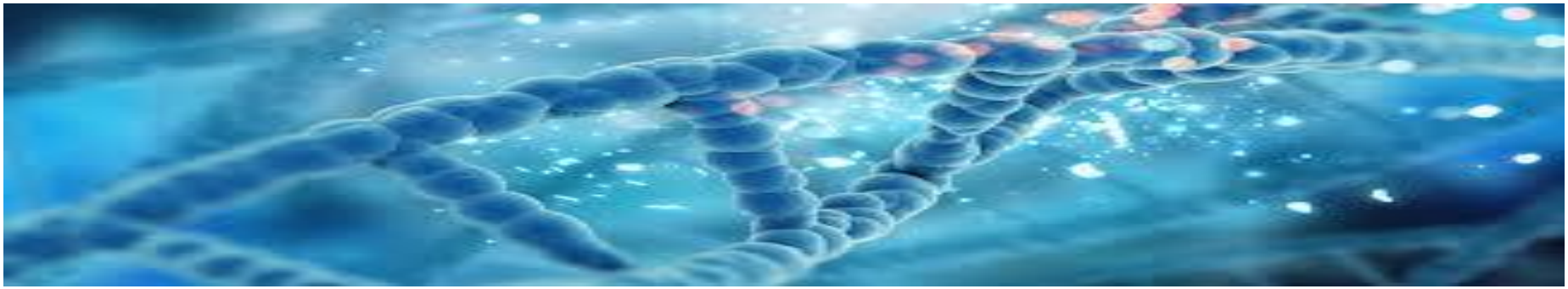
Gene regulation makes cells different

Each cell type in your body has a different set of active genes—despite the fact that almost all the cells of your body contain the exact same DNA. These different patterns of gene expression cause your various cell types to have different sets of proteins, making each cell type uniquely specialized to do its job.



For example, one of the jobs of the liver is to remove toxic substances like alcohol from the bloodstream. To do this, liver cells express genes encoding subunits (pieces) of an enzyme called alcohol dehydrogenase. This enzyme breaks alcohol down into a non-toxic molecule. The neurons in a person's brain don't remove toxins from the body, so they keep these genes unexpressed, or "turned off." Similarly, the cells of the liver don't send signals using neurotransmitters, so they keep neurotransmitter genes turned off



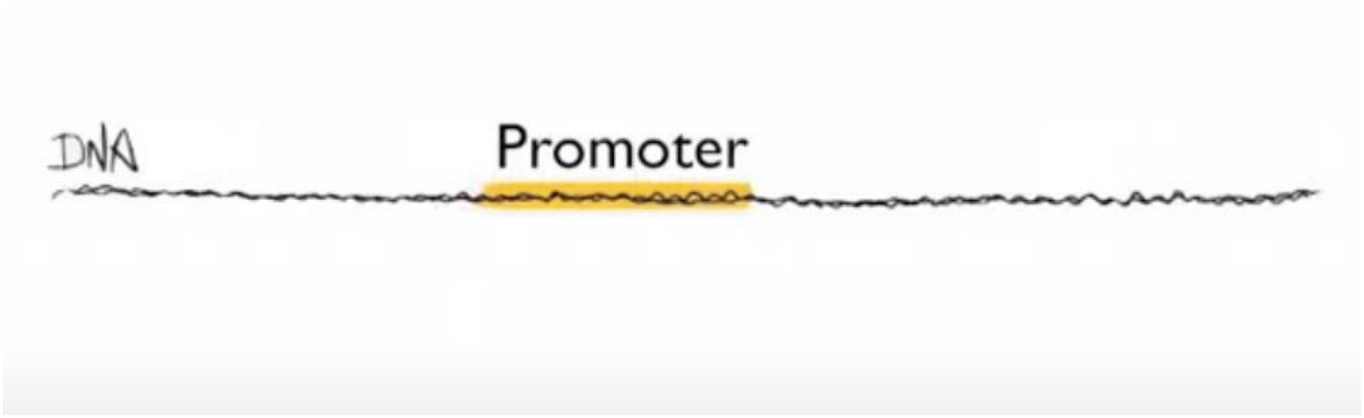


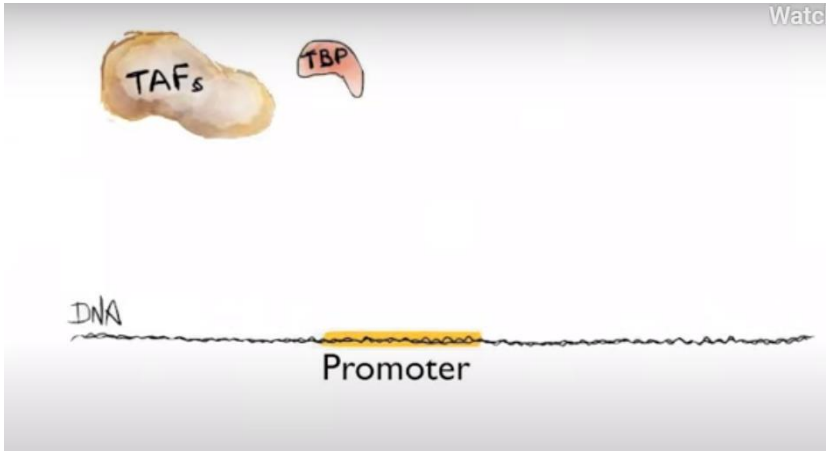
Gene regulation stages

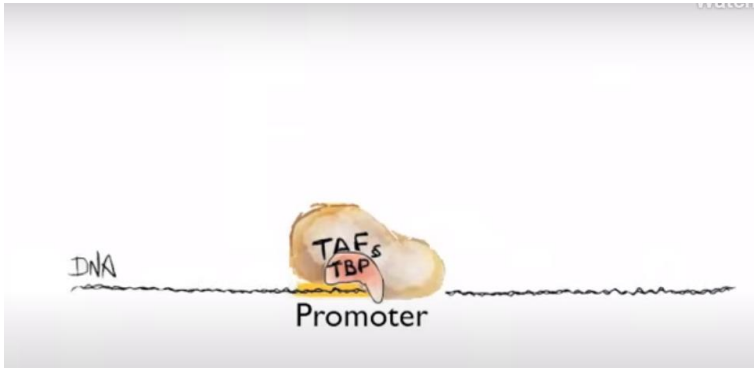
Gene regulation is the process of turning genes on or off. Gene regulation can occur at any point of the transcription-translation process but most often occurs at the transcription level. Gene expression is regulated to ensure that the correct proteins are made when and where they are needed.

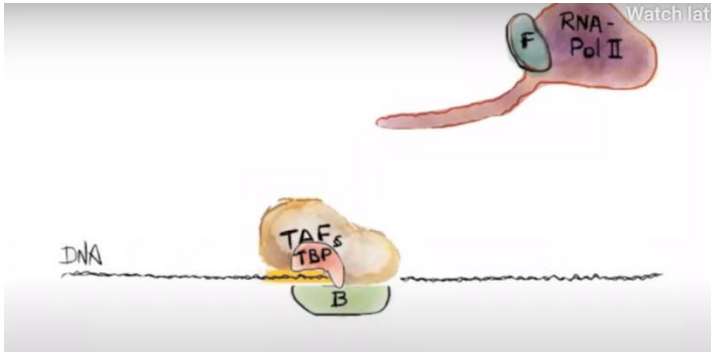
Proteins that can be activated by other cells and signals from the environment are called transcription factors. Transcription factors bind to regulatory regions of the gene and increase or decrease the level of transcription. Other mechanisms of gene regulation include regulating the processing of RNA, the stability of mRNA and the rate of translation.

Gene expression is regulated by numerous factors, including **polymerase recruitment**, **epigenetic signaling**, and **transcription factors (TFs)** that regulate the activity of gene promoters and enhancers. Enhancers are regulatory sequences in the genome that affect gene expression of a nearby gene. The exact definition of enhancers and the mechanism by which they regulate gene expression is still a matter of active research. The current view is that they act by recruiting specific TFs and polymerase to a distal site, which then activate gene expression through a mechanism that involves physical contact with the gene promoter. More recently, it has been proposed that enhancers can form highly active genomic clusters (super enhancers or stretch enhancers, which may act in a phase-separated assembly of molecules. Enhancers can regulate gene expression by recruiting TFs and the transcriptional machinery and subsequently forming a loop with the promoter region of the target gene.

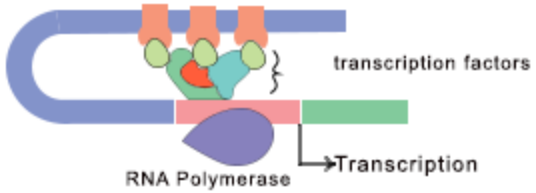

DNA
Promoter

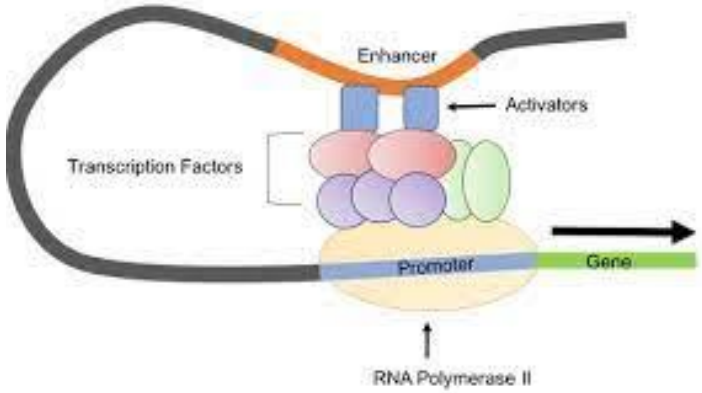

TAFs
TBP
DNA
Promoter

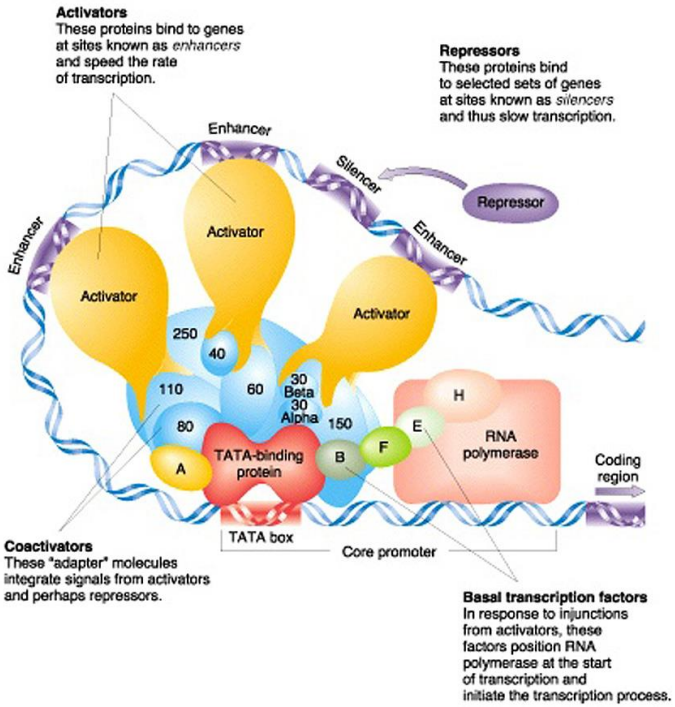

DNA
TAFs
TBP
Promoter


F
RNA - Pol II
DNA
TAFs
TBP
B

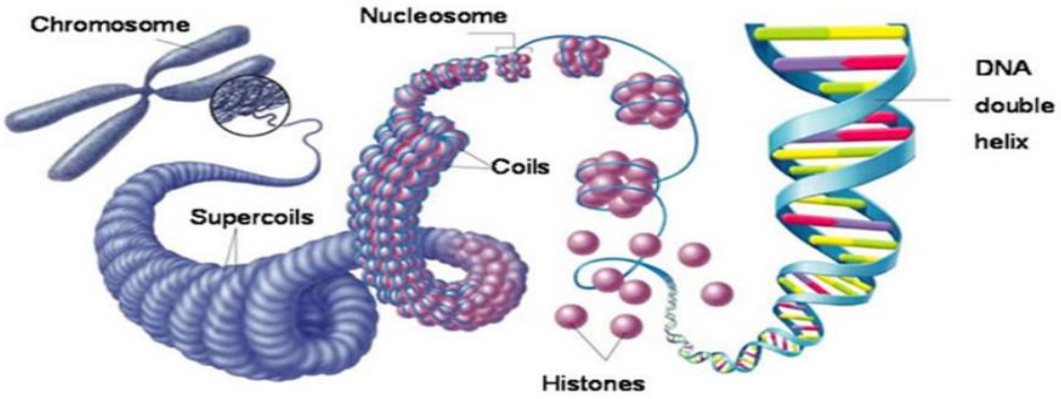
Transcription Regulation







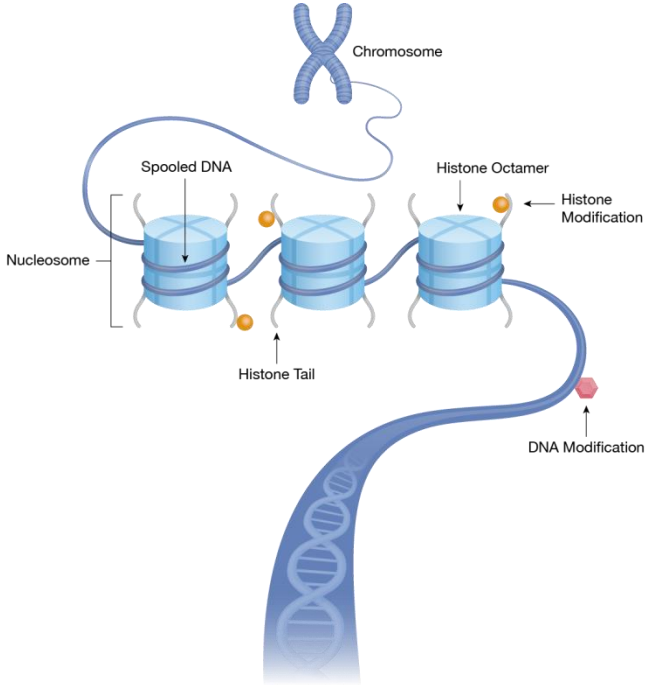
Epigenetics generally refers to changes in gene expression and chromatin organization that are not due to alterations in the DNA sequence. Epigenetic modifications typically occur by changes in DNA methylation, histone covalent modifications .



DNA (the instructions) can be found in two forms:

- **Chromatin**: loose, uncoiled form.
- **Chromosome**: tightly wound, condensed form.

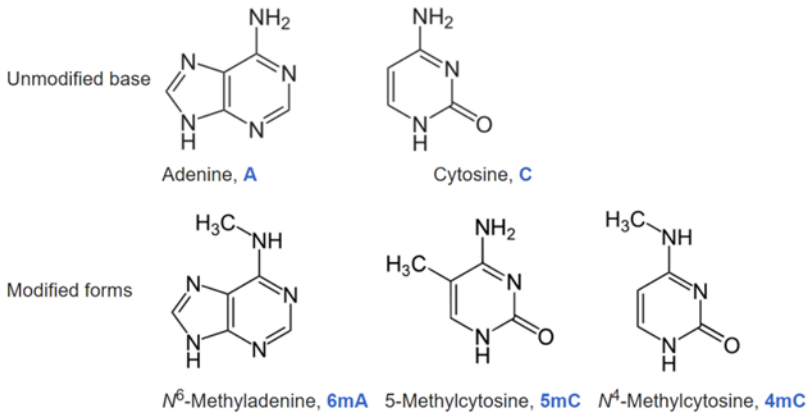
An epigenetic change is a modification to DNA that occurs when a chemical compound or protein attaches to a gene and alters gene expression. Epigenetic changes can be passed down through inheritance or can occur through exposure to environmental substances, as a result of lifestyle behaviors or due to increasing age. They occur on genomic DNA and histones and their chemical modifications regulate gene expression.



One example of epigenetic change is methylation. Methylation occurs when small molecule methyl groups are added to DNA. The addition of these groups to DNA results in the gene being turned off, and thus the protein made from that gene is not produced.

When located in a gene promoter, DNA methylation typically acts to repress gene transcription

Two nucleobases have been found on
which natural, enzymatic DNA methylation takes place:
adenine and **cytosine**



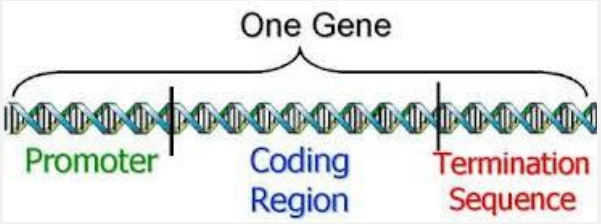
How do histones play a role in gene expression?

Histones are highly alkaline proteins found in eukaryotic cell nuclei and play an important role in gene regulation. An active gene is less bound by histone, whereas an inactive gene is highly bound by histone.

Histone modifications. These modifications include acetylation, methylation, phosphorylation, that can cause either activation or repression of transcription,

Acetylation of the histone increases transcriptional activity of the gene promoter region.

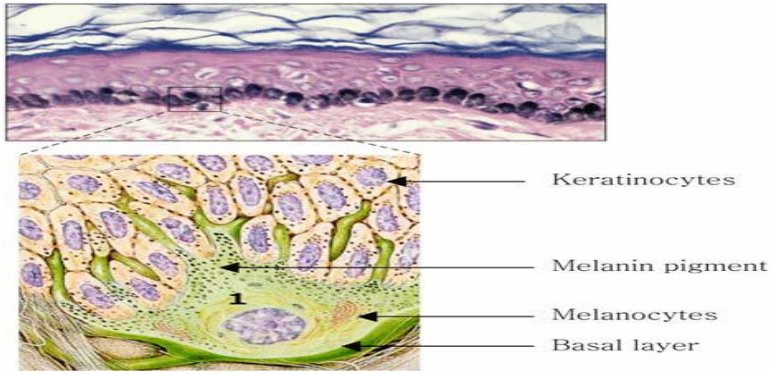
methylation, phosphorylation, that can cause either activation or repression of transcription.



phytochemicals affecting the epigenome. natural phytochemicals can largely modulate mammalian epigenome through regulation of mechanisms and proteins responsible for chromatin remodeling. Phytochemicals are mainly contained in fruits, seeds, and vegetables as well as in foods supplements.

1- Anti-melanogenic activity of plant extract

Skin represents the primary line of defense against environmental stressors, including chemical stimuli, microbial insults, allergens, and ultraviolet (UV) radiation. Protection from UV rays is essentially based on melanogenesis, the process leading to the synthesis of pigments called melanin, the main substance that influences skin color. Melanin protects the skin can absorb UV and visible light and shows antioxidative and radical scavenging abilities, Therefore, reduced melanogenesis is also a major risk factor for melanoma and other skin cancers.



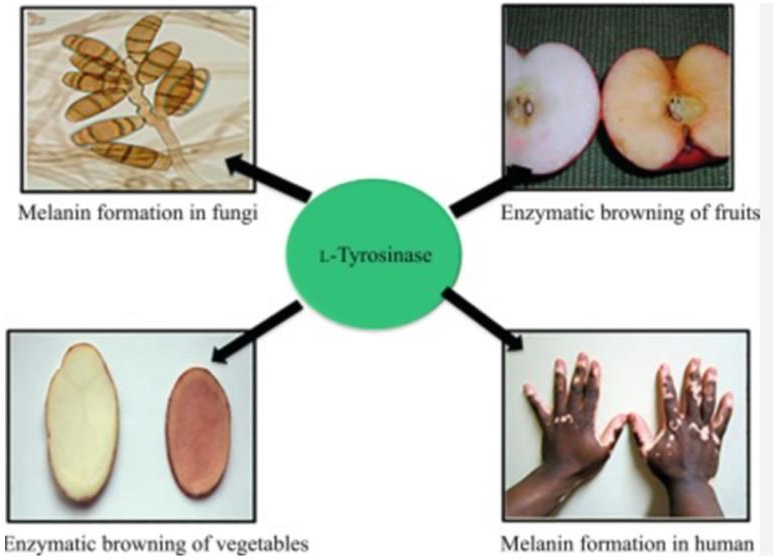
Melanin production is controlled by several enzymes including tyrosinase (TYR), which plays a key role in the melanin biosynthesis. Typical age spots are one of the major changes due to an increased tyrosinase activity and melanin production associated with aging.

Several extracts from natural resources have been reported to exhibit anti-melanogenic activity. Extracts with the anti-melanogenic activity can be used as skin whitening cosmetics agents.

1- cosmetic activities of extracts

2- anti-aging potential of the extracts

Tyrosinase is an enzyme present in plant and animal tissues that catalyzes the production of melanin and other pigments from tyrosine by oxidation. In humans, the tyrosinase enzyme is encoded by the **TYR gene**. Inhibition of tyrosinase has been a long-time target in the skin health research, cosmetics



Tyrosinase activity assay

1. **Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

Tyrosinase mRNA RT-PCR Analysis

1. **Tyrosinase Inhibitor Screening Kit**

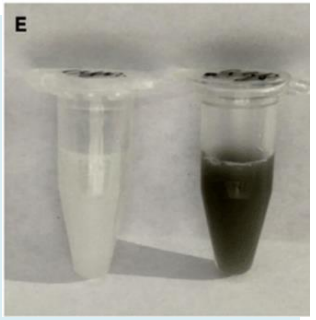
Tyrosinase Inhibitor Screening Kit (Colorimetric) provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of tyrosinase inhibitors. Tyrosinase catalyzes the oxidation of tyrosine, producing a chromophore that can be detected at 475 nm.

A chromophore is the part of a molecule responsible for its color.

Tyrosinase Activity Assay Kit

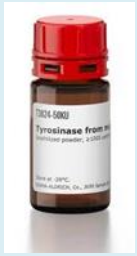
$399.00

Tyrosinase Inhibitor Screening Assay Kit



1-The test samples

2- DOPA (dihydroxyphenylalanine)

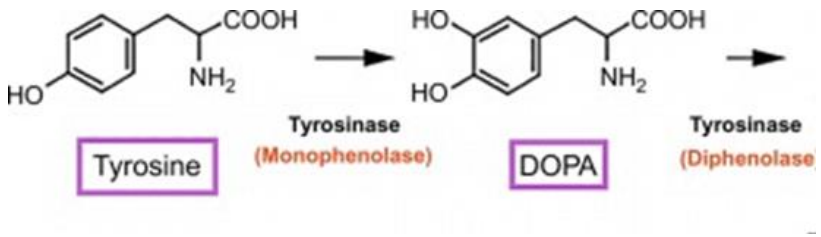


3- tyrosinase enzyme (Mushroom Tyrosinase)

4- phosphate buffer solution (PBS) of pH 6.8, respectively.

5- 96-well plates

During the reaction, DOPA was converted to dopachrome, which resulted in a change in color from colorless to orange. This change was measured through absorbance at 475 nm.
Instead of the sample solution, 50% DMSO was used as the control. The reaction mixture without enzyme served as a blank. In the presence of kojic Acid, a reversible inhibitor of tyrosinase, the rate of oxidation of the substrate is decreased.



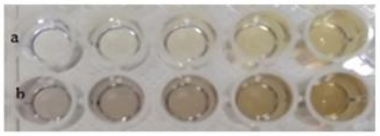


Figure 1. Tyrosinase inhibition assay (a) Test with Bacopa monnieri methanol extract and (b) control without Bacopa monnieri extract.

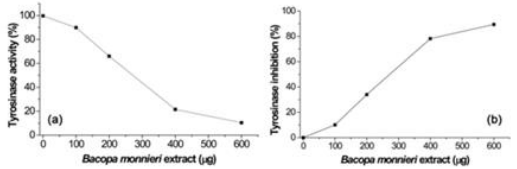
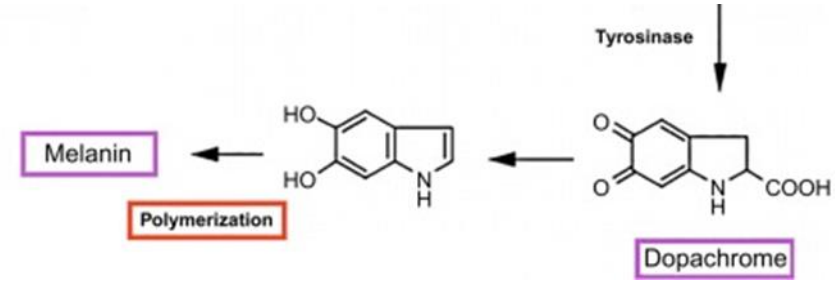


Figure 2. Effect of different concentrations of methanol extract of Bacopa monnieri on (a) mushroom tyrosinase activity (IU) and (b) percent inhibition of mushroom tyrosinase activity.

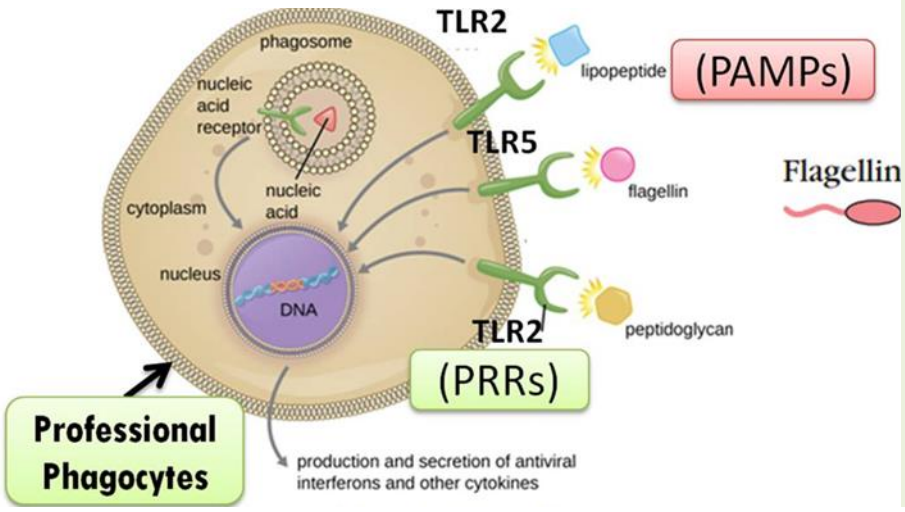


2- Plant-Derived Anti-Inflammatory Compounds

Plant-derived bioactive compounds can be classified as phenolic compounds, including flavonoids and tannins, glycosides, alkaloids, and terpenoids. When consumed by humans, they get involved in different biological processes inside the body. Nowadays, natural products are becoming a promising source for the treatment of several inflammatory conditions. Evidence suggests that phytochemicals can attenuate the expression of pro inflammatory genes, and promote anti-inflammatory genes

The innate immune response begins when pattern recognition receptors (PRRs) expressed on immune cells. They are proteins expressed, mainly, by cells of the innate immune system, such as dendritic cells, macrophages, monocytes, neutrophils and epithelial cells, to identify two classes of molecules: pathogen-associated molecular patterns (PAMPs), which are associated with microbial pathogens, and damage-associated molecular patterns (DAMPs), which are associated with components of host's cells that are released during cell damage or death.

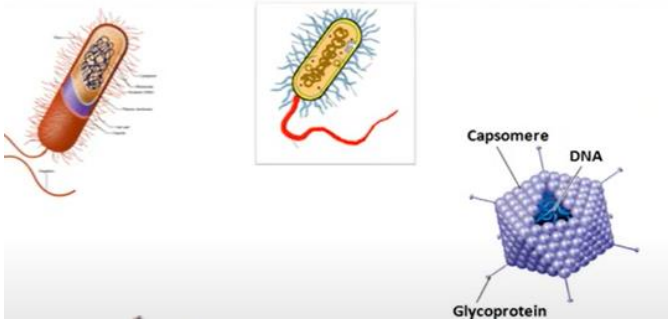
Pattern Recognition Receptors (PRR)

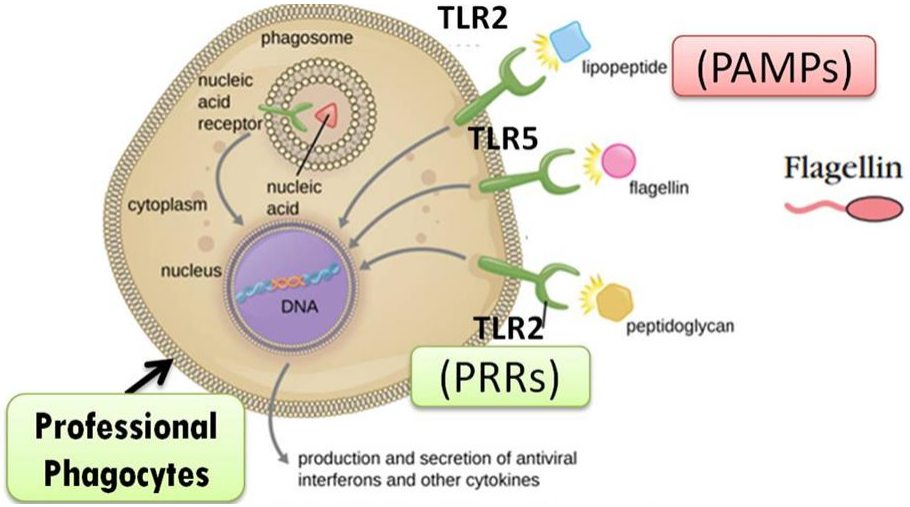


these leukocytes, release more pro inflammatory cytokines, such as TNF-α, IL-6, IL-12, and type I this enables macrophages to secrete various physiologically active substances and pro-inflammatory cytokines, such as nitric oxide (NO), tumor necrosis factor (TNF-α), interleukin (IL)-1β, and IL-6, and interferons (IFNs) to maximize the immune response

Pathogen-Associated Molecular Patterns (PAMPs)

Pattern Recognition Receptors (PRR)



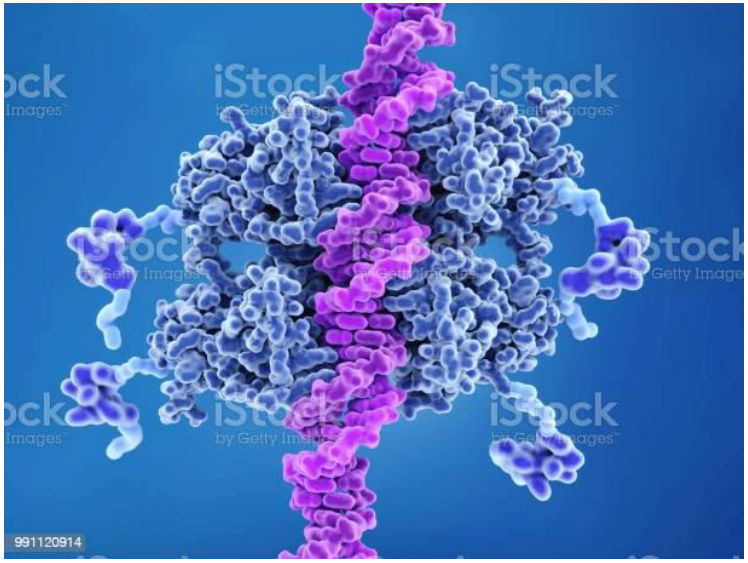


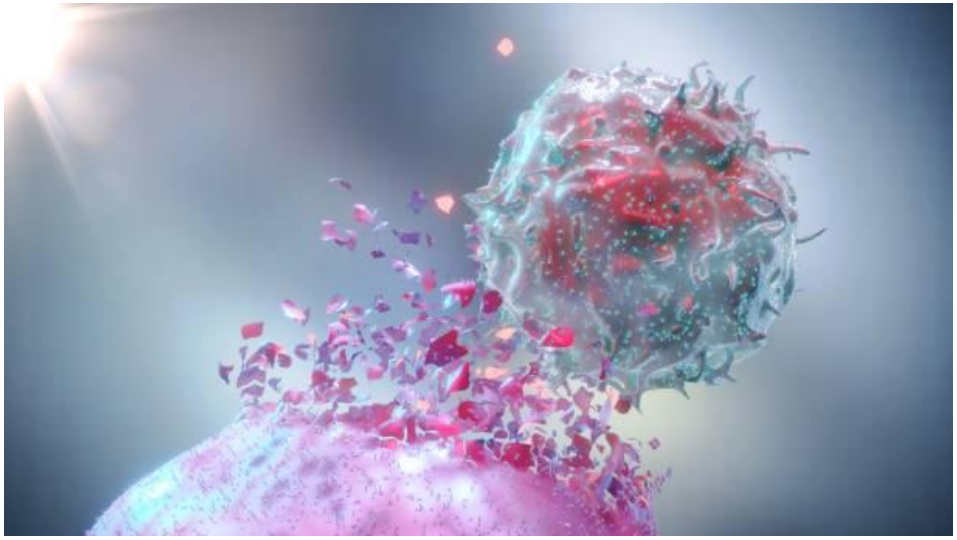
leukocytes, including macrophages, neutrophils, and dendritic cells phagocytose microbial elements and release more proinflammatory cytokines, such as TNF-α, IL-6, IL-12, and type I and interferons (IFNs). When activated, Toll-like receptors (TLRs), in turn, activate various genes that function to moderate host defense, including inflammatory cytokines

3- Apoptotic Effects of plant compounds

Apoptosis, a type of cell death mechanism, is controlled by the interactions between several molecules and responsible for the elimination of unwanted cells from the body. Apoptosis can be triggered by intrinsically or extrinsically through death signals from the outside of the cell. Any abnormality in apoptosis process can cause various types of diseases from cancer to auto-immune diseases.

The most studied genes related to apoptosis are the tumour suppressor gene p53, the anti-apoptotic gene bcl-2 (B cell lymphoma), tumor necrosis factor (TNF) and the pro-apoptotic gene bax



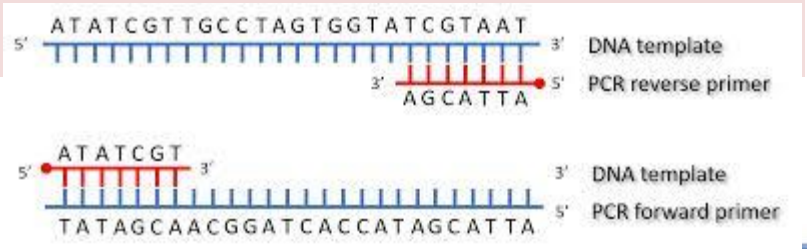


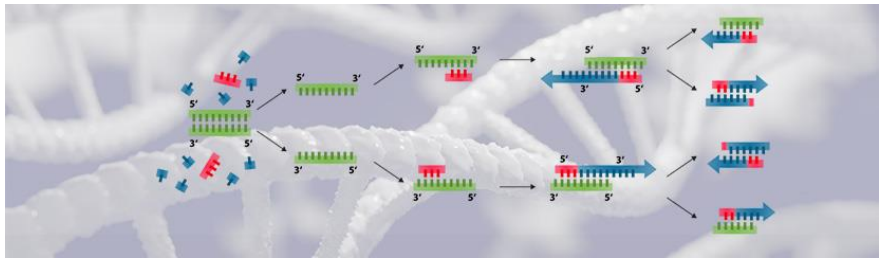
Gene-Expression Experimental Design

Reverse transcription quantitative PCR (qPCR) is a common approach to measure mRNA and offers an accurate and sensitive method for gene-expression analysis

1- determined type of bioactivity
2- determined the target genes
3- choose specific primers for amplification each gene

primers hybridizes with the sample DNA and defines the region that will be amplified, resulting in millions and millions of copies in a very short time. (primers. - short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.)





4- Types of cells

anti-inflammatory effect

macrophage cell line

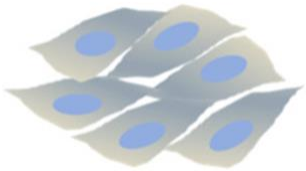
TNF-α
IL-6
IL-12
IFNs

anti-cancer effect

Cancer cells

p53,
bcl-2,
TNF
bax

anti- melanogenice ffect



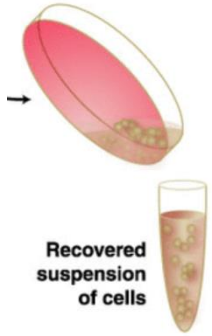
Melanoma cells

Tyr

5- Prepare different concentration from isolated plant compound

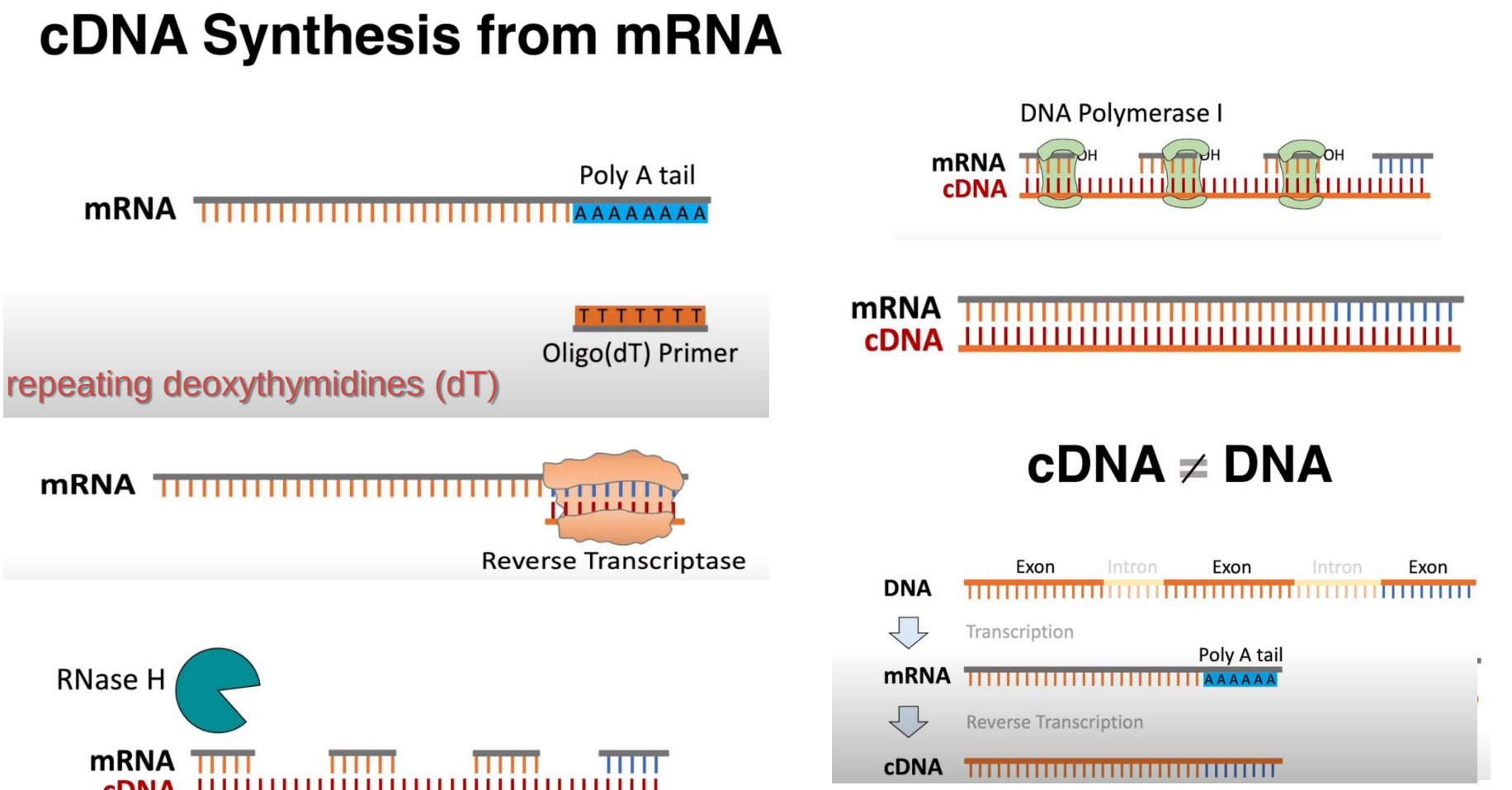
6- Cells are treatment with plant compound, or with (positive control)

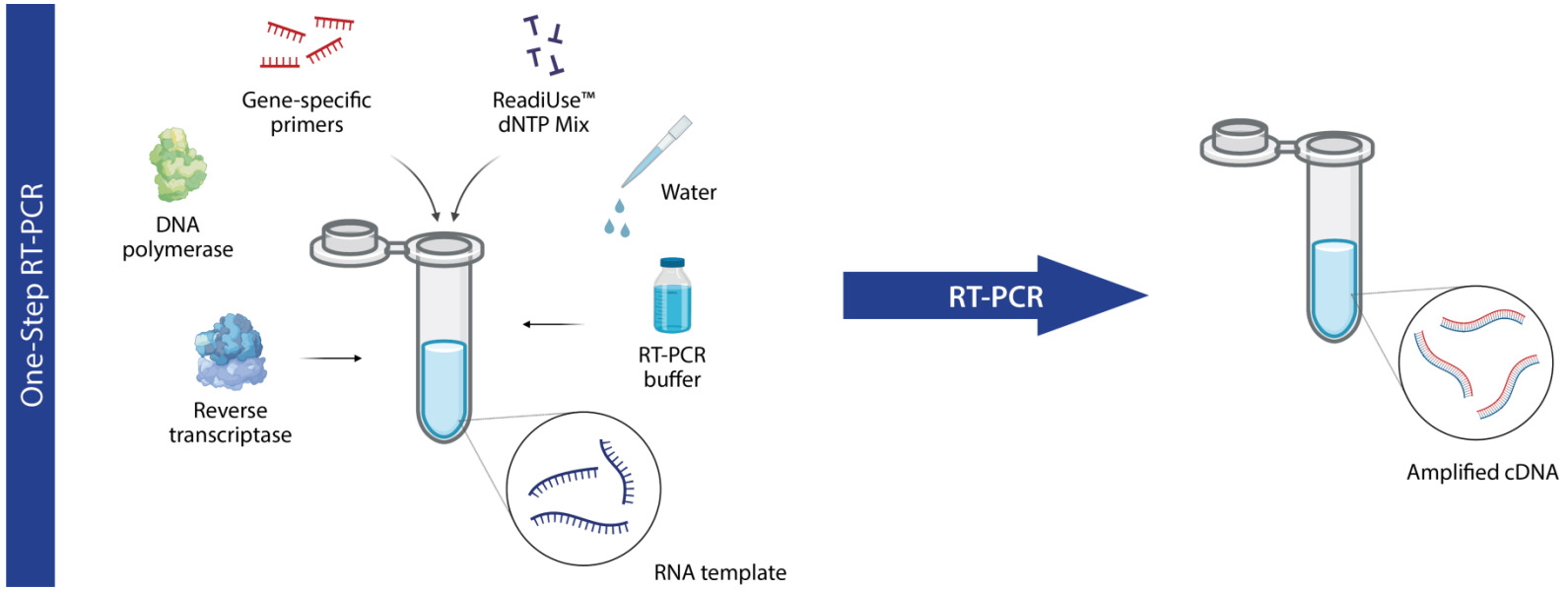
7- the total RNA of the cells was isolated



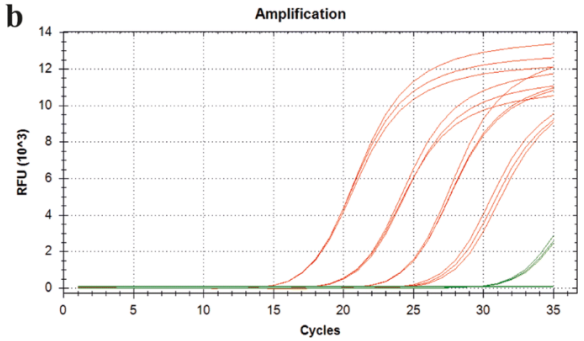
8-cDNA Synthesis

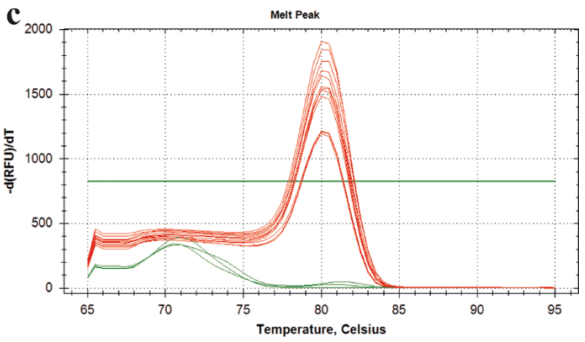
The synthesis of complementary DNA (cDNA) from RNA is an essential first step in gen expression analysis. We use an enzyme called "**reverse transcriptase**" to create a complementary DNA (cDNA) sequence from the RNA fragment.




One-Step RT-PCR
Gene-specific primers
ReadiUse™ dNTP Mix
DNA polymerase
Water
RT-PCR buffer
Reverse transcriptase
RNA template
RT-PCR
Amplified cDNA

9- Quantitative reverse transcription PCR (RT-qPCR) involves the detection and quantification of RNA

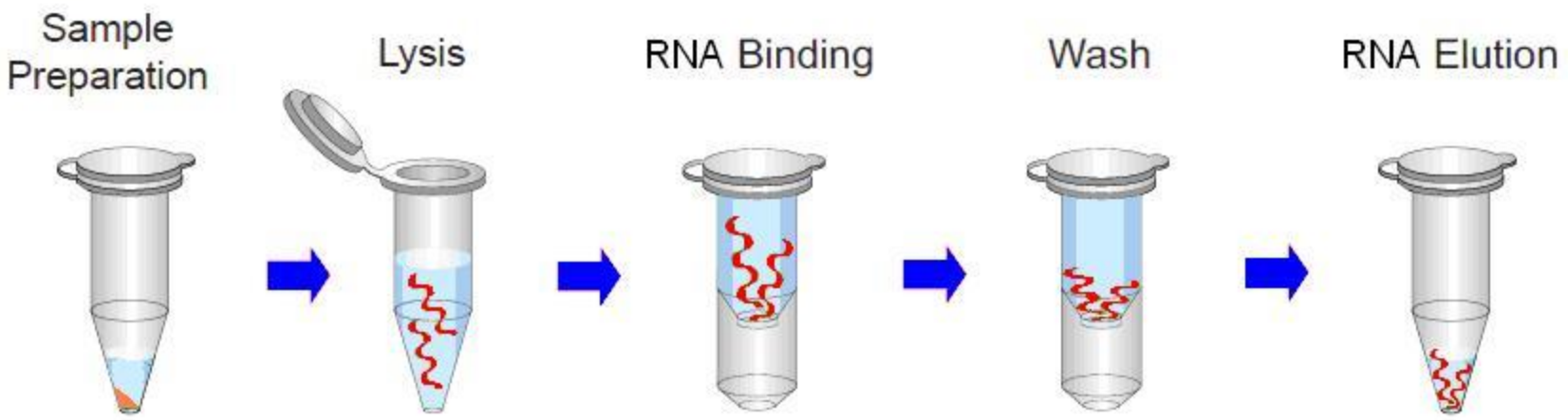




DNA or RNA Extraction

The entire process completes in following steps:

1. cell lysis
2. Isolation of nucleic acid
3. DNA or RNA precipitation
4. DNA or RNA washing
5. DNA or RNA dissolving



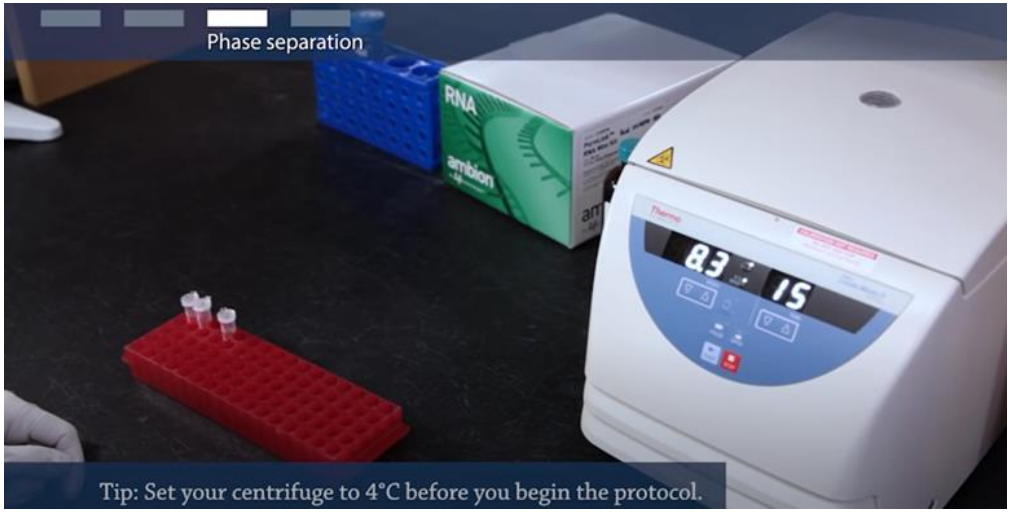
Sample sizes can be up to 200 mg or 5 x 10^8 cells; see the online product manual for details.

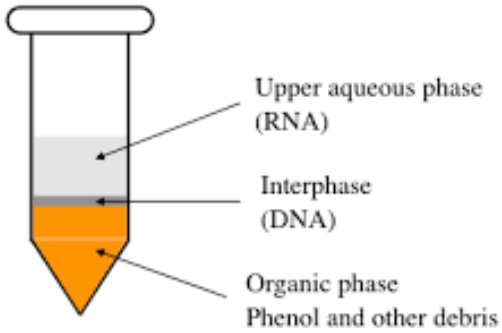
Step 1

Cell lysis

Cell lysis is a process of breaking cell wall/membrane and nuclear membrane using chemicals, physical or enzymatic techniques

Lysis buffer, and Proteinase K

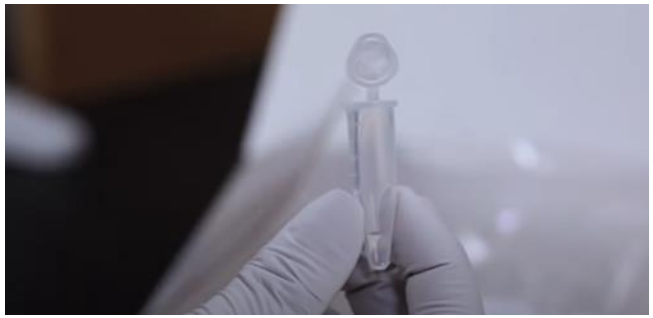




2- RNA binding using silica spin column

3- washing: Remove inhibitors and contaminants by washing steps

4-Elution: elute pure RNA in Rnase-free water



DNA store -20 ℃
RNA store -80 ℃

Agarose gel electrophoresis.