

Cancer : A Disease of Altered Gene Expression

Cancer occurs when genes that **control cell activity** become abnormally activated or silenced. This can happen due to **genetic mutations or changes in gene regulation**, such as how **DNA is modified**, **how RNA is processed**, or **how proteins are made and modified**.

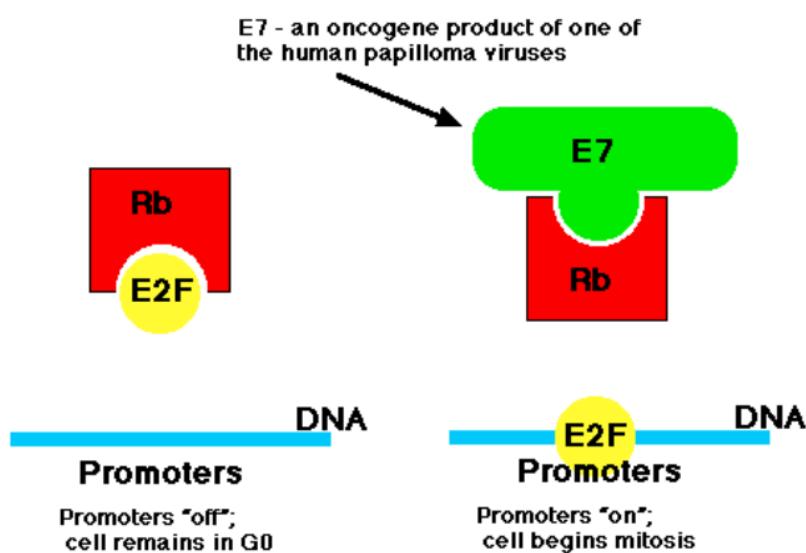
Scientists have found **different types of gene alterations in cancer**, including changes in:

- **Epigenetics** (e.g., histone acetylation, which silences genes)
- **Transcription** (activation of genes by specific proteins)
- **RNA stability** (leading to longer-lasting RNA)
- **Protein translation and**
- **modification** (affecting protein function)

Tumor Suppressor Genes, Oncogenes, and Cancer

- **Tumor suppressor genes** (like *p53*) normally prevent uncontrolled cell growth. When these genes mutate, they can no longer stop excessive cell division, leading to cancer.
- **Proto-oncogenes regulate normal cell growth**, but when mutated, they become **oncogenes** that drive uncontrolled cell division.

Example: The *myc* oncogene, when overactive, causes Burkitt's Lymphoma by transforming normal B cells into cancerous ones, leading to tumor formation.



- **Cancer:** A disease where cells grow uncontrollably.

1. Cancer and Epigenetic Changes

Epigenetics in cancer refers to **changes in gene activity that do not involve mutations in DNA but still contribute to cancer**. These changes can **silence tumor suppressor genes** or **activate oncogenes** through:

- **DNA methylation** (adding methyl groups to DNA, which turns genes off)
- **Histone modifications** (changing proteins that package DNA)
- **Dysregulation of DNA-binding proteins**

How Epigenetics Silences Genes in Cancer

- In cancer cells, **CpG islands** (DNA regions rich in cytosine and guanine) become **methylated**, turning off genes.
- **Histones** (proteins that help package DNA) lose their **acetyl groups**, further silencing genes.
- This combination of **DNA methylation** and **histone deacetylation** leads to gene silencing, promoting cancer.

Potential for New Cancer Treatments

- Since epigenetic changes are **reversible**, scientists are developing **drugs** that can **reactivate silenced genes** and restore normal cell function.
- Understanding epigenetics may also help treat other diseases like allergies, inflammation, and autism.

2. Cancer and Transcription factors

proteins that control when and how genes are turned on or off. Some of these factors are **proto-oncogenes** or **tumor suppressors**, which help regulate cell growth and division. When cancer develops, these controls can be disrupted.

How Transcription Control is Altered in Cancer

- **Mutations** can increase the activity of transcription factors, leading to **uncontrolled gene expression** and abnormal cell growth.
- Changes in **promoter or enhancer DNA regions** can make transcription factors bind more strongly, leading to **excessive gene activation**.

Targeting Transcription in Cancer Treatment

- Scientists study how transcription factors work to develop drugs that can **turn off cancer-related genes**.
- In **breast cancer**, **EGFR (epidermal growth factor receptor)** is often **overexpressed**, activating a chain of proteins that promote cancer growth.
- **New drugs** that block EGFR are used to **slow down tumor growth**.

3. Cancer and Post-Transcriptional Control

Post-transcriptional control happens after a gene is transcribed into RNA but before it is translated into protein. This process determines the stability, processing, and final fate of RNA molecules. **RNA-binding proteins (RBPs)** help regulate different steps, such as:

- **Alternative splicing** (producing different RNA versions from the same gene)
- **RNA degradation** (breaking down unnecessary RNA)
- **Exporting RNA from the nucleus**
- **Storing or destroying RNA in special cellular structures**

How This Affects Cancer

- **MicroRNAs (miRNAs)** are small RNA molecules that bind to other RNA molecules and destroy them.
- **Too many miRNAs** can reduce protein production, disrupting normal cell function.
- **Different cancers** have unique miRNA patterns—for example, breast cancer cells have different miRNAs than lung cancer cells or normal breast cells.

Potential Cancer Treatments

- Since **some miRNAs are only found in cancer cells**, scientists are exploring drugs that **block or turn off these miRNAs** to slow cancer growth.

4. Protein Modifications and Cancer

Cancer cells often show **changes in protein production and modification**, such as:

- **Increased protein translation** (making more of a protein)
- **Changes in phosphorylation** (adding chemical groups to proteins)
- **Alternative splicing** (producing different versions of a protein)

Example: Colon Cancer and the c-Flip Protein

The c-Flip protein regulates **cell death** and has two forms:

- **Long form (c-FLIPL)**
- **Short form (c-FLIPS)**

In **normal cells**, both forms help control cell death. However, in **colon cancer cells**, the **long form (c-FLIPL)** promotes **cell growth instead of cell death**, leading to cancer progression.

New Cancer Treatments: Targeted Therapy

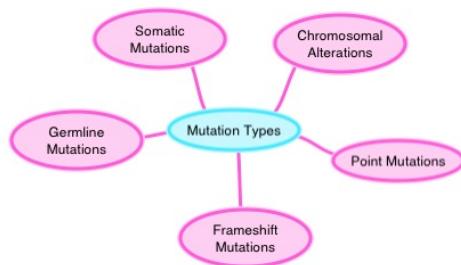
Scientists are **designing drugs** based on the **specific gene expression patterns** of individual tumors. This approach is called **personalized medicine** and helps create treatments that:

- **Target cancer cells** while sparing healthy ones
- **Exploit gene mutations or protein overproduction**

Example:

- **Anti-EGF receptor drugs** are used to treat breast cancer tumors that produce **too much EGF protein**.
- More **targeted therapies** are being developed as scientists continue to study gene expression changes in cancer.

Gene expression refers to how certain genes are activated in specific cells at certain times, which determines how cells function. Gene expression is often measured by **quantifying proteins or mRNA levels**. Common protein measurement methods include **Western blotting** and **ELISA**.



Mutations

A mutation is a change in the DNA or RNA sequence. While mutations are often linked to science fiction, they happen naturally in everyone. Most people have many mutations, and they are essential for evolution as they create new genetic variations. Although most mutations have no impact, some can be beneficial, and even harmful mutations rarely cause drastic changes.

Types of Mutations

- Germline Mutations: These occur in reproductive cells (sperm or eggs). They are significant because they can be passed on to offspring, affecting every cell in the next generation.
- Somatic Mutations: These happen in other body cells and are not passed to offspring. They usually have little effect, as they only affect the individual where they occur.

Chromosomal Alterations

These mutations change the structure of chromosomes, often when a part of a chromosome breaks off and reattaches incorrectly. They can be serious and may result in the death of an organism or lead to abnormalities, like **Down Syndrome**, which is caused by a chromosomal duplication.

Point Mutations

A point mutation changes a single nucleotide in the DNA sequence. These mutations are typically less serious than chromosomal alterations. There are three types of point mutations:

- **Silent Mutation: No effect; the change doesn't alter the protein.**

- **Missense Mutation:** Changes one amino acid, which may or may not affect the protein's function.
- **Nonsense Mutation:** A change that creates a stop codon, usually leading to a nonfunctional protein.

Type	Description	Example	Effect
Silent	mutated codon codes for the same amino acid	CAA (glutamine) → CAG (glutamine)	none
Missense	mutated codon codes for a different amino acid	CAA (glutamine) → CCA (proline)	variable
Nonsense	mutated codon is a premature stop codon	CAA (glutamine) → UAA (stop)	usually serious

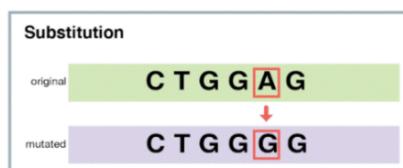
Frameshift Mutations

A frameshift mutation occurs when nucleotides are added or removed from the DNA sequence, shifting the reading frame. This alters how the codons are read and can drastically change the protein produced.

- Germline mutations affect reproductive cells and can be passed to offspring.
- Somatic mutations affect body cells and cannot be inherited.
- Chromosomal alterations change chromosome structure and can cause severe effects.
- Point mutations alter a single nucleotide, which can have varying effects.
- Frameshift mutations change the reading frame of the sequence, leading to major changes in the protein.

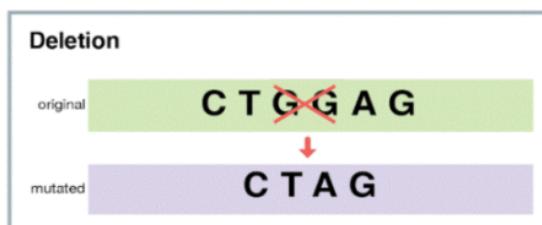
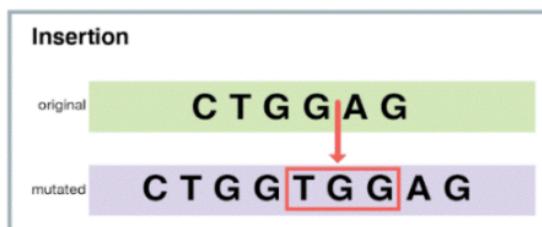
DNA can change in several ways, leading to different types of mutations. Here's a quick overview:

1. **Substitution:** One base is replaced with another (e.g., changing A to G). This can:
 - Change a codon to code for a different amino acid, altering the protein (e.g., sickle cell anemia).
 - Change a codon to one that still codes for the same amino acid, causing no change (silent mutation).
 - Change a codon to a “stop” codon, leading to an incomplete protein that may not work.



2. **Insertion:** Extra base pairs are added into the DNA sequence.

Deletion: A section of DNA is lost or removed.



3. **Frameshift:** Insertions or deletions can shift the reading of the gene, causing the codons to be read incorrectly. This often results in a non-functional or incomplete protein.

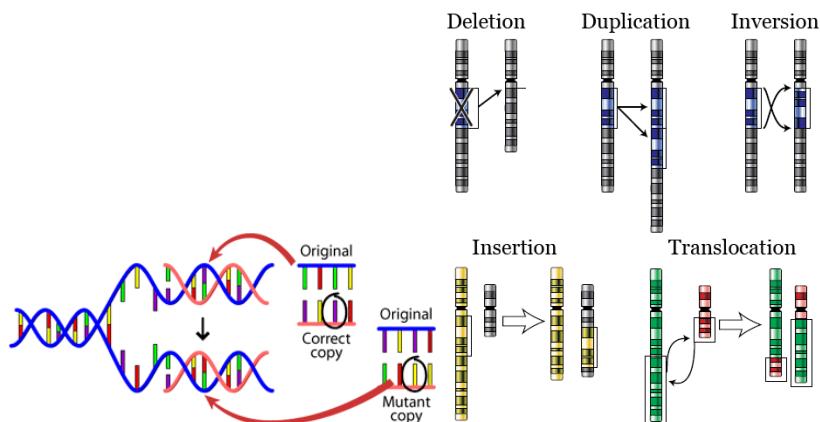


AUG-AAU-ACG-GCU = start-asparagine-threonine-alanine

Now, assume an insertion occurs in this sequence. Let's say an **A** nucleotide is inserted after the start codon **AUG**:

AUG-AAA-UAC-GGC-U = start-lysine-tyrosine-glycine

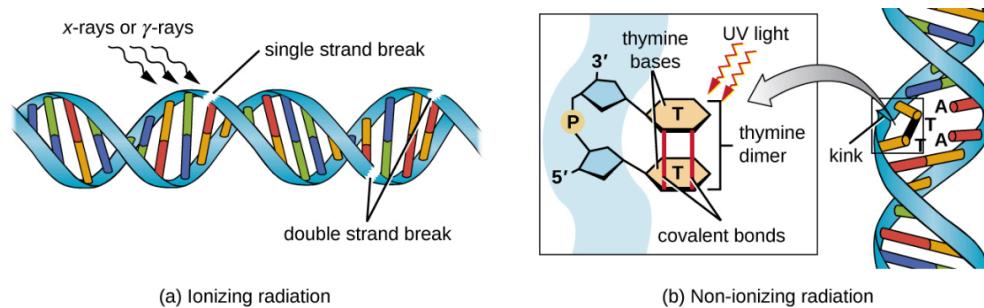
While there are other types of mutations, these are the main ones to understand.



Causes of Mutations

Mutations can happen for several reasons:

- DNA copying errors:** When a cell divides, it copies its DNA, but sometimes the copy is imperfect, leading to a mutation.
- External influences:** Exposure to chemicals or radiation can damage DNA. The cell tries to fix this, but the repair might not be perfect, causing a mutation.



Effects of Mutations

- Somatic mutations:** These occur in non-reproductive cells and do not get passed to offspring. For example, a mutation causing a tulip's petal to have two colors won't affect the plant's seeds. Somatic mutations can also cause diseases like cancer.

Germ line mutations: These happen in reproductive cells (eggs and sperm) and can be passed to offspring, affecting evolution.

Effects of Germ Line Mutations



Neutral or no effect: Some mutations don't affect the organism. For example, a mutation might slightly change a cat's ear shape but have no impact on its health.

Detrimental effect: Some mutations harm the organism. For example, Marfan syndrome, caused by a mutation in connective tissue, leads to heart problems.

Beneficial effect: Some mutations help an organism. For example, insects might develop resistance to pesticides like DDT due to a mutation.

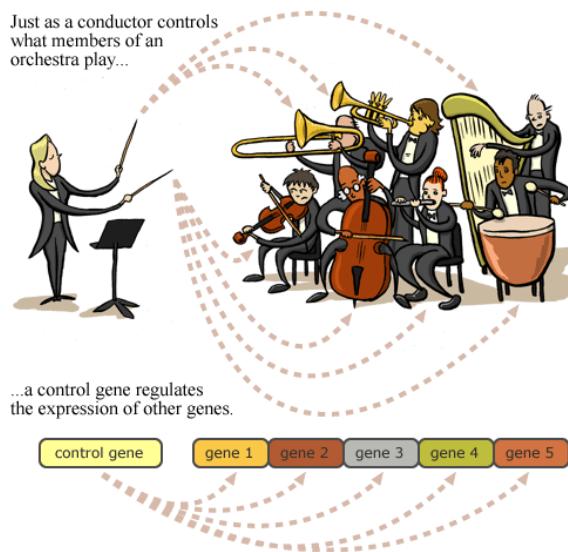
A Summary of Mutagenic Agents

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
Nucleoside analogs			
2-aminopurine	Is inserted in place of A but base pairs with C	Converts AT to GC base pair	Point
5-bromouracil	Is inserted in place of T but base pairs with G	Converts AT to GC base pair	Point
Nucleotide-modifying agent			
Nitrous oxide	Deaminates C to U	Converts GC to AT base pair	Point
Intercalating agents			
Acridine orange, ethidium bromide, polycyclic aromatic hydrocarbons	Distorts double helix, creates unusual spacing between nucleotides	Introduces small deletions and insertions	Frameshift
Ionizing radiation			
X-rays, γ -rays	Forms hydroxyl radicals	Causes single- and double-strand DNA breaks	Repair mechanisms may introduce mutations
X-rays, γ -rays	Modifies bases (e.g., deaminating C to U)	Converts GC to AT base pair	Point
Nonionizing radiation			
Ultraviolet	Forms pyrimidine (usually thymine) dimers	Causes DNA replication errors	Frameshift or point

Mutations in Control Genes

Some mutations happen in genes that control when and where other genes are active. These mutations can have big effects because they can change how the entire body is built. For example, Hox genes control where body parts like heads and limbs develop. A mutation in these genes can cause major changes in an organism's body structure over generations.

- <https://evolution.berkeley.edu/dna-and-mutations/a-case-study-of-the-effects-of-mutation-sickle-cell-anemia/>



A beneficial mutation called CCR5-delta 32 provides resistance to HIV infection. The mutation affects a protein called CCR5 on T cells, which HIV needs to enter the cell. People with this mutation are less likely to get infected with HIV because the virus can't bind to the altered receptor. This mutation is most common in northern Europeans and may have been selected in the past due to protection against diseases like the plague and smallpox. It might have spread during Viking invasions.

Although this discovery is promising for HIV research, such as developing drugs that block CCR5, testing for the mutation is not widely recommended. Some individuals with the mutation have still contracted HIV, so relying on the mutation alone is not a foolproof method of protection. However, targeting CCR5 remains a potential strategy for new HIV treatments.

Why study Gene Expression?

Understanding Gene Expression and Function

To understand **how genes work**, scientists often study what happens when a **gene is missing or altered**. This approach, called classical genetics, **involves observing mutant organisms with genetic changes and analyzing how their traits (phenotypes) are affected**. By identifying the responsible genes, researchers **gain insight into gene function**.

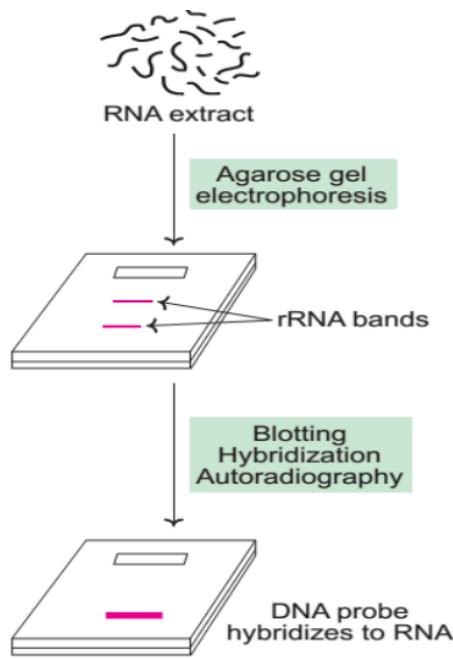
With advancements in **genome sequencing**, scientists can also start with a **DNA sequence and predict its function by comparing it to known genes**. To confirm its role, they may create mutants that lack or modify the gene and study how this affects the organism.

Mutants can be generated using chemicals, radiation, or insertional mutagenesis—where foreign DNA disrupts a gene, making it easier to identify. This method has been widely used in bacteria, yeast, fruit flies, and plants. Common

Sources of Variation in Gene Expression (GE)

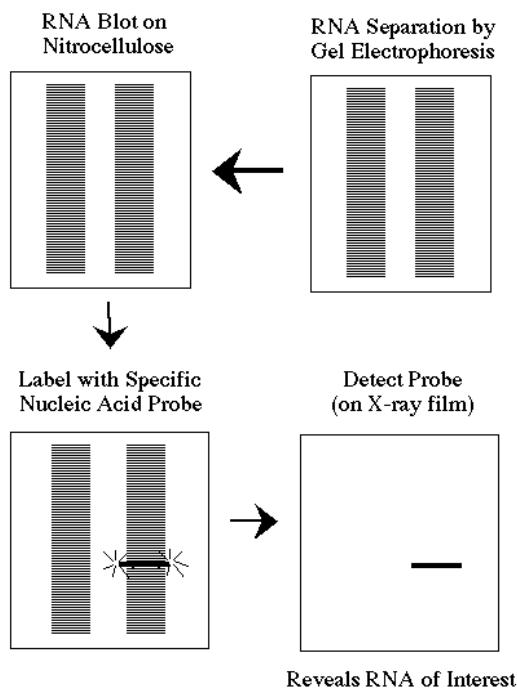
Several factors influence gene expression (GE), which researchers must consider when designing and analyzing GE studies. These factors include:

- **Tissue Specificity:** GE varies between tissues and cell types. Some genes are specific to certain tissues (e.g., PLIN1 in fat cells), while others, like housekeeping genes (e.g., GAPDH), are consistently expressed across tissues.
 - **Host Age:** GE changes with age, with some genes consistently increasing or decreasing in expression. These changes may be due to DNA damage and occur gradually. Researchers should account for age in their analyses.
 - **Host Gender:** GE differs between males and females, affecting both autosomal and sex chromosome genes. Researchers should control for gender when analyzing GE data.
 - **Time of Sample Collection:** GE fluctuates over a 24-hour period and across seasons. For consistency, researchers should collect samples at the same time of day and record collection times.
 - **Environment:** Factors like pollution, stress, and diet can alter GE. In disease studies, researchers may need to account for environmental influences.
 - **Inherited Variation:** Genetic differences affect GE and disease risk. Studies show a strong hereditary component in GE variations. Researchers should consider genetic factors in their analysis.
 - **Transcript vs. Protein Levels:** Changes in mRNA levels do not always reflect protein levels due to mRNA degradation and other cellular processes. Measuring both mRNA and protein is important for accurate conclusions.
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Identify this Technique?

Measuring Gene Expression: Blotting Techniques



Blotting is a widely used method to identify specific DNA, RNA, or protein fragments. It involves fixing nucleic acids or proteins onto a solid membrane (nitrocellulose or nylon) and detecting them through hybridization.

Common Blotting Techniques:

1. **Southern Blotting** – Detects DNA (named after Ed Southern, 1975).
 2. **Northern Blotting** – Detects RNA.
 3. **Western Blotting** – Detects proteins using specific antibodies.
 4. **Dot Blotting** – Detects DNA or RNA without electrophoresis.
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RNA Measurement Techniques

Method	Technique	Processing Step	Strengths	Limitations
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Northern Blot	Hybridization-based	Labeled RNA bound to paper	Simple, inexpensive, highly specific	Risk of degradation, low throughput
qPCR	PCR-based quantification	RNA → cDNA	Fast (8–12 hrs), easy to use	Only quantifies known genes
Microarray	Hybridization-based	RNA → cDNA	High throughput, good for GWAS	Slow (72 hrs), needs special software
RNA-Seq	Sequencing-based	RNA → cDNA or labeled RNA	High accuracy, high throughput, good for GWAS	Expensive, slow (48 hrs), data-heavy

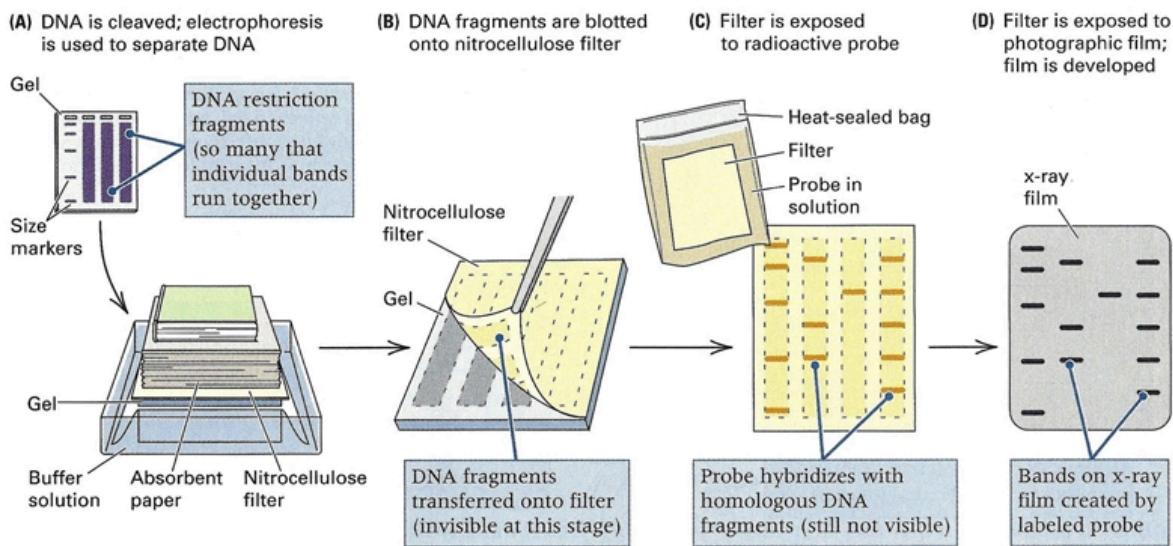
Simplified Explanation of Southern Blotting

1. DNA Migration & Transfer:

- DNA, being negatively charged, moves towards the positive electrode during electrophoresis. Smaller fragments move faster.
- The separated DNA is treated with a mild alkali to denature it (make it single-stranded) and then transferred onto a nitrocellulose or nylon membrane, creating an exact replica of the gel pattern.

2. DNA Fixation on Membrane:

- The DNA is permanently attached to the membrane by:
 - **Oven baking (80°C)** for nitrocellulose.
 - **UV cross-linking** for nylon membranes (bonds thymine residues to the membrane).



3. Hybridization & Detection:

- A labeled probe (radioactive or non-radioactive) complementary to the target DNA is added.
- The probe binds to matching DNA sequences on the membrane.
- After washing off unbound probes, the membrane is exposed to X-ray film.
- The developed film shows specific DNA bands, revealing the presence of the target DNA.

Key Points:

- **Capillary Action Transfer:** Buffer pulls DNA from the gel onto the membrane.
- **Membrane Choice:** Nylon is preferred over nitrocellulose due to its higher strength and better DNA binding.
- **Final Detection:** X-ray film shows the hybridized DNA fragments.

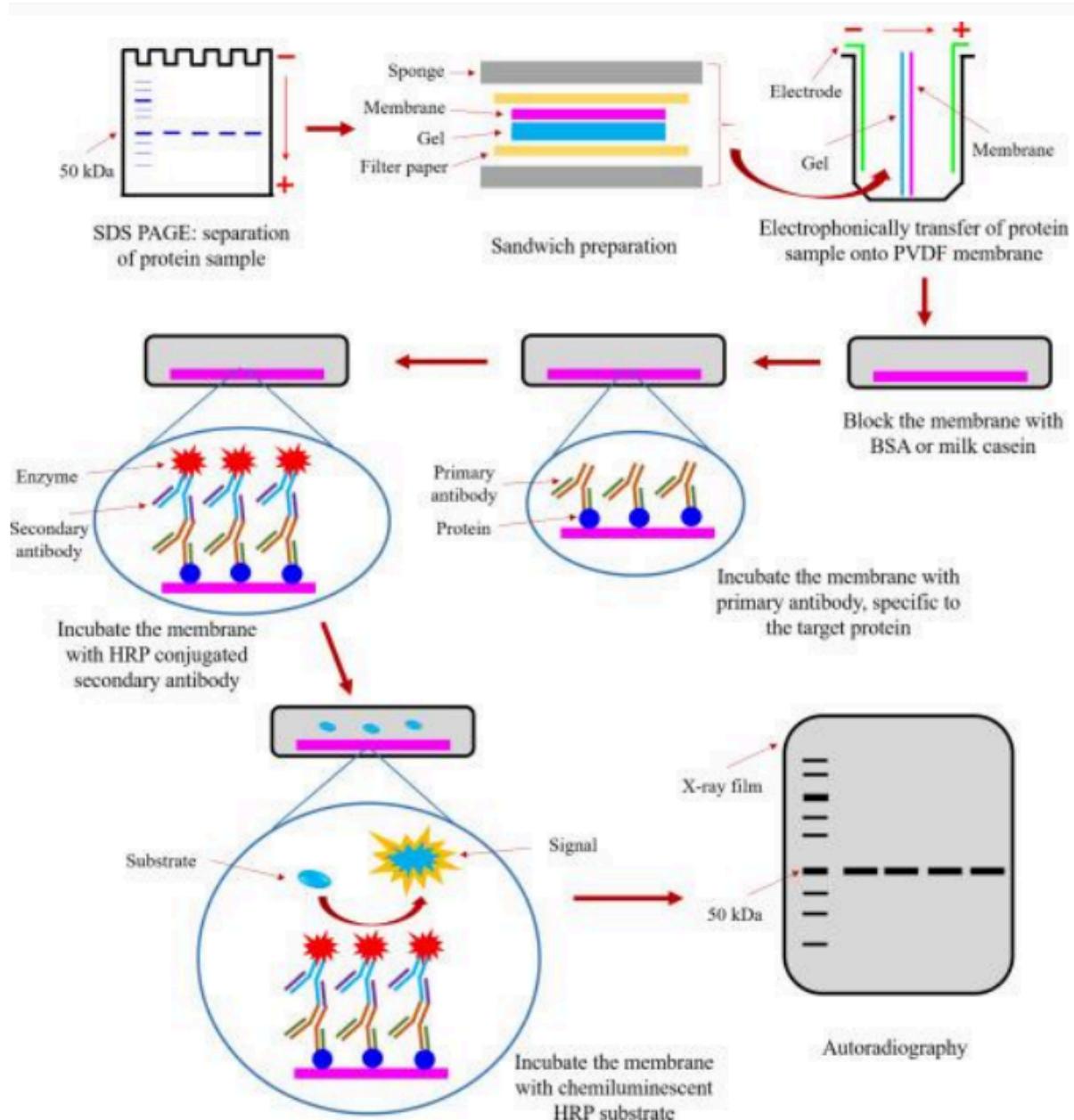
Measuring Protein Expression: Western Blotting

1. What is Western Blotting?

- A technique used to transfer proteins from polyacrylamide gels to a membrane (**nitrocellulose or nylon**).
- The term "**Western Blotting**" was introduced by Burnette in 1981.
- The transferred proteins bind strongly to the membrane for further analysis.

2. How Are Proteins Detected?

- **Antibodies** are commonly used to detect specific proteins (antigens).
- **Lectins** can identify glycoproteins.
- Detection can be done using labeled probes (radioactive, enzyme, or fluorescent tags).



Polyvinylidene fluoride (PVDF) membranes are a popular choice for many membrane processes, including Western blotting, membrane distillation, and water treatment. horseradish peroxidase (HRP). HRP is an enzyme that's commonly used to amplify signals in a variety of applications, including western blotting, ELISA, and immunohistochemistry.

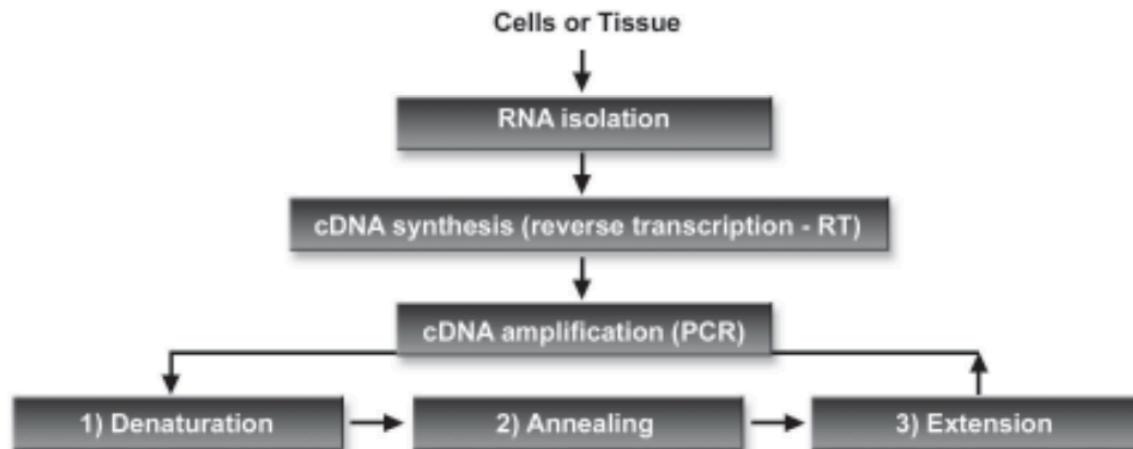
3. Sandwich Detection Method (Enhanced Sensitivity)

- Instead of labeling the primary probe, a second labeled molecule is used for detection.
- Examples include:
 - Species-specific antibodies (e.g., rabbit anti-mouse antibodies).
 - Protein A (from *Staphylococcus aureus*), which binds to IgG antibodies.
 - Streptavidin, which binds to biotin-labeled antibodies.
- This method improves **sensitivity** because multiple second molecules bind to a single probe, amplifying the signal.

Key Advantage:

- A single labeled second molecule can be used as a universal detector for different primary probes, making the technique versatile and highly sensitive.

Measuring Protein Expression: RT PCR



Here's a simplified version:

RT-PCR Overview

Objective:

- Amplifies a specific RNA segment, producing billions of copies.
- Used for diagnosing infections, gene studies, and gene expression analysis.

Principle:

- Converts RNA to complementary DNA (cDNA) using reverse transcriptase.
- cDNA undergoes PCR cycles (denaturation, annealing, elongation) to amplify DNA copies.

Key Components of RT-PCR

1. **RNA Sample:** Typically mRNA, converted into cDNA.
2. **Reverse Transcriptase Enzyme:** Converts RNA into cDNA.
3. **DNA Polymerase (e.g., Taq Polymerase):** Amplifies cDNA.
4. **Primers:** Short DNA sequences to initiate amplification. Types include:
 - **Random Primers:** Bind at various RNA sites.
 - **Oligo (dT) Primers:** Target mRNA poly(A) tail.
 - **Sequence-Specific Primers:** Target a specific gene.
5. **dNTPs (DNA Building Blocks):** A, T, C, G nucleotides for DNA synthesis.

6. **PCR Buffers & Chemicals:** Maintain reaction stability.
7. **Thermocycler (PCR Machine):** Controls temperature for reaction cycles.

qPCR: SYBR Green I (SG) is a fluorescent dye that binds to DNA, while TaqMan is a method that uses a fluorogenic probe to detect a target gene. Both are used in real-time PCR (qPCR) to analyze DNA

Applications of RT-PCR

1. Studying Gene Expression

- Helps analyze gene activity by amplifying small mRNA samples.
- Used to study drug-resistant genes in pathogens.

2. Identifying Unknown Species

- Detects viruses like HIV, SARS, dengue, and HCV.
- Identifies microorganisms and higher organisms by analyzing rRNA and mRNA.

3. Diagnosing Infectious Diseases

- Used in clinical labs to detect viral, bacterial, fungal, and parasitic infections.
- Helps in cancer and genetic disease diagnosis.

4. Gene Insertion & Gene Therapy

- Produces cDNA from mRNA for insertion into prokaryotes.
- Monitors gene therapy by analyzing specific gene expressions.

5. Detecting Mutations & Cancer Cells

- Identifies tissue-specific mutations and unique cancer-related mRNAs.
- Detects unwanted changes in mRNA sequences.

6. Genetic Engineering & Viral Studies

- Analyzes modified DNA and its transcribed RNA.
- Amplifies target RNA for research.

7. Measuring mRNA Expression Knockdown

- Uses RT-qPCR to measure gene silencing by siRNA.

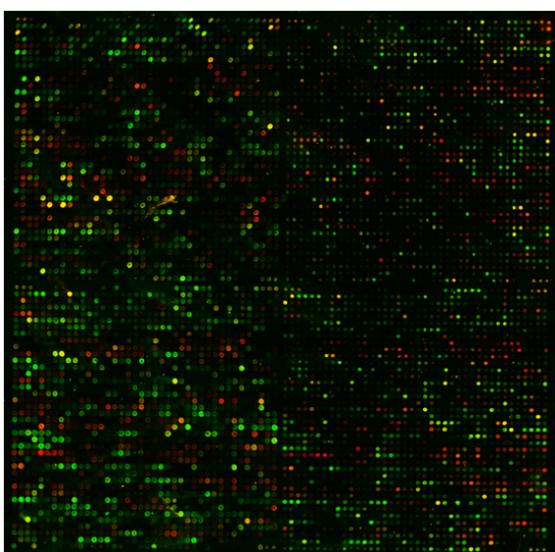
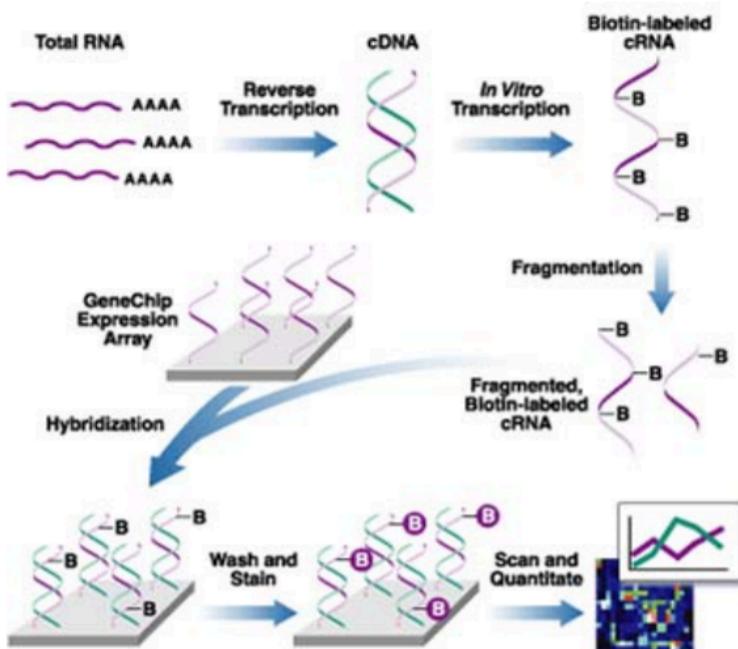
Measuring Gene Expression: MicroArray

A **microarray** is a collection of single-stranded DNA (ssDNA) probes fixed onto a solid surface like a glass or nylon slide. These probes are arranged in a grid pattern, with each spot containing millions of copies of a unique DNA sequence.

Applications of Microarrays:

- **Gene expression studies:** By labeling mRNA from cells and hybridizing it to a microarray, scientists can measure which genes are active and at what levels.
- **Mutation detection:** Microarrays can identify **single nucleotide polymorphisms (SNPs)** or mutations in genes, useful in disease research.
- **Medical diagnostics:** Helps classify diseases, understand biological processes, and identify drug targets.
- **Food safety:** Detects contamination in food by identifying unwanted DNA from other organisms.

Microarrays use **hybridization** to detect specific DNA or RNA in a sample. Unlike a **Southern blot**, which uses one probe to detect DNA, a microarray uses **millions of probes** on a chip to analyze a sample. The **sample DNA is labeled**, and when it binds to a probe, the hybridization is detected. This allows researchers to determine which DNA sequences are present in the sample and in what quantity.



How Gene Expression Microarrays Work:

1. **mRNA Isolation:** Extract mRNA from cells of interest.
2. **Labeling & Hybridization:** Convert mRNA into labeled cDNA and allow it to bind to probes on the microarray.
3. **Detection & Analysis:** A laser scans the chip, and software quantifies gene expression based on signal intensity. *Fig: yeast gene expression microarray*

For example, in **yeast gene expression studies**:

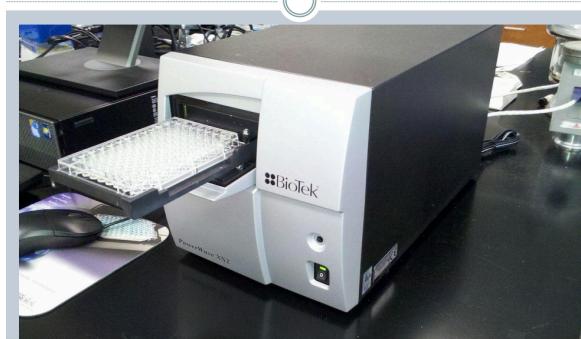
- **Red spots:** Genes expressed only in oxygen-rich conditions.
- **Green spots:** Genes expressed only in oxygen-poor conditions.
- **Yellow spots:** Genes expressed in both conditions.
- **Black spots:** No expression in either condition.

Microarrays have revolutionized gene expression analysis, enabling scientists to study thousands of genes at once, identify disease markers, and explore new drug targets.

Measuring Protein Expression: ELISA

ELISA is a safe, sensitive, and widely used technique for **medical diagnostics, drug testing, and disease detection** without the risks of radiation.

ELISA Reader Spectrophotometer
A microplate reader with a 96-well plate in the sample drawer



Development of ELISA/EIA Test Kits



Simplified Version:

Materials Needed for ELISA Testing:

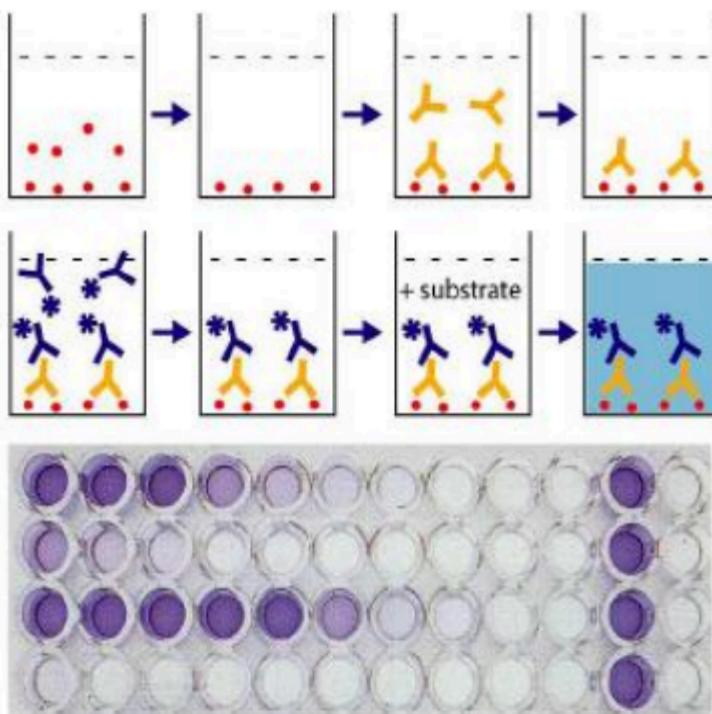
1. Equipment:

- **ELISA Reader:** Measures color intensity at specific wavelengths (450 nm & 650 nm).
- **Pipettes:** Available in fixed or adjustable volumes, single or multi-channel.
- **Washing System:** Can be manual (washing row by row), semi-automated (washing a strip or plate at a time), or fully automated (processing multiple plates).

2. Reagents (Included in the Kit):

- **Coated Plates:** 96-well polystyrene plates coated with antigens or antibodies to capture the target molecules.
- **Controls:** Positive and negative controls help standardize results.
- **Conjugates:** Enzyme-labeled antibodies that bind to the target analytes.
- **Wash Concentrate:** A buffered solution to remove unbound materials.
- **Stop Solution:** Stops the enzyme reaction and prevents further color change.

Principle of ELISA:



- **Process:**

- Serum samples are added to wells coated with antigen or antibody.
- The plate is washed to remove unbound components.
- Enzyme-linked secondary antibodies are added to detect bound antigens or antibodies.
- After incubation, unbound secondary antibodies are washed away.
- A substrate is added, reacting with the enzyme to produce a color.
- The color intensity is measured at **450 nm**, indicating the quantity of antigens or antibodies in the sample.

- **Interpretation:**

- The **stronger the color**, the **higher** the concentration of the detected antigen or antibody.

The Gene Expression Omnibus (GEO) is a public database hosted by NCBI, designed to store **high-throughput genomic data**, such as **gene expression profiles** from **microarray and sequencing technologies**. It allows researchers to **submit and access data related to functional genomics**. GEO supports various data types like **mRNA expression**, **genomic DNA**, and **proteomic data**. Users can query data based on keywords, organisms, or other parameters and use tools to analyze and visualize gene expression patterns. GEO also offers tools like **GEO2R** for differential gene expression analysis, **ClueGO** for functional network analysis, and **FunRich** for visualizing enrichment and network data. As of 2018, it contained over 2.4 million samples and a vast number of gene expression profiles for numerous organisms. Researchers use GEO for data analysis and to explore experiments related to diseases, including cancer.

<https://www.ncbi.nlm.nih.gov/geo/geo2r/>

Expression data for HT29 cells treated with 5-aza-deoxy-cytidine [RNA-Seq]

Accession number: GSE41586

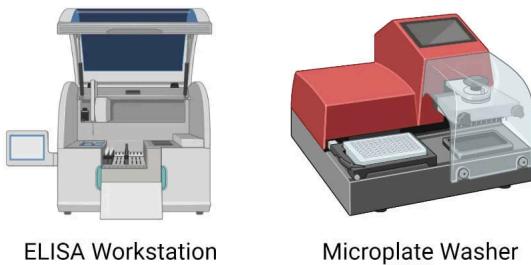
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41586>

The study compared RNA-Seq and microarray platforms using **HT-29 colon cancer cells treated with three concentrations of 5-aza-deoxy-cytidine**. Results showed strong correlation but some biases between the platforms. DESeq performed best for detecting differentially expressed genes, with high consistency between RNA-Seq and microarray methods.

Before Grouping

Samples		Define groups		Selected 0 out of 9 samples			
Group	Accession	Title	Source name	Cell line	Cell type	Treatment	Columns
-	GSM1019735	HT29 at 0 µM of 5-Aza, biological rep1	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control	
-	GSM1019736	HT29 at 0 µM of 5-Aza, biological rep2	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control	
-	GSM1019737	HT29 at 0 µM of 5-Aza, biological rep3	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control	
-	GSM1019738	HT29 at 5 µM of 5-Aza, biological rep1	HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low	
-	GSM1019739	HT29 at 5 µM of 5-Aza, biological rep2	HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low	
-	GSM1019740	HT29 at 5 µM of 5-Aza, biological rep3	HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low	
-	GSM1019741	HT29 at 10 µM of 5-Aza, biological rep1	HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high	
-	GSM1019742	HT29 at 10 µM of 5-Aza, biological rep2	HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high	
-	GSM1019743	HT29 at 10 µM of 5-Aza, biological rep3	HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high	

Analyze with GEO2R



Enzyme immunoassays (EIAs) use enzymes to detect and measure immune reactions. One common type, Enzyme-linked immunosorbent assay (ELISA), is widely used in clinical testing. In ELISA, a solid surface (like a microtiter well) is used to attach one component of the reaction, allowing separation of bound and free labeled substances.

In a typical ELISA test, a sample containing the target antigen is added to a surface-bound antibody, which binds to the antigen. After washing, an enzyme-labeled antibody is added, forming a complex. The addition of a substrate causes a color change, which indicates the amount of antigen present.

ELISA can also quantify antibodies in a sample, where antigen is bound to the surface, and an enzyme-labeled antibody specific to the analyte antibody is used.

ELISA is used for various applications, such as detecting antibodies against viruses, autoantigens, and measuring hormones or tumor markers. It is also widely used in home tests and screenings due to its ability to produce visible results.

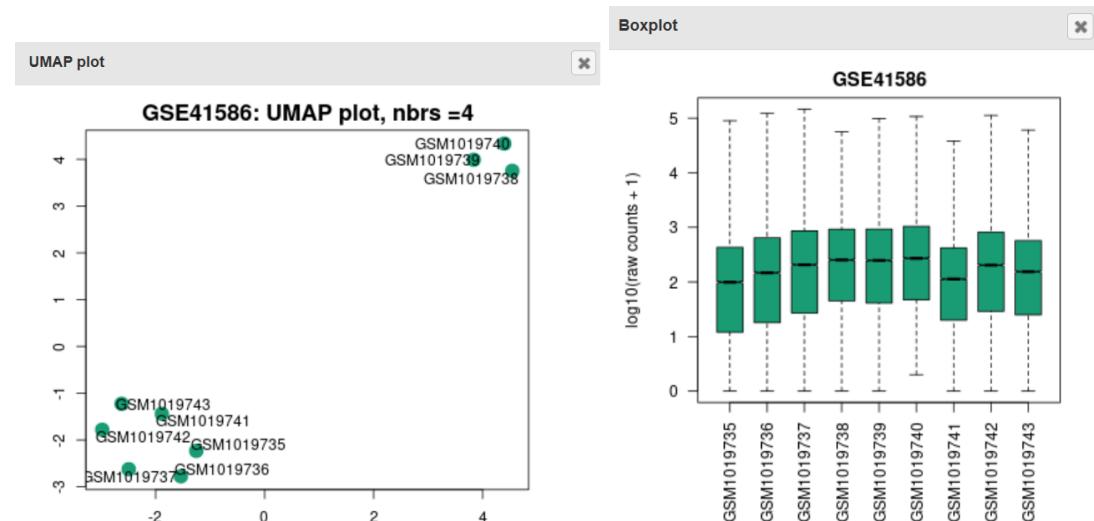
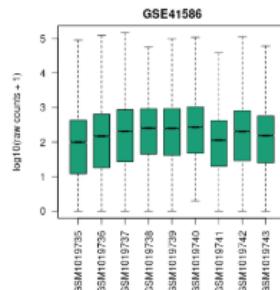
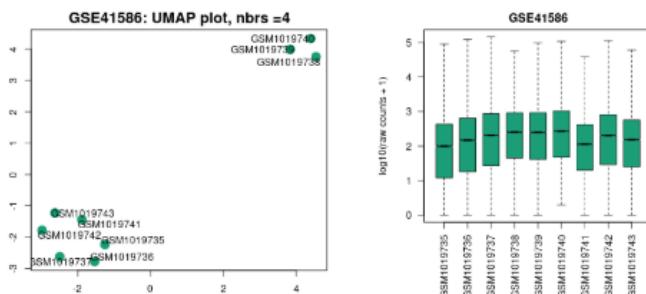
There are four main types of ELISA:

1. Direct ELISA – Uses a single enzyme-conjugated antibody that binds directly to the antigen.
2. Indirect ELISA – Uses two antibodies: one for the antigen and a second enzyme-conjugated antibody that binds to the first antibody.
3. Sandwich ELISA – Uses two antibodies: a capture antibody bound to the surface and a detection antibody to detect the antigen.
4. Competitive ELISA – Measures the presence of antibodies by competition with an enzyme-conjugated antibody.

ELISA is useful for detecting diseases, measuring hormone levels, screening donated blood, and drug abuse testing. However, various factors like plate quality, buffer contamination, and enzyme conjugate issues can affect results.

Reanalyze if you changed any options.

Visualization ?

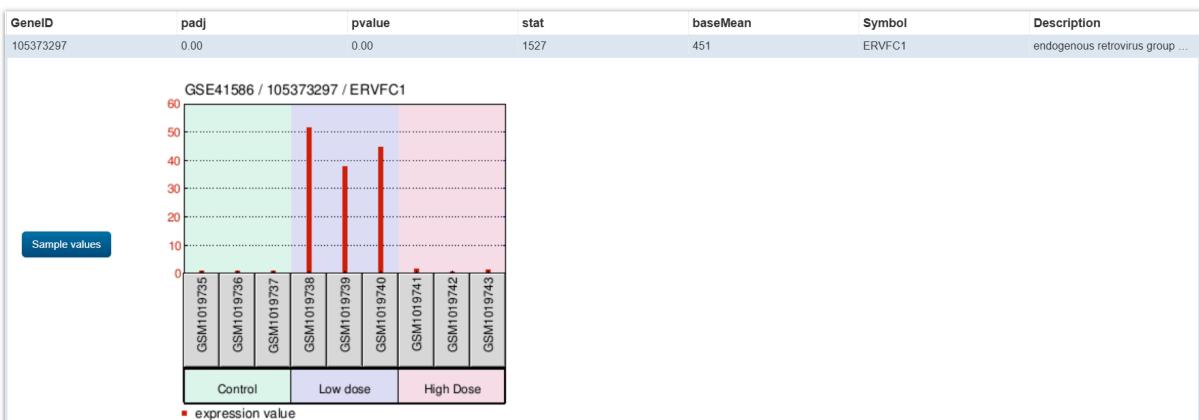


Analyze with GEO2R > Grouped under 3 concentrations

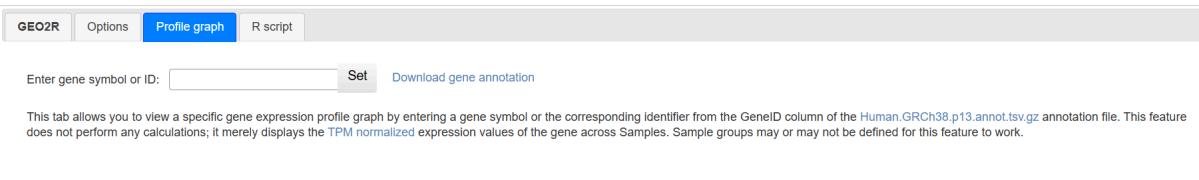
Samples		Define groups		Selected 9 out of 9 samples			
Group	Accession	Enter a group name:	List	Source name	Cell line	Cell type	Treatment
Control	GSM1019735	x Cancel selection	Control (3 samples)	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control
Control	GSM1019736		Low dose (3 samples)	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control
Control	GSM1019737		High Dose (3 samples)	HT29 treated with 0 µM of 5-Aza	HT29	colon cancer	control
Low dose	GSM1019738	HT29 at 5 µM of 5-Aza, biological rep1		HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low
Low dose	GSM1019739	HT29 at 5 µM of 5-Aza, biological rep2		HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low
Low dose	GSM1019740	HT29 at 5 µM of 5-Aza, biological rep3		HT29 treated with 5 µM of 5-Aza	HT29	colon cancer	5-Aza low
High Dose	GSM1019741	HT29 at 10 µM of 5-Aza, biological rep1		HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high
High Dose	GSM1019742	HT29 at 10 µM of 5-Aza, biological rep2		HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high
High Dose	GSM1019743	HT29 at 10 µM of 5-Aza, biological rep3		HT29 treated with 10 µM of 5-Aza	HT29	colon cancer	5-Aza high

We can do this “define” upto 10 groups.

Analyze> We get a list of genes with there expression levels in each group.



Red bars represent the expression in each group.

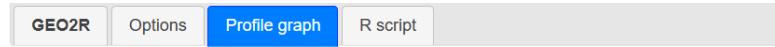


Top differentially expressed genes ?

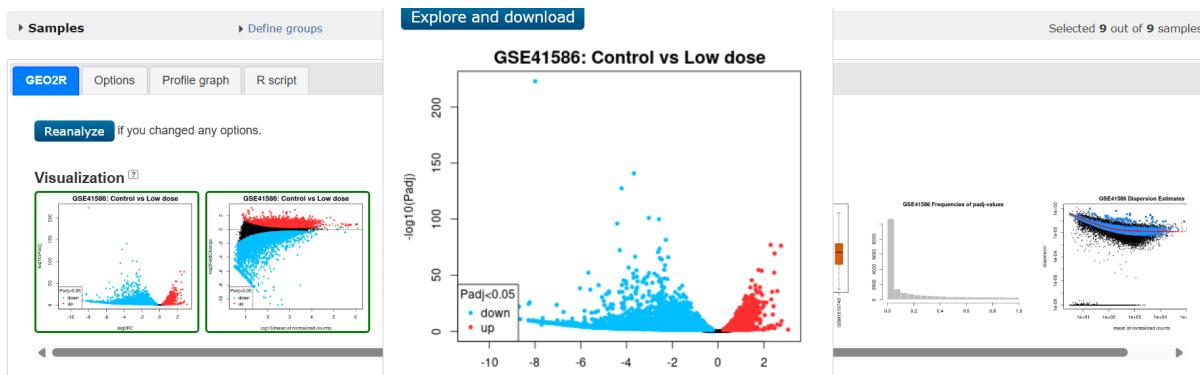
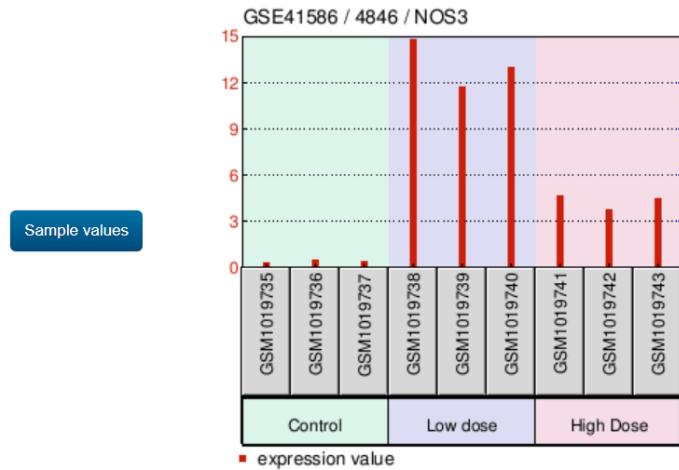
[Download full table](#) [Select columns](#)

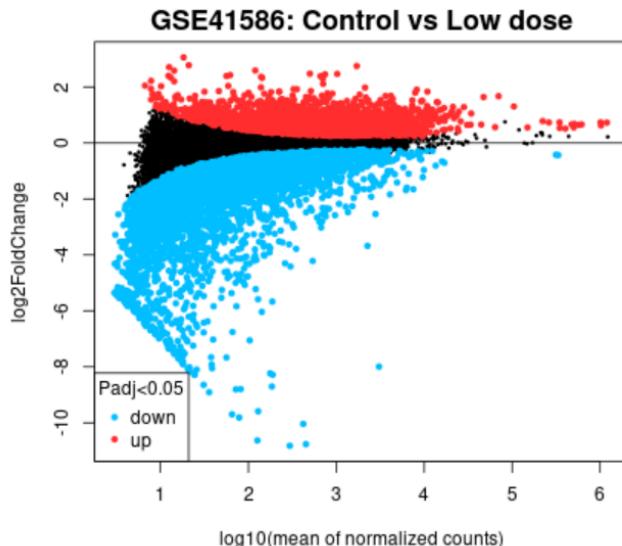
GenelID	padj
105373297	0.00

If I am interested in: Nitric Oxide Synthase 3 (NOS3)



Enter gene symbol or ID: NOS3 Download gene annotation



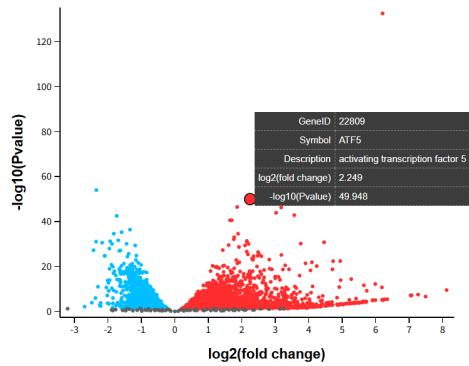


Red: Increased Expression

Blue: Decreased Gene Expression

Grey is threshold

High Dose vs Control, Padj<0.05



Select contrast to display

Control vs Low dose

Low dose vs High Dose

High Dose vs Control

[Download significant genes](#)

Explore and download: Click on each point to know the gene information, we can also download the significant genes in High/Low dose.

GEO2R Options Profile graph R script

Apply adjustment to the P-values. [More...](#)

Benjamini & Hochberg (False discovery rate)

Benjamini & Yekutieli

Bonferroni

Hochberg

Holm

Hommel

Plot displays. [More...](#)

Significance level cut-off
(enter number between 0 and 1)

0.05

Log 2 fold change threshold

0

Volcano and Mean-difference plot contrasts
(select up to 5)
0 selected ([clear](#))

Control vs Low dose

Control vs High Dose

Low dose vs High Dose

If you edit *Options* after performing an analysis, click *Reanalyze* to apply the edits:

Reanalyze

Options tab is available to change the parameters and reanalyse.

What ever changes we made in the analysis are automatically scripted and available to download:

GEO2R Options Profile graph R script

```
# Version info: R 4.2.2, Biobase 2.58.0, GEOquery 2.66.0, limma 3.54.0
#####
# Differential expression analysis with DESeq2
library(DESeq2)

# load counts table from GEO
urlId <- "https://www.ncbi.nlm.nih.gov/geo/download/?format=file&type=rnaseq_counts"
path <- paste(urlId, "acc=GSE41586", "file=GSE41586_raw_counts_GRCm38.p13.NCBI.tsv.gz", sep="")
tbl <- as.matrix(data.table::fread(path, header=T, colClasses="integer"), rownames="GeneID")

# load gene annotations
apath <- paste(urlId, "type=rnaseq_counts", "file=Human.GRCh38.p13.annot.tsv.gz", sep="")
annot <- data.table::fread(apath, header=T, quote="", stringsAsFactors=F, data.table=F)
rownames(annot) <- annot$GeneID

# sample selection
gsms <- "000111222"
sm1 <- strsplit(gsms, split="")[[1]]

# group membership for samples
gs <- factor(sm1)
groups <- make.names(c("control","low dose","High Dose"))
levels(gs) <- groups
sample_info <- data.frame(Group = gs, row.names = colnames(tbl))
```