Fundamentals of Genetics I

Molecular and Cytogenetics

Foundation of Medical Sciences HS501

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Mission: To advance the science and art of health through education, service, scholarship and social accountability

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Learning Objectives

1. Describe DNA and RNA structures.
2. Describe DNA replication, transcription and protein translation.
3. Describe different types of gene mutations.
4. Describe mutagens and DNA repair mechanism
5. Describe several mutation detection mechanisms

Outline

- Intro to human genetics
- DNA and RNA structures
- Flow of genetic information and its regulation
- Genetic Variations: its origin and detection.

Genetics

Study of genes, genetic variations in disease and inheritance patterns.

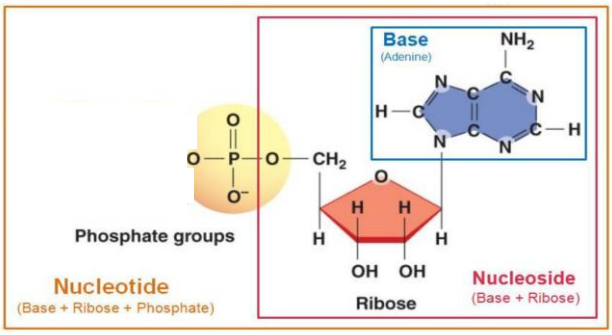
Understanding of the genetic basis of different health conditions helped the development of novel diagnostic technologies, disease prevention and management, personalized medicine, and targeted therapies

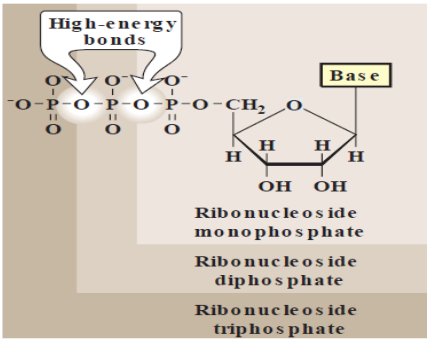
Disciplines

1- Molecular genetics, studies variability in DNA and RNA & impact on human health.
2- Cytogenetics, studies the structure and function of chromosomes in health and disease.
3- Population genetics, quantitative analysis for the distribution of genetic variation in populations. Determines the frequency of genetic diseases and carrier state. Important for risk prediction.
4- Medical genetics, studies the etiology, pathogenesis of diseases with genetic origin.
5- Behavior genetics, concerned with genetic factors that may lead to behavioral disorders.

Organization of Genetic Material
Nucleotide and Nucleosides

Nucleosides, nucleotide and nucleic acid





If the sugar is **ribose**, the nucleotide is a **ribonucleotide,** and it becomes a component of **RNA.**
If the sugar is **deoxyribose**, the nucleotide is named **deoxyribonucleotide,** and it becomes a component of **DNA**.

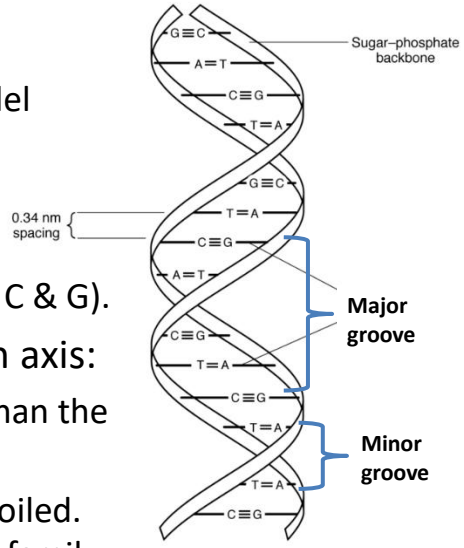
The second and third phosphate bonds to form ADP and ATP are named **high energy bonds** and each cell should have a minimal amount of ATP to enable its survival.

Functions of Nucleotides

- Precursors for nucleic acid synthesis.
- ATP is the most common energy source.
- Co-factors for multiple enzymatic pathways (NAD, FAD,..) and serve as a source of phosphate for kinases.
- Participate in metabolic reactions (like, glycogen synthesis).

DNA

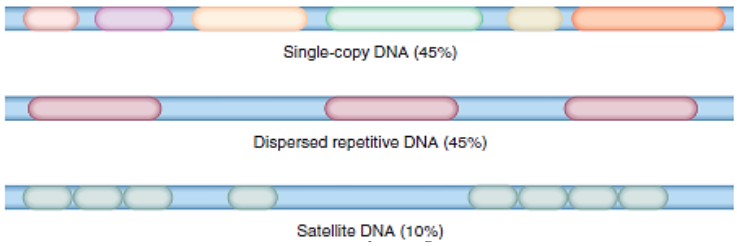
- Double helix:
  - Complementary strands that run in anti-parallel orientation.
  - backbone is made up of phosphodiester bond (phosphate-sugar bonds).
  - Held by hydrogen bonds between bases (A, T, C & G).
- DNA helix is supercoiled around a common axis:
  - **Positive supercoiling** = DNA is coiled tighter than the Watson-Crick model.
  - **Negative supercoiling**= DNA is more loosely coiled. Done the help of enzymes like topoisomerase family.



The Watson-Crick Model of the Structure of DNA

Types of DNA

- **Single-copy DNA:** around half the genome. Account for protein-coding genes with both intron and exon populations.
- **Dispersed Repetitive DNA:** Several functions like gene regulation, drive evolution and variation.
- **Satellite DNA:** Satellite repeats clustered in certain chromosomal location. Functions to keep genome stability as well as chromosome and heterochromatin structure.



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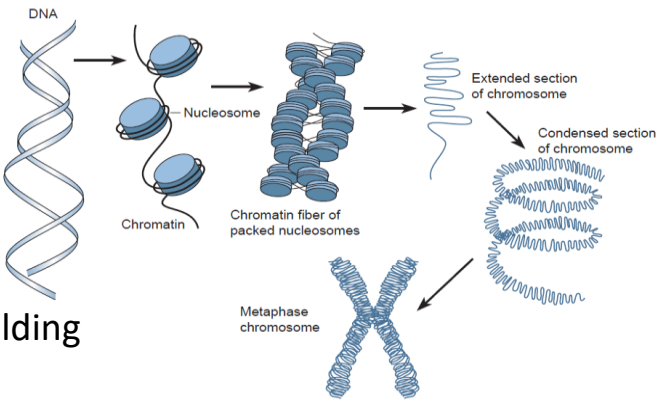
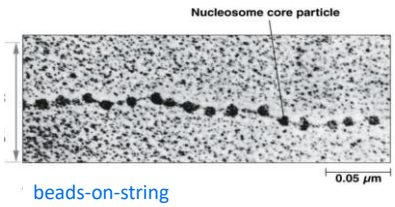
- Negatively charged DNA is condensed by wrapping around a core of positively charged histone forming the **nucleosome**.



- Nucleosome further condense yielding chromatin:
  - **Euchromatin:** Nucleosomes are loosely packed. Regions of DNA frequently used to make proteins.
  - **Heterochromatin:** nucleosomes are densely packed = genes rarely used by the cell.
- Chromatin is condensed to form the chromosome.



beads-on-string

- A somatic cell reproduce by mitosis and contains 23 chromosome pairs (diploid), 22 autosomes and a pair of sex chromosomes, either XX or XY.
- A Germ cell is produced by meiosis and contain haploid number of chromosomes, one copy of each autosome and one sex chromosome.

  Euploidy = chromosome number that is multiple of 23.

  Aneuploid = chromosome number that is not multiple of 23.

Chromosome Structure

Chromosome has a short arm (p) and a long arm (q) separated by a centromere. Centromere ensure correct chromosomal segregation to daughter cells during division. mitosis or meiosis.

1) **Metacentric** centromere in the middle of the chromosome.
2) **Submetacentric:** centromeres between the middle and the tip of the chromosome.
3) **Acrocentric:** centromere at the tip of the chromosome.

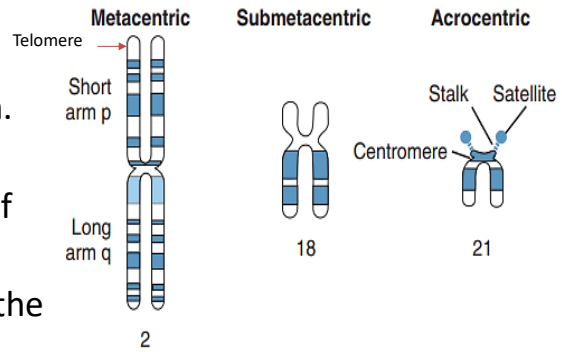
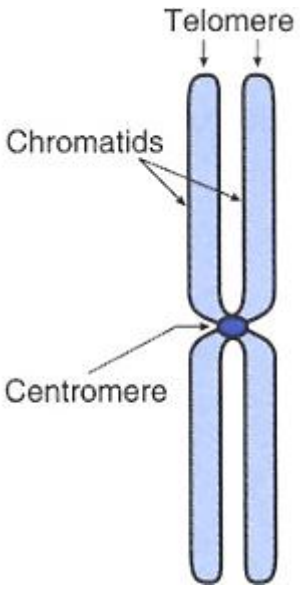


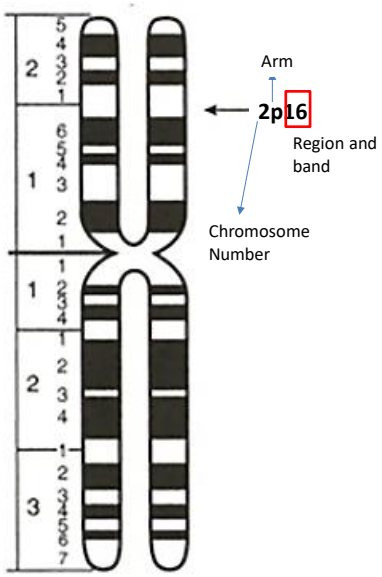
FIG 6-2 Metacentric, submetacentric, and acrocentric chromosomes.

Jorde et al, Medical genetics, 5th edition

- Each human chromosome is capped by a telomere.
- Telomere is made up of several repeats of unique DNA sequence, “TTAGGG”.
  - Function to maintain the chromosomal stability during replication and prevent its degradation by nucleases.
  - Telomerase: responsible for the *de novo* elongation of telomeres during replication.
  - Dysregulation of telomerase play a role in cell aging and cancer development.



- **Karyotyping:** number and morphology of chromosomes.
- **G-banding**: staining chromosomes during mitosis (with Giemsa) yields a characteristic staining pattern that aided in mapping. Used to identify chromosomal anomalies.
- Example *gene x* is located in 2p16



Chromosome 2

DNA replication

- DNA synthesis occurs in a 5’-3’ direction. It starts at “origin of replication” in a bidirectional manner creating a leading and lagging strands.
- Enzymes include **DNA helicase, primase polymerase, ligase**.

1- DNA helicase separate the two strands.

2- DNA Primase, synthesize short RNA fragments that are used as a primer necessary for DNA polymerase function

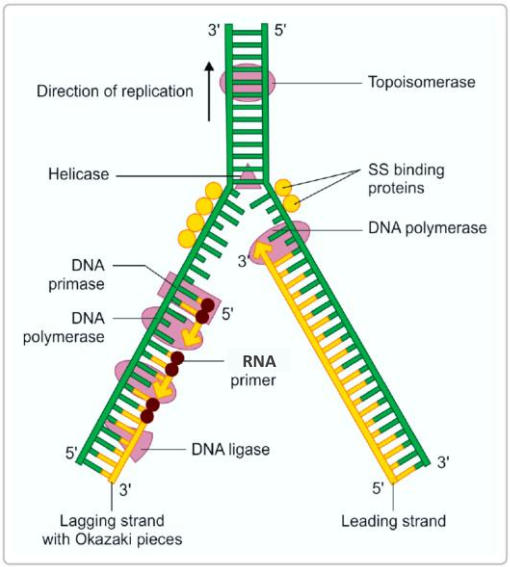


Figure 24.10: Lagging strand and Okazaki pieces

3- DNA polymerase:
On the leading strand, DNA polymerase adds nucleotides in a 5'-3' direction.
In the lagging stand (3'-5'), DNA polymerase replicates the Okazaki fragments (in 5'-3' direction. Several types of polymerases, among them proofreading polymerase (exonucleases).

4- Ribonuclease H1 (RNase H1) removes the RNA primers.

5- DNA ligase joins Okazaki fragments.

6- DNA topoisomerase family transiently cleave one or both DNA strands to remove the DNA supercoils and tangling during replication. Members of this family include DNA topoisomerases & DNA gyrase.

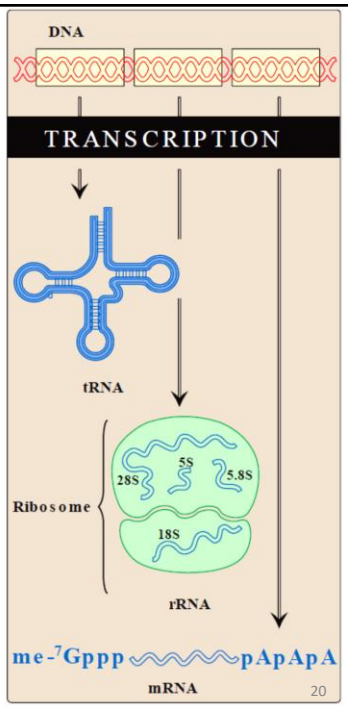
Clinical significance:

Replication errors lead to DNA mutations and several pathologies.

Targets for anticancer medications and antibiotics

RNA

- Single stranded, made up of adenine, guanine, cytosine and **uracil** nucleotides.
- Ribose sugar.
- RNA molecules often form secondary structures (like proteins).
- Types: ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA) and regulatory RNA.



Messenger RNA

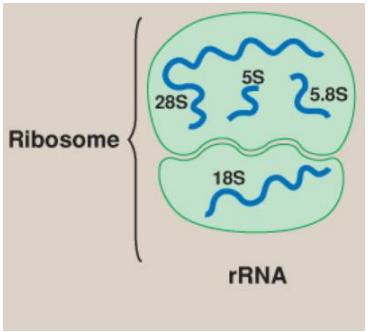
The genetic information present in DNA is **transcribed** into mRNA by RNA polymerase II. mRNA carries genetic code to produce a protein from a specific DNA sequence. mRNA has a short half-life and is often degraded quickly.

Ribosomal RNA

Synthesized by RNA polymerase I (28S, 18S and 5S).

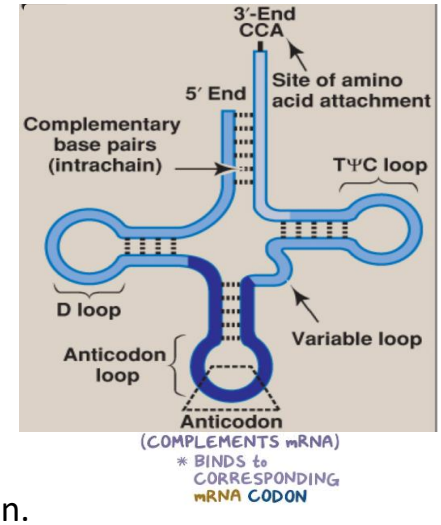
- Eukaryotic RNA: 80s, two subunits (40S and 60S)
- Prokaryotic RNA: 70S (50S & 30S).

rRNA binds the mRNA and tRNA to enable protein biosynthesis. Most abundant RNA type.



Transfer RNA

Synthesized by RNA polymerase III. tRNA carries amino acids to the ribosomal site of protein synthesis.



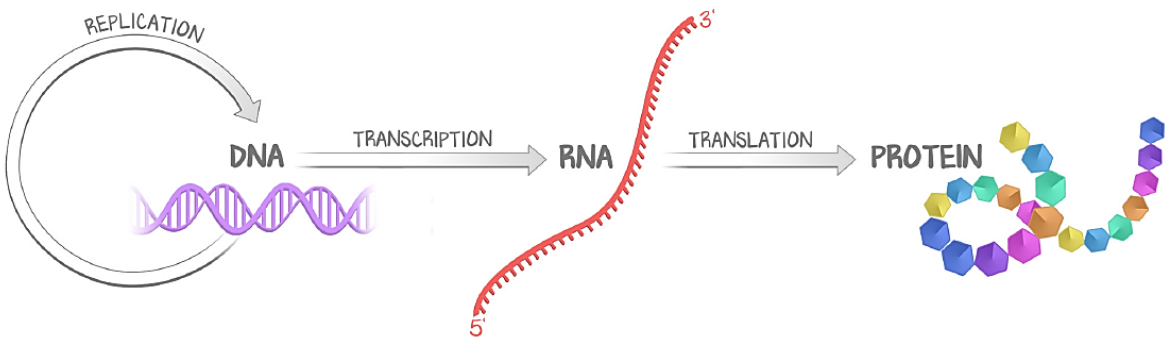
Regulatory RNA

Small nuclear RNA: These are involved in mRNA intron splicing.

Antisense RNA: complementary to mRNA strand. Upon binding, it can suppress the protein translation.

MicroRNA (miRNA): Another method to suppress protein translation.

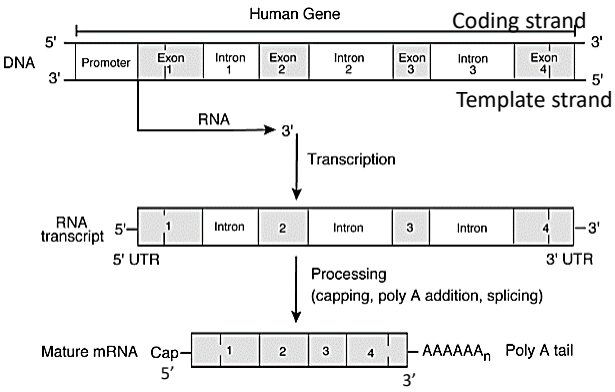
The flow of genetic information from genes to proteins "Central Dogma"

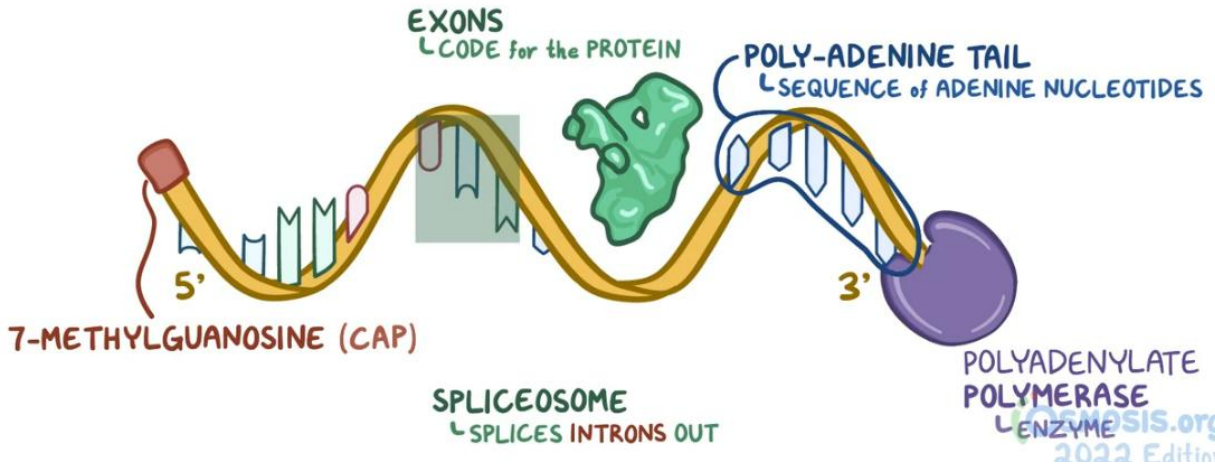


Transcription

A promoter region in the DNA sequence initiates the gene transcription.

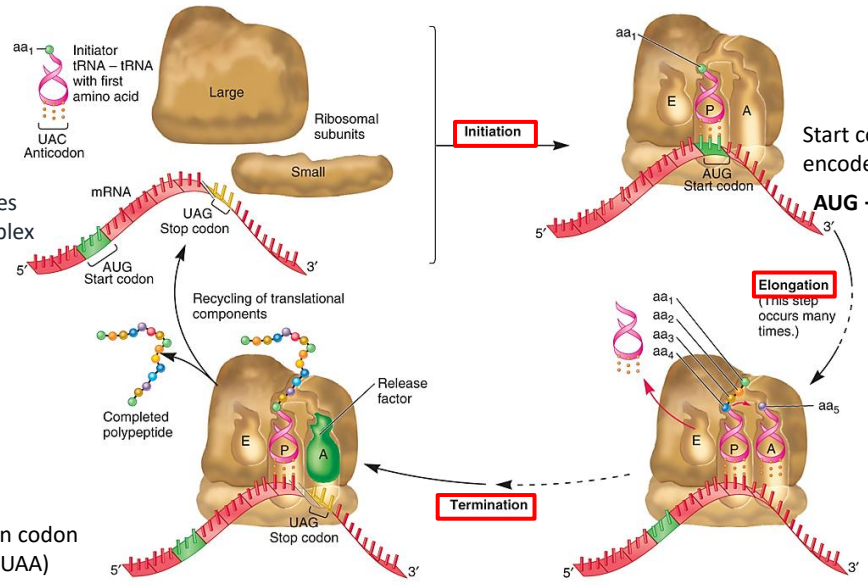
- TATAAT or TATA box is a famous promotor, located 25 bp upstream of transcription start point.
- In the nucleus, the template DNA strand (3'-5') is transcribed into a complementary pre-messenger RNA (pre-mRNA) by RNA polymerase from start to stop codon.





Following transcription termination, a cap is added to the 5' end, a poly (A) tail is added to the 3' end and the introns (noncoding regions) are spliced in the nucleus. Then, mRNA goes to the cytoplasm (mature mRNA) to initiate protein translation.

Protein Translation



In cytoplasm, mRNA assembles ribosomal complex

Start codon "AUG" encodes for methionine

AUG → methionine

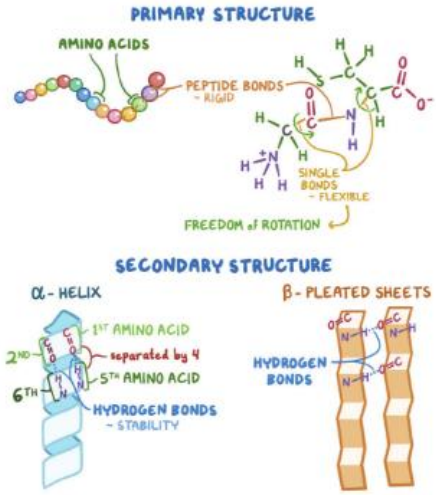
The termination codon (UGA, UAG, or UAA)

A = aminoacyl site, P =peptidyl site; E= exit site

Protein Structure and Folding

Primary structure:
The linear amino acids chain joined together by a peptide bond via a peptidyl transferase.

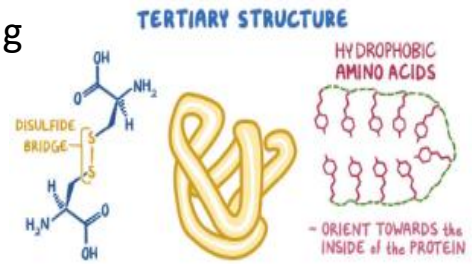
Secondary structure:
1- Alpha-helix: the primary structure fold in a spring or spiral-like structure.
2-Beta-pleated sheet: Hydrogen bonding is formed between two or more polypeptide chains.



Lippincott's Illustrated Reviews, Fifth Edition

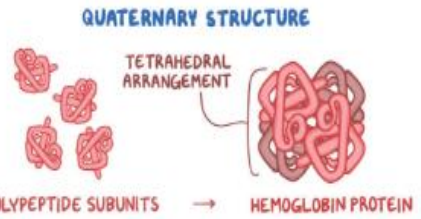
Tertiary structure:

Formed by further interactions, and bonding between the primary and secondary structures yielding a biologically active 3D structure. Stabilized by disulfide bonds.



Quaternary structure:

The association of several polypeptide chains into a packed arrangement. For example, hemoglobin is formed from two types of globulin chains (2 alpha and 2 beta chains).



Regulation of Gene Expression

1- Transcription

a- The wrapping or degree of condensation of chromatin. Decondensed or **euchromatin** is transcriptionally active while, **heterochromatin** which is highly condensed is transcriptionally inactive.

b- **Transcription factors** can enhance DNA transcription in the nucleus.

2- Post-transcription regulation

a) RNA splicing refers to the removal of introns. Depending on the cellular needs, the cell can decide if it wants to translate the whole mRNA or part of it. This leads to alternative splicing variants of the same mRNA molecule.

b) RNA processing include the addition of the7-methyl-guanine molecule at its 5’ end and poly-A tail at the 3’ end to increase the RNA stability.

3- Translation

Initiation factors regulate the initiation of protein translation. Therefore, modifying the initiation factors can inhibit or induce protein translation.

RNA splicing, Misfolded proteins and human diseases

- Alternative splicing yield diverse protein products from a single gene. However, splicing errors are linked to several genetic disorders.

- Properly folded proteins is essential for protein and cellular functions. Misfolded proteins can happen spontaneously or due to gene mutation (& other stressors). Accumulation of misfolded proteins lead to ER stress and is associated with several pathological conditions, like cancer, Alzheimer's, Parkinson disease, etc.

Epigenetics

- Mechanisms to upregulate or silence gene expression patterns without changing the DNA sequence.
- Epigenetics is affected by environmental factors and some epigenetic changes can be inherited (epigenetic inherence).
- Epigenetic mechanisms:
  - DNA methylation, by adding methyl groups to the DNA. Methylation within promoter regions results in gene silencing.
  - histone modification via several mechanisms. Histone acetylation increase gene transcription.

Genetic Variation
Origin and Detection Methods

Gene mutation

- **Mutation:** alteration in the DNA sequence that gives rise to a nonfunctional or an aberrant gene product in an individual.
- Mutation can be
  - Germline mutation: mutation in the gametes and can be passed to the offspring.
  - Somatic mutation: mutation in somatic cell and are not inherited.
- Polymorphism refers to DNA variants at the population level.

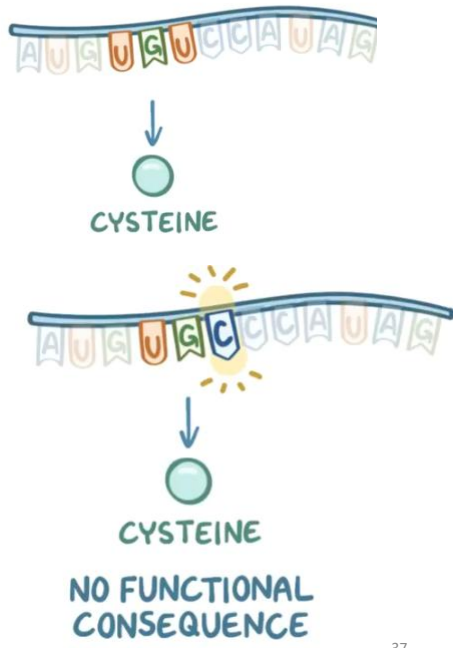
Impact of Gene Mutation

1. No effect (silent mutation)
2. Affects structure.
3. Affects function:
   a. Loss of function mutations
   b. Gain of function mutation

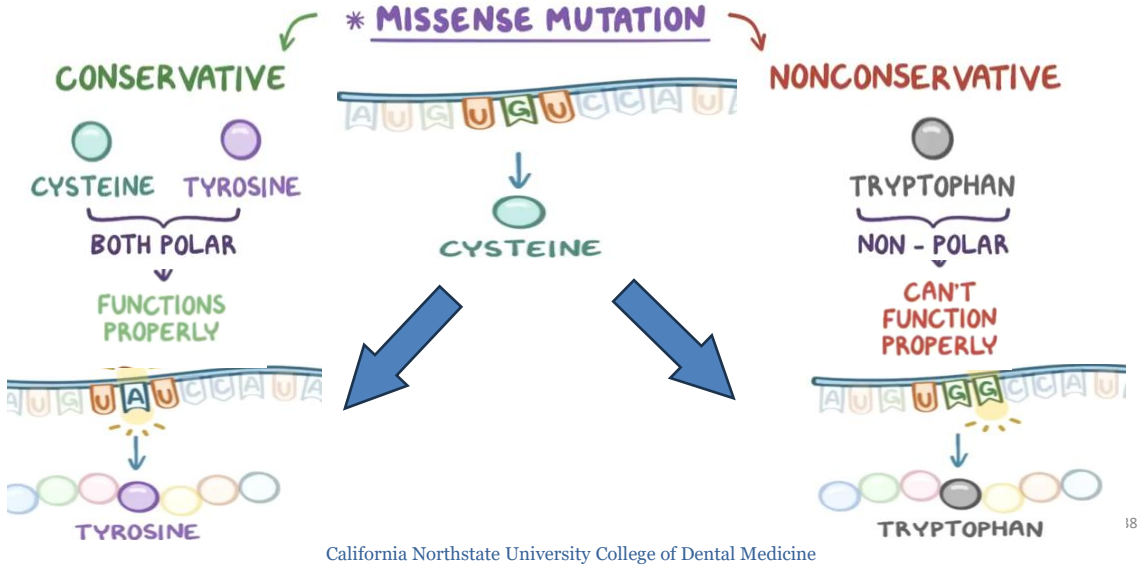
Types:

A) Point mutations, one base in the DNA is replaced by another

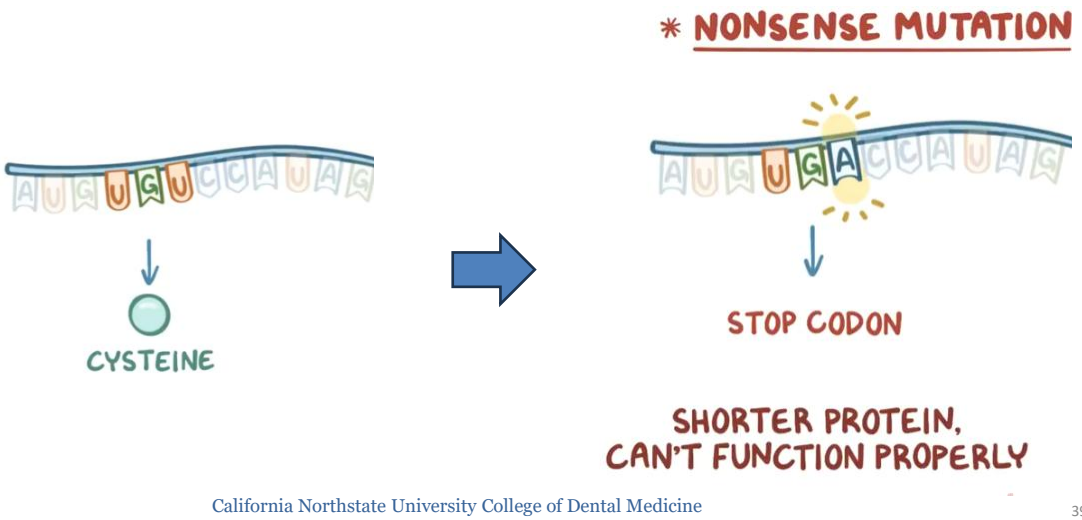
(1) Silent: mutation that results in an amino acid as the original. Like CGA and CGG are two codons for arginine or UGU to UGC , both are codons for cysteine.



(2) Missense: swaps one amino acid for another.

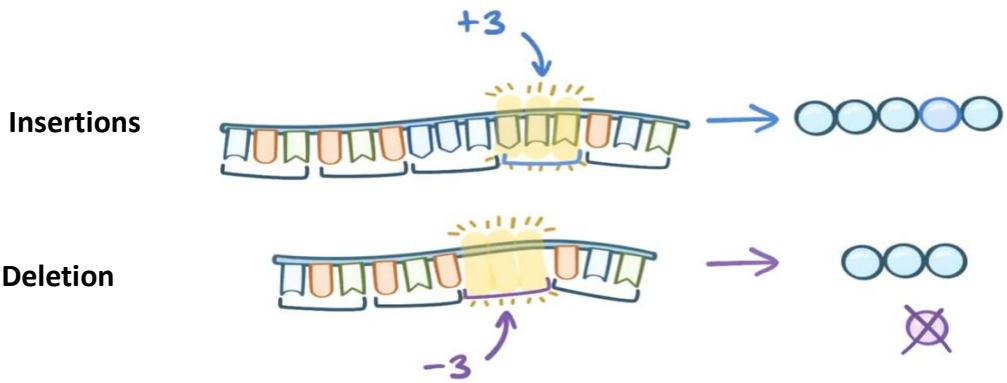


(3) Nonsense: replaces the amino acid by stop codon leading to premature termination of protein synthesis, like UGU to UGA changes cysteine to a stop codon.

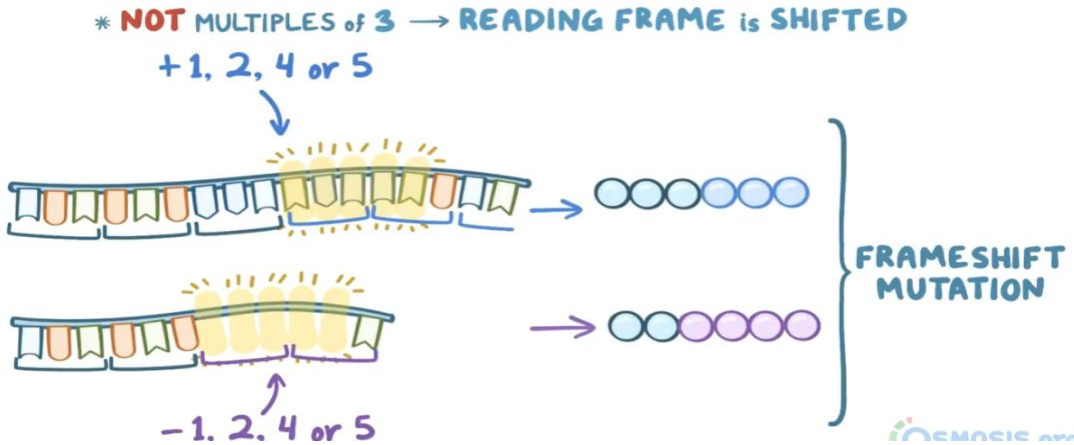


B) Insertions of several nucleotide bases (multiples of 3), resulting in a polypeptide chain with more amino acids than normal.

C) Deletions of several nucleotide base (multiples of 3) are removed from the DNA leading to a protein with fewer amino acids than normal.



D) Frameshift mutations: insertion or deletion of a number of bases that are not multiples of 3. This completely shifts the reading frame or translation frame yielding a completely different polypeptide chain.



Causes of Mutation

DNA damage can be :

A) Spontaneous, like errors in replication and proofreading.

B) Induced by various environmental insults (mutagens).

Mutagens

- A mutagen is any agent that can cause or significantly increase the risk of changing the DNA sequence.
- Most mutations arise spontaneously, usually as a result DNA replication error or secondary to exposure to mutagens.
- Mutations in somatic cells can lead to cancer.

1- Chemical mutagens:

- Alkylating agents: ability to cause DNA damage via crosslinking the guanine nucleotides. Can be also used as anti-cancer medications.
- Reactive oxygen species: free radicles that can decrease the DNA stability.
- Base analogs: agents that structural similarity to DNA nucleotides. Therefore, they will be incorporated into DNA during replication leading to subsequent damage.
- DNA intercalating agents: Cause DNA damage by inserting themselves between two nucleotide base pairs.

2-Ionizing radiation:

- Type of electromagnetic radiation that can cause DNA damage via removing electrons from the atomic structure, leading to DNA nucleotide and double strand damage. Ionizing radiation can also be used in targeted cancer treatment and radiography (x-rays). Can reach germline cells.

3- Nonionizing radiation (like UV):

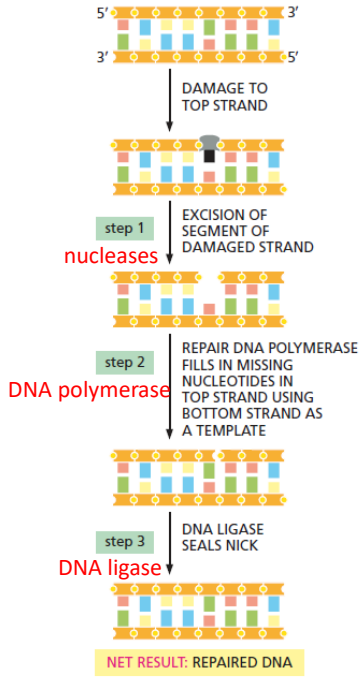
- Type of electromagnetic radiation that causes DNA damage via bonding adjacent thymine nucleotides (forming **thymine dimers**). Excessive sun exposure can lead to DNA damage in the melanocytes and consequently development of melanomas. Can't reach germline cells.

DNA Repair

Single strand DNA damage is corrected using several enzymes (nucleases, DNA polymerase and DNA ligase) via mismatch repair or base-or nucleotide excision repair mechanism that involves removal, replacement of damaged area and DNA ligation.

- Nucleotide excision repair (NER) for UV-induced damage (thymine or cytosine dimers).
- Mismatch repair is mainly for replication errors.

Double strand DNA damage is corrected with the aid of the second chromatid.



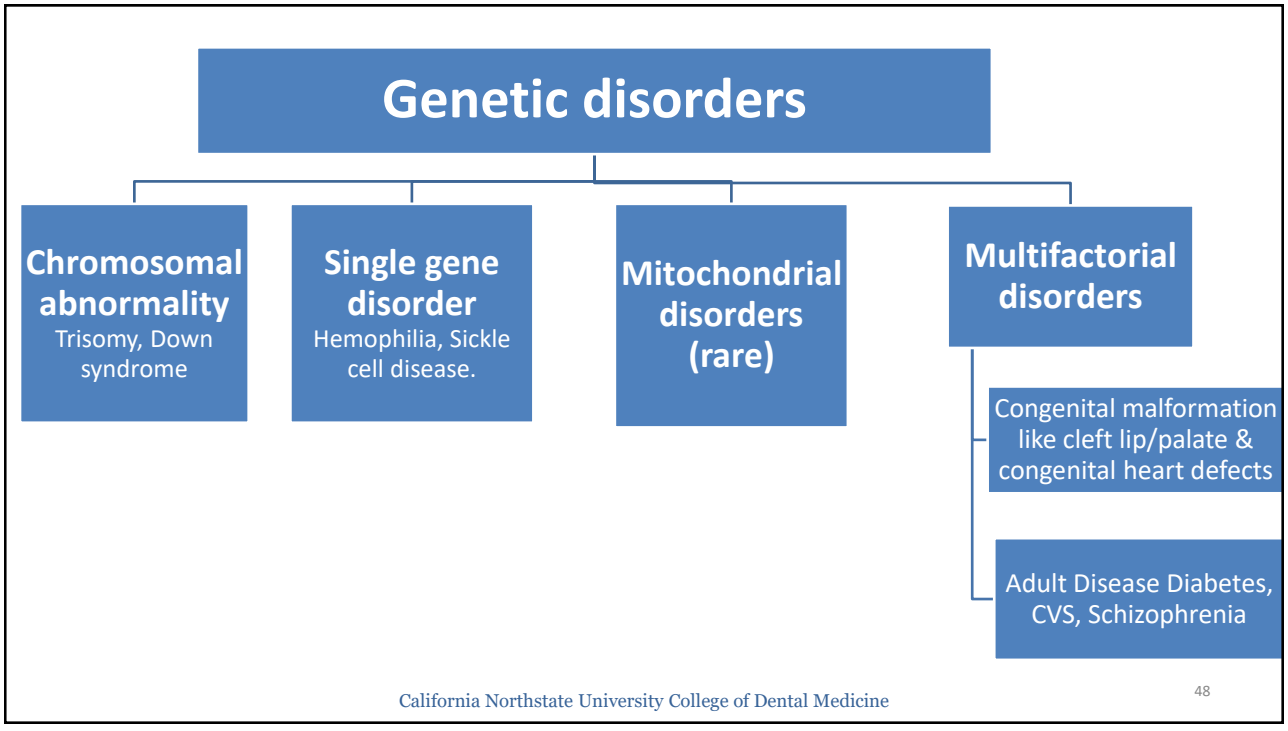
- If the DNA repair mechanisms are no longer effective, the cell will undergo:

  1. Senescence (aging cell): A cell enters a dormant state that is irreversible.

  2. Apoptosis: A cell undergoes programmed cell death.

  3. Cancer: A cell can undergo unregulated cell division, resulting in neoplasia and tumor growth.

Failure will lead to DNA mutation and chromosomal aberration.


Genetic disorders
Chromosomal abnormality
Trisomy, Down syndrome
Single gene disorder
Hemophilia, Sickle cell disease.
Mitochondrial disorders (rare)
Multifactorial disorders
Congenital malformation like cleft lip/palate & congenital heart defects
Adult Disease Diabetes, CVS, Schizophrenia
California Northstate University College of Dental Medicine
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Detection techniques
"Molecular genetic testing"

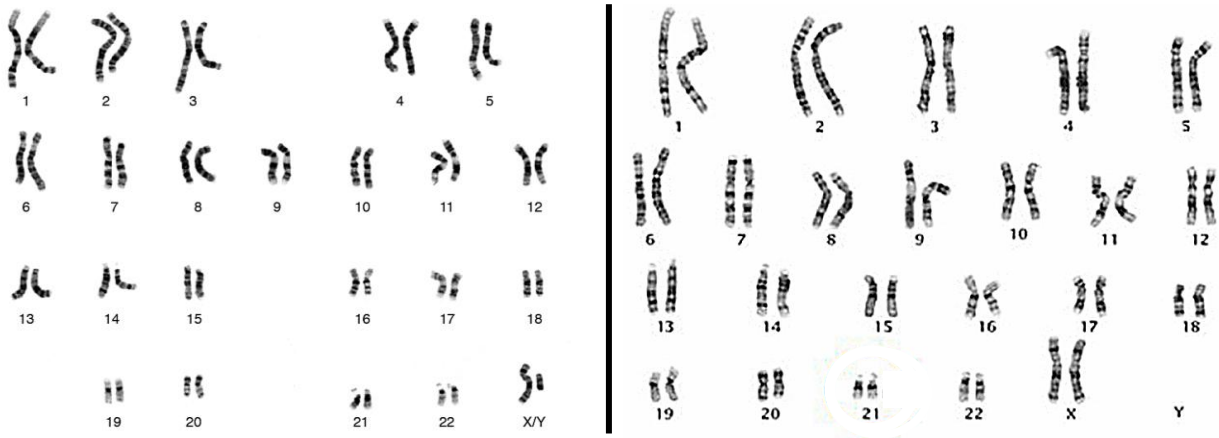
- DNA-related:

Karyotyping, DNA sequencing.

- Protein-related:

Electrophoresis, Western Blotting.

Karyotyping



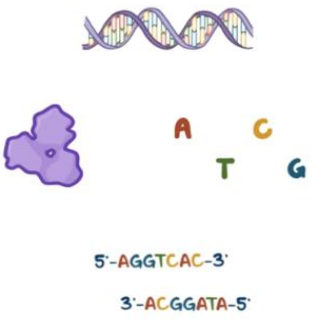
The human genome contains 23 pairs of chromosomes with around 3 billion nucleotides.

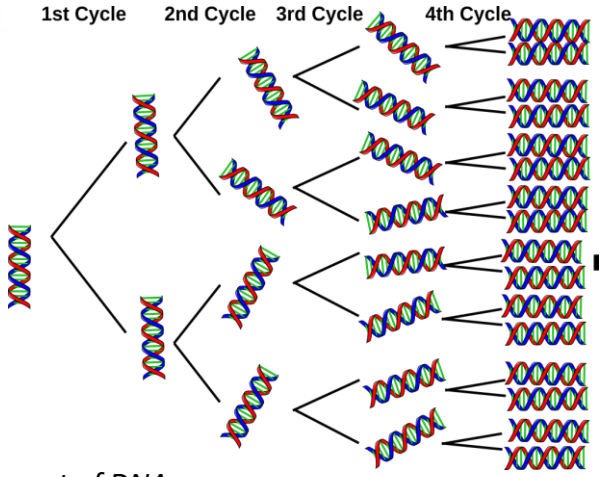
Karyotype (genome.gov)

POLYMERASE CHAIN REACTION

~ TECHNIQUE used to MULTIPLY DNA

THERMAL CYCLER

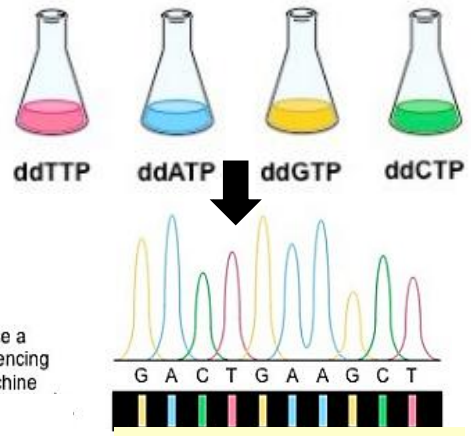




Polymerase chain reaction is used to amplify a segment of DNA.
RT-PCR is the most sensitive technique for mRNA detection and quantitation.

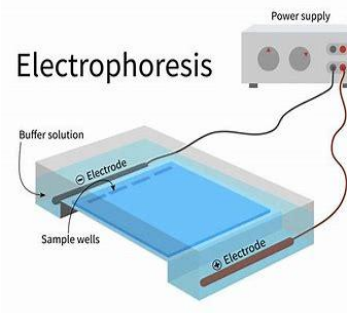
DNA sequencing

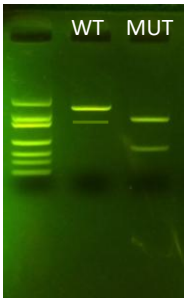
PCR mix= DNA template + primer+ dNTP + chain terminator dideoxynucleotide



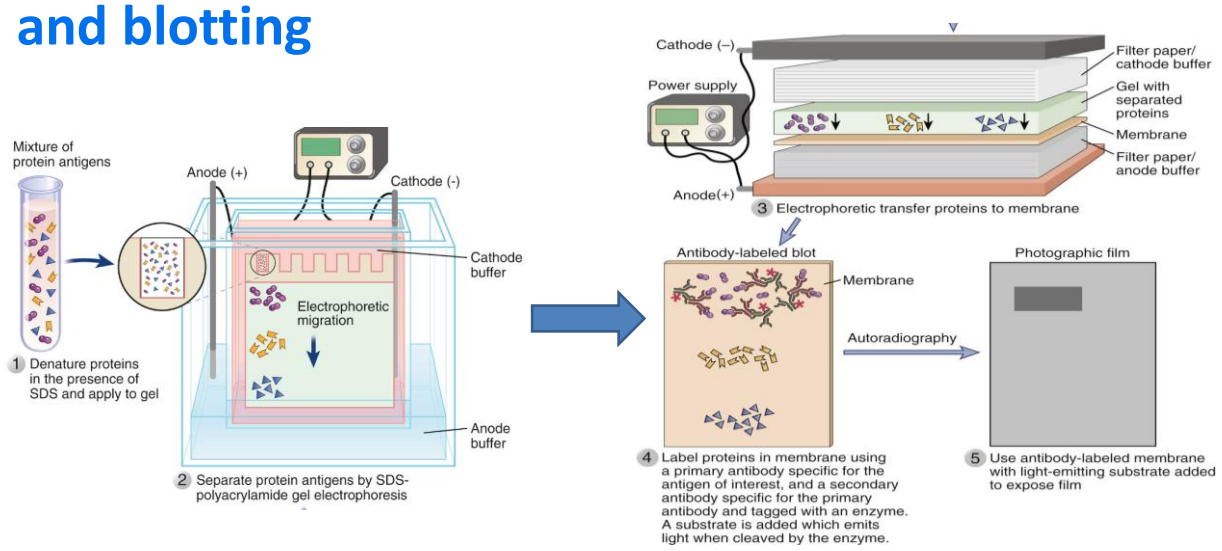
WT Hemoglobin CTG ACT CCT GAG GAG AAG TCT
Mut Hemoglobin CTG ACT CCT GTG GAG AAG TCT

Single Strand Confirmation Polymorphism





Protein electrophoresis and blotting



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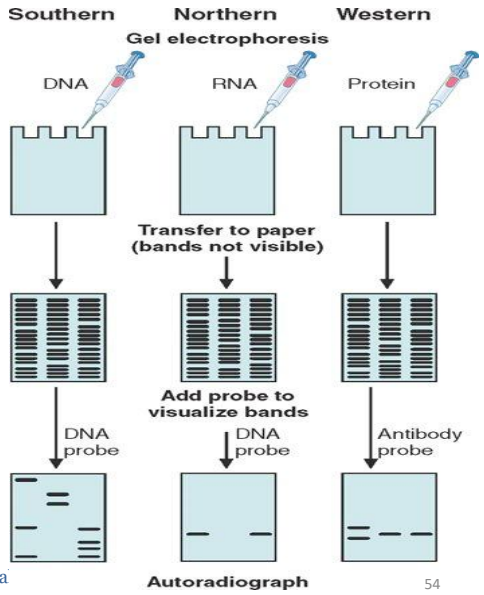
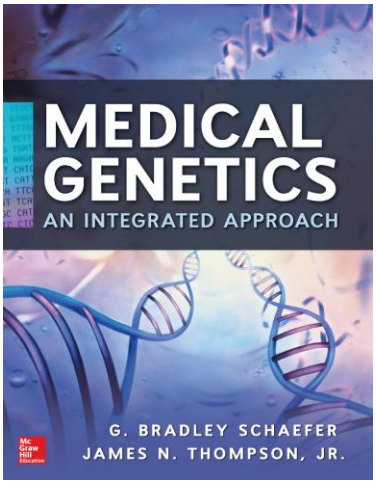
Southern, Northern and Western blots

- Detect DNA, RNA and protein, respectively.
- Southern and northern blots both utilize DNA probes to detect bands of interest.
- Southern blot identify cloned genes, identifying presence of mutations
- Northern blotting define a particular gene's expression pattern between tissues, organs, developmental stages.



Resources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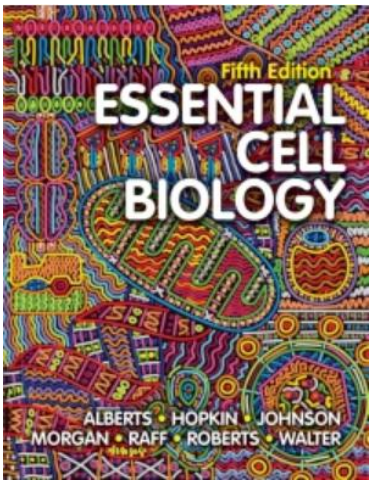
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