Adalae: DNA Notes Key
SPRING SEMESTER 2024

INSTRUCTOR:

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Vocabulary / Key Terms/ Concepts	**DNA**
Agarose Gel *Allele* *Anode* *Autosomes* *Band Migration Distance* *Cathode*	**Student Expectations:** • **Identify the components of DNA** ☐ Double helix strand of linked nucleotides – Nucleotides are subunits made up of three parts: a phosphate group, Deoxyribose, and a nitrogen base. ☐ 4 nitrogen bases – adenine and guanine (purines) – cytosine and thymine (Pyrimidines) ☐ nitrogen bases occur in pairs on opposite strands: adenine pairs with thymine and cytosine pairs with guanine ☐ the sugar-phosphates are the backbone of the ladder while the nitrogen base pairs form the rungs of the ladder • **Know that traits are determined by proteins that are built according to instructions coded in DNA**

Centromere *Centrosome* *Chromatid* *Chromatin* *Chromosome* *DNA Band* *DNA Fragment Size* *DNA Marker* *DNA Molecules* *Electrophoresis* *Electrophoretic Apparatus*	• **Summarize the process of DNA replication** ☐ Enzymes work to unwind and separate the double helix and add complementary nucleotides to the exposed strands ☐ The result is two exact copies of the cell's original DNA. Each new double helix is composed of one original DNA strand and one new DNA strand. ☐ Understand that enzymes proofread the newly synthesized DNA correcting mistakes • **Understand the purpose and significance of gel electrophoresis in biology research and DNA analysis.** • **Explain the principles of gel electrophoresis, including the relationship between charge, size, and migration of DNA molecules.** • **Describe the step-by-step procedure of gel electrophoresis, from preparing the agarose gel to visualizing DNA bands.** • **Analyze and interpret gel electrophoresis results, including identifying DNA bands, determining fragment size, and using DNA markers as references.** • **Explore various applications of gel electrophoresis, such as comparing DNA samples for genetic variation, DNA fingerprinting in forensic investigations, and studying gene expression and protein analysis.** • **Recognize common issues that may arise during gel electrophoresis and apply troubleshooting strategies to address them effectively.** • **Understand the ethical considerations related to DNA analysis and research, including privacy, consent, and responsible use of genetic information.** • **Demonstrate knowledge of safety measures and proper handling of chemicals and biohazardous materials associated with gel electrophoresis.**

Ethidium Bromide

Gel Electrophoresis

Gel Image Analysis

Gene

Helicase

Histone Proteins

Kinetochore

Lagging Strand

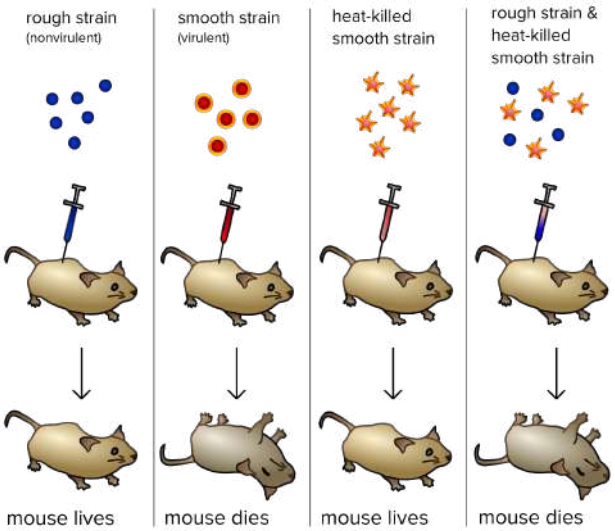
Leading Strand

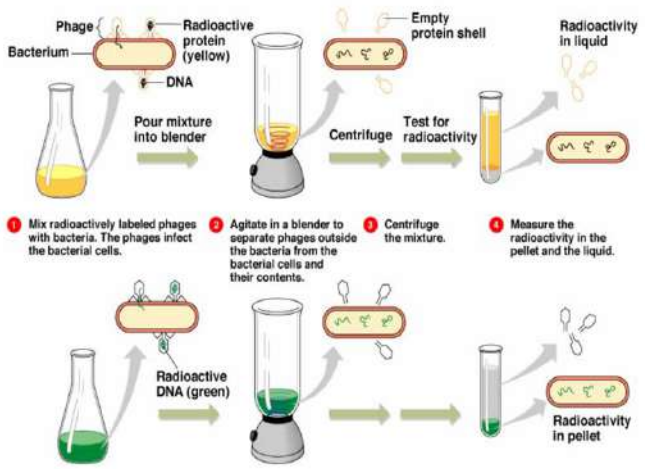
Ligase

Loading Buffer

History of DNA

- Early scientists thought protein was the cell's hereditary material because it was more complex than DNA
- **Proteins** were composed of **20 different amino acids in** long **polypeptide** chains
- **Griffith Transformation:**
  - ➔ Fred Griffith worked with virulent S and non-virulent R strain Pneumococcus bacteria
  - ➔ He found that R strain could become virulent when it took in DNA from heat-killed S strain
  - ➔ **Study suggested that DNA was probably the genetic material**
- **Hershey & Chase**
  - ➔ **Chromosomes** are made of both **DNA** and **protein**
  - ➔ Experiments on bacteriophage viruses by Hershey & Chase proved that **DNA was** the cell's **genetic material**





Locus

Migration

Mismatch Repair

Mutation

Noncoding DNA

Nucleosome

Nucleotide Excision Repair

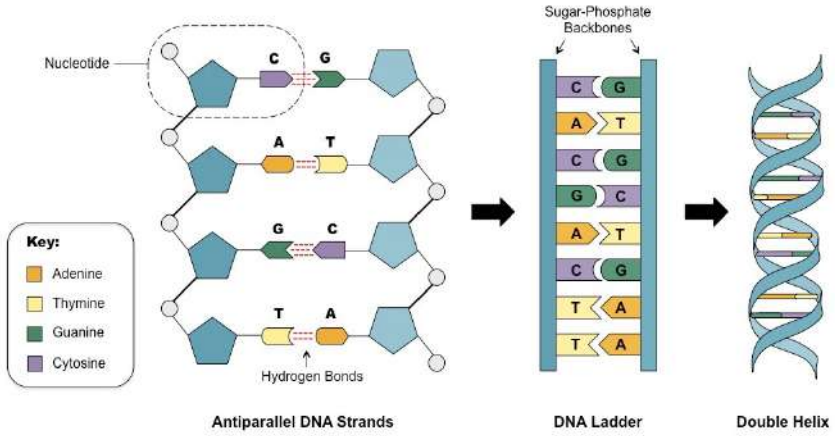
Okazaki Fragment

Ploidy

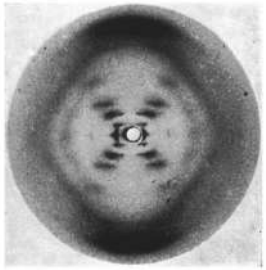
Primase

DNA Structure:

- **Erwin Chargaff** showed the amounts of the four bases on **DNA (A,T,C,G)**
- **In a body or somatic cell:**
  - ➔ **A = 30.3%**
  - ➔ **T = 30.3%**
  - ➔ **G = 19.5%**
  - ➔ **C = 19.9%**



- **Chargaff's Rule:**
  - ➔ **Adenine** must pair with **Thymine**
  - ➔ **Guanine** must pair with **Cytosine**
  - ➔ **The bases are held together by weak hydrogen bonds**
  - ➔ **DNA's First Photograph**
    - ☐ **Rosalind Franklin** took diffraction x-ray photographs of DNA crystals
    - ☐ In the 1950's, **Watson & Crick** built the first model of DNA using Franklin's X-rays



- **Structure**
- Two strands coiled called a **double helix**
- **Sides** made of a **pentose sugar Deoxyribose** bonded to **phosphate (PO4)** groups by **phosphodiester bonds**
- **Center** made of **nitrogen bases** bonded together by weak **hydrogen bonds**
- **Helix**

Primer

Proofreading

Replication Fork

Replication Origin

Sample Wells

Single-Strand Binding Protein

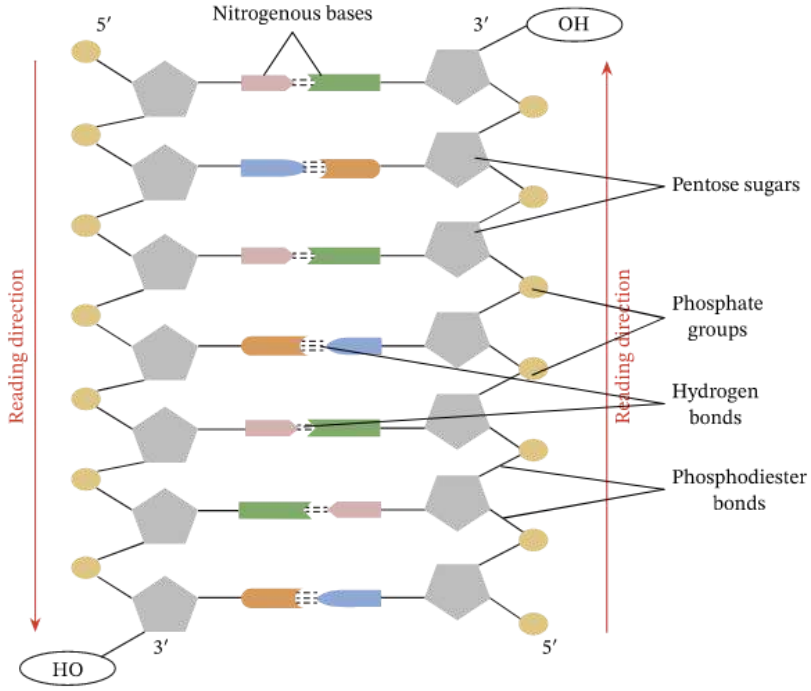
Size Separation

Sliding Clamp

Telomerase

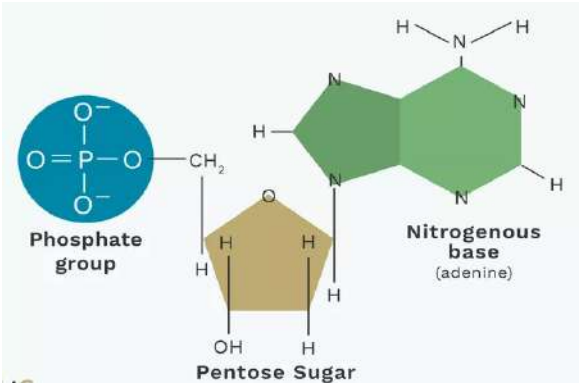
Telomere

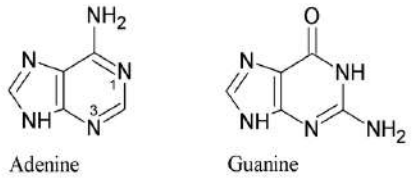
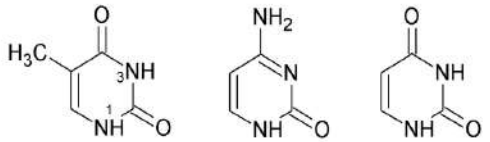
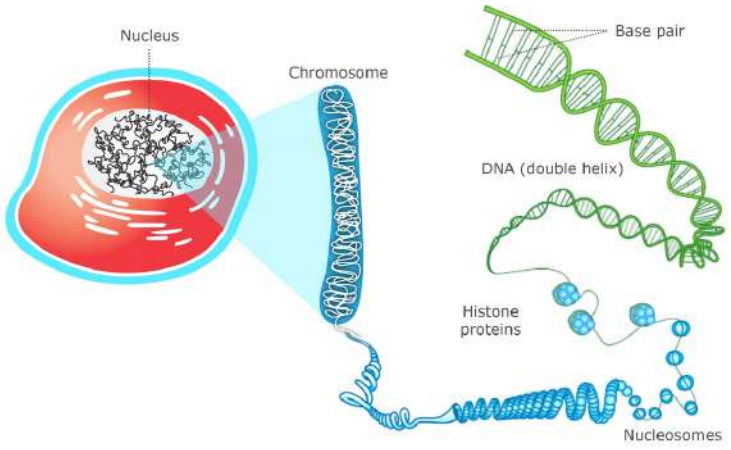
- ➔ **Most DNA** (B-DNA) has a right-hand twist with 10 base pairs in a complete turn
- ➔ **L**eft twisted DNA is called Z-DNA or southpaw DNA
- ➔ Hot spots occur where right and left twisted DNA meet producing mutations



Nucleotides

- **DNA** Stands for **Deoxyribonucleic acid**
- Made up of subunits called **nucleotides**
- **Nucleotide** made of:
  - ➔ **Phosphate group**
  - ➔ **5-carbon sugar**
  - ➔ **Nitrogenous base (**genetic code**)**
- **Double** ring **PURINES**
  - ➔ **Adenine (A)**
  - ➔ **Guanine (G)**



Telomere *Topoisomerase* *Transformation* *UV Transilluminator* *Voltage*	• **Single** ring **PYRIMIDINES** ➔ **Thymine (T)** ➔ **Cytosine (C)** • **Base Pairings** ➔ **Purines** only pair with **Pyrimidines** ➔ **Three hydrogen bonds** required to bond **Guanine** *to* **Cytosine** ➔ **Two hydrogen bonds** are required to bond **Adenine** to **Thymine** ➔ These are what allows for DNA to be copied exactly Purines  Pyrimidines  **Introduction to DNA and Chromosomes** • **DNA (deoxyribonucleic acid):** ➔ **Genetic material** found in the nucleus of cells. ➔ Carries the instructions for the development, functioning, and reproduction of living organisms. • **Chromosomes:** ➔ Thread-like structures made 

up of DNA molecules and proteins.

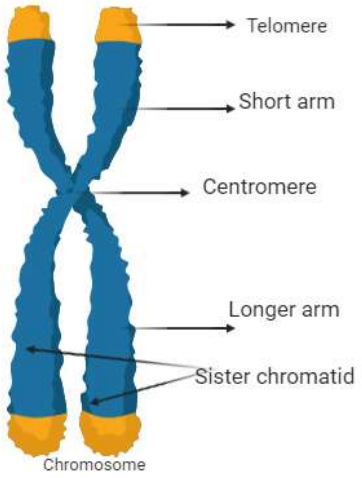
➔ Contains genes, which are segments of DNA that code for specific traits.

Organization of DNA into Chromatin

- **Nucleosomes:**
  - ➔ DNA wrapped around a group of proteins called **histones.**
  - ➔ Bead-like structures formed by **nucleosomes** along the DNA.
- **Chromatin:**
  - ➔ Further folding and packaging of nucleosomes.
  - ➔ Forms fibers that help compact and organize DNA within the nucleus**.**

Chromosome Structure

- **Chromosomes:**
  - ➔ Condensed and organized structures of DNA and proteins.
  - ➔ Visible during cell division.
- **Sister chromatids:**
  - ➔ Two identical copies of a chromosome.
  - ➔ Held together by a region called the **centromere**.
- **Centromere:**
  - ➔ Specialized region of a chromosome where sister chromatids are joined.
  - ➔ Essential for proper alignment and separation of

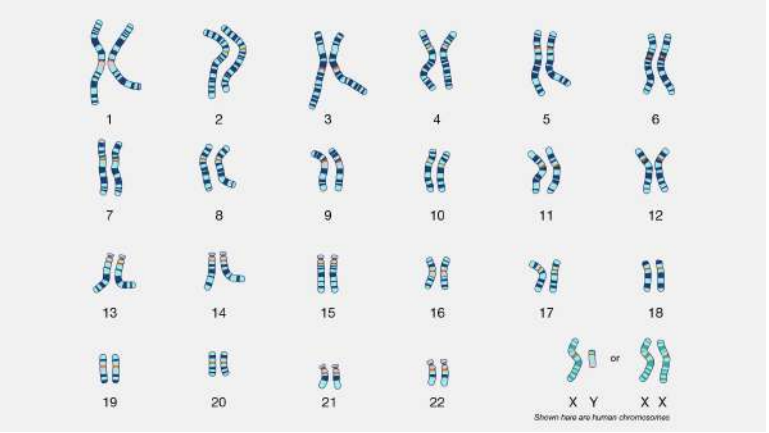


chromosomes during cell division.

- **Telomere:**
  - ➔ Protective cap at the ends of chromosomes.
  - ➔ Composed of repetitive DNA sequences and specialized proteins.
  - ➔ Helps maintain chromosomal stability and prevents degradation of DNA during replication and cell division.

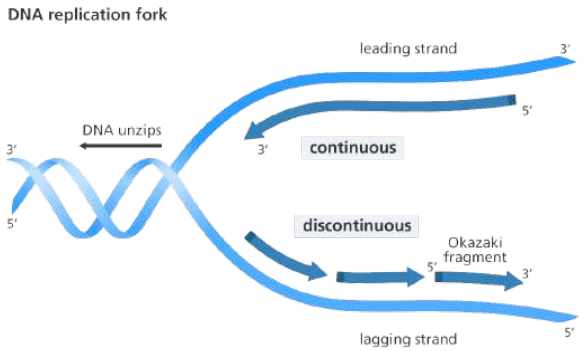
Organization of Chromosomes

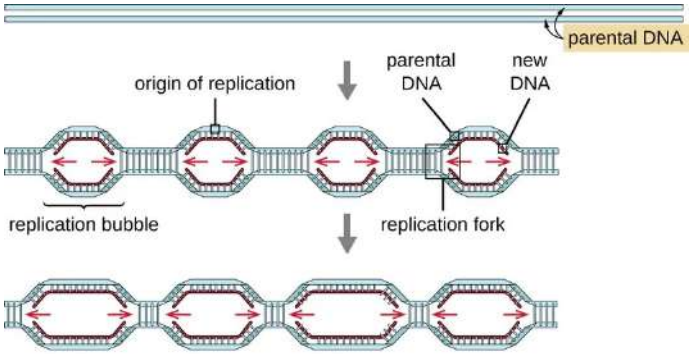
- **Chromosome territories:**
  - ➔ Specific regions in the nucleus where each chromosome is located.
  - ➔ Helps maintain the organization and accessibility of genetic material.
- **Karyotype:** we will talk more when we get to Genetics
  - ➔ The complete set of chromosomes in an individual, arranged and classified based on their size, banding patterns, and centromere positions.
  - ➔ Used for genetic analysis and identifying chromosomal abnormalities.



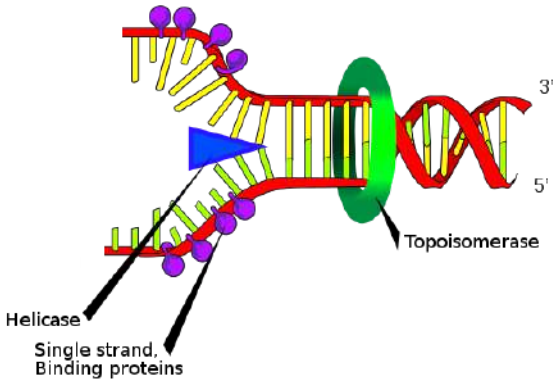
DNA Replication:

- **DNA** has to be **copied** before a cell **divides**
- DNA is copied during the **S or synthesis phase** of **interphase**
- New cells will need **identical DNA** strands
  - ➔ Occurs in the Nucleus of eukaryotes
  - ➔ **Replication Fork -**
    - ❑ Begins at **Origins of Replication** - Two strands open forming **Replication Forks:** (Y-shaped region)
    - ❑ New strands grow at the forks
  - ➔ **Replication Bubbles**
    - ❑ As the 2 DNA strands open at the origin, Replication Bubbles form
    - ❑ **Eukaryotic** chromosomes have **MANY** bubbles
    - ❑ **Prokaryotes** (bacteria) have a **single** bubble

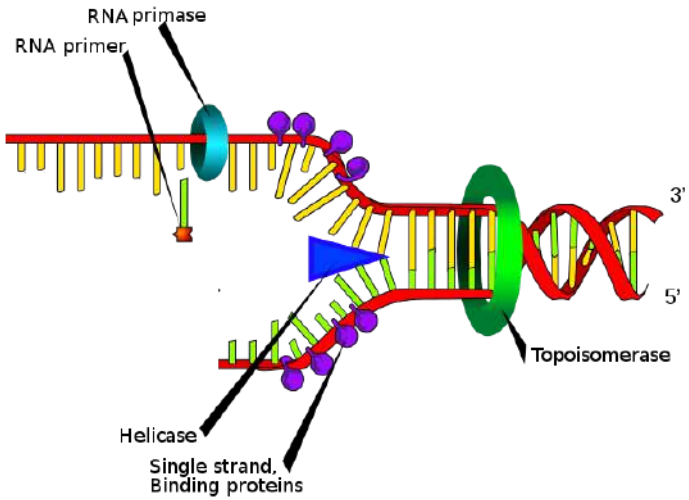




- **Sequence:**
  - ➔ **Helicase**
    - ☐ **unwinds** and **separates** the 2 DNA strands by **breaking** the weak **hydrogen bonds**.
  - ➔ **Single-Strand Binding Proteins** attach and **keep** the 2 DNA strands **separated** and **untwisted**
  - ➔ **Topoisomerase**
    - ☐ **attaches** to the 2 forks of the bubble to **relieve stress on the DNA molecule as it separates**

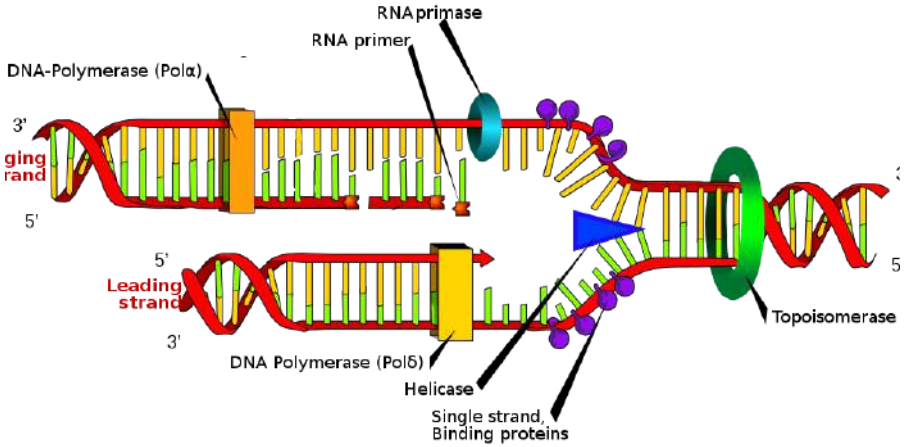


- ➔ **RNA Primers and Primase**
  - ☐ Before new DNA strands can form, there must be **RNA primers present to start** the addition of new **nucleotides**
  - ☐ Primase is the enzyme that **synthesizes** the **RNA Primer**
  - ☐ **DNA polymerase** can then **add** the new **nucleotides**



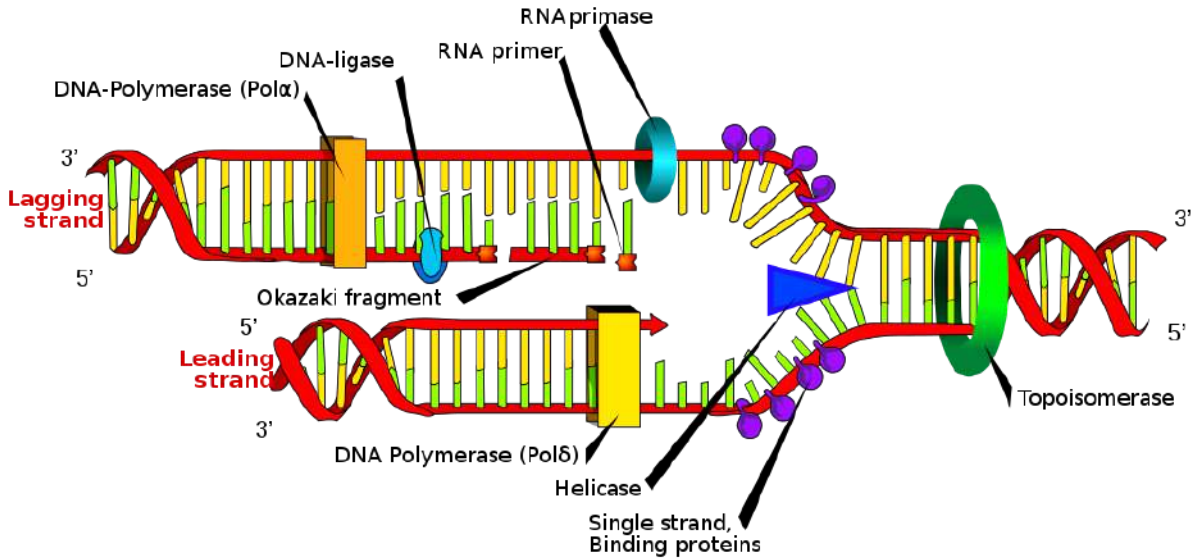
➔ **DNA Polymerase**

- ☐ DNA polymerase can only add nucleotides to the 3’ end of the DNA
- ☐ This causes the NEW strand to be built in a 5’ to 3’ direction

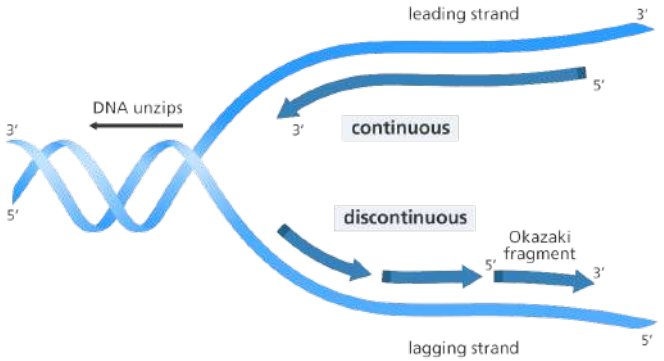


➔ **DNA Ligase**

- ☐ **Okazaki Fragments** - *series of short segments on the lagging strand*
- ☐ Okazaki Fragments must be *joined together* by an *enzyme* – **DNA Ligase**

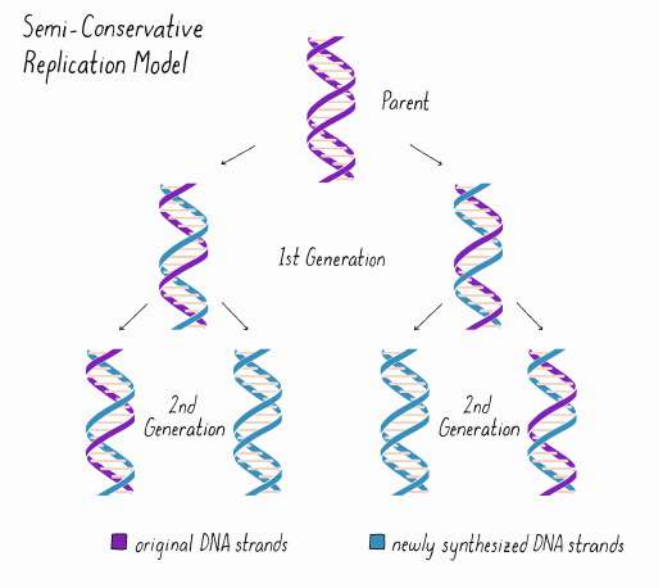


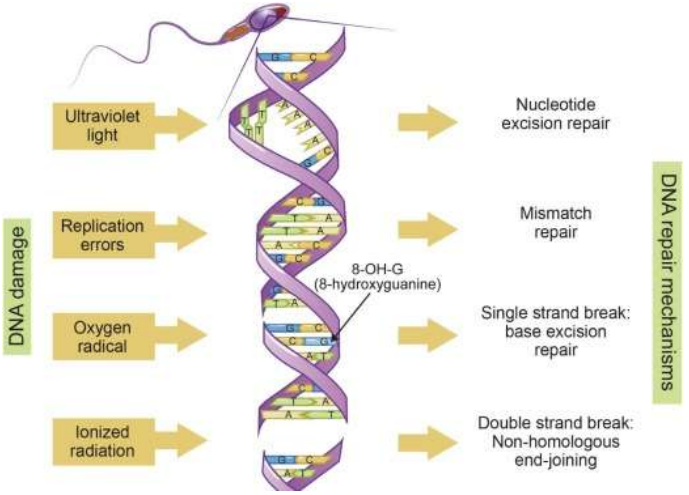
- **The Leading Strand - (continuous)**
  - ➔ is synthesized as a single strand from the point of **origin toward the opening replication fork**
- **The Lagging Strand: (discontinuous)**
  - ➔ The Lagging Strand is synthesized discontinuously against overall direction of replication
  - ➔ This strand is made in MANY short segments It is replicated from the **replication fork toward the origin**
  - ➔ **Okazaki Fragments** - series of short segments on the lagging strand



Semiconservative Model for Replication

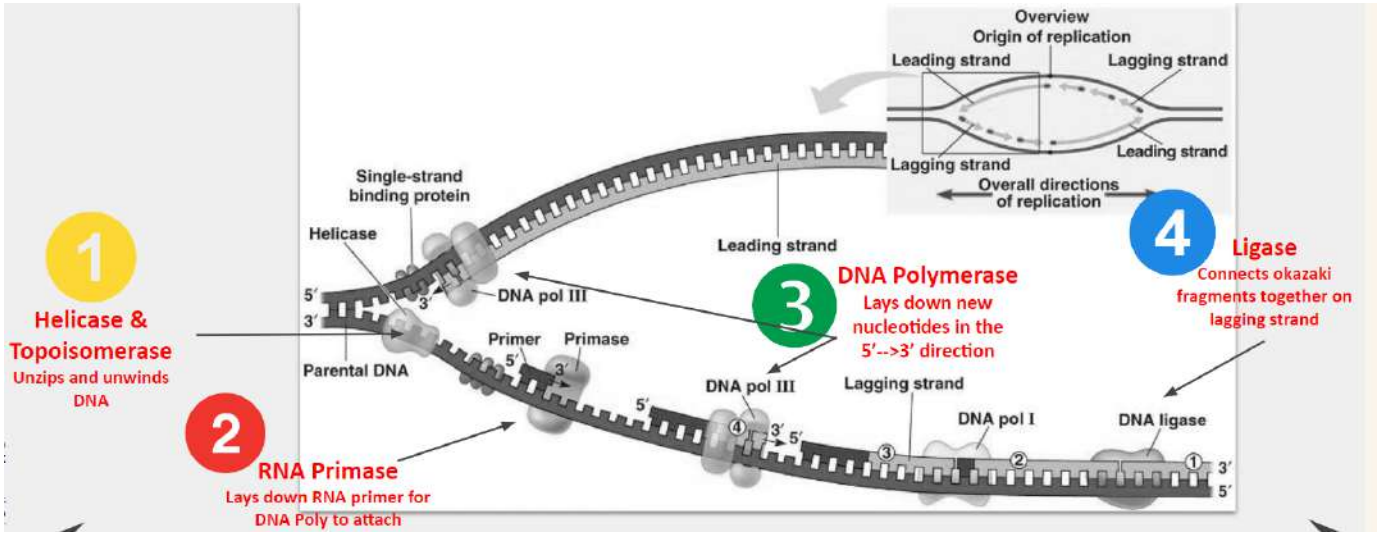
- Idea presented by Watson & Crick
- The two strands of the parental molecule separate, and each acts as a template for a new complementary strand
- New DNA consists of 1 PARENTAL (original) and 1 NEW strand of DNA
- **Proofreading DNA**
  - ➔ DNA polymerase initially makes about 1:10,000 base pairing errors
  - ➔ Enzymes proofread and correct these mistakes
  - ➔ The new error rate for DNA that has been proofread is 1 in 1 billion base pairing errors
- **DNA Damage & Repair:**
  - ➔ Chemicals & ultraviolet radiation damage the DNA in our body cells
  - ➔ Cells must continuously repair DAMAGED DNA
  - ➔ Excision repair occurs when any of over 50 repair enzymes remove





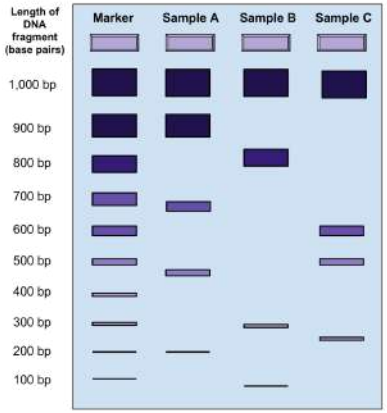
damaged parts of DNA

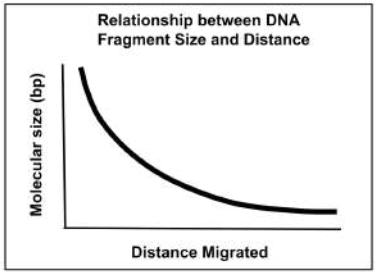
➔ DNA polymerase and DNA ligase replace and bond the new nucleotides together



Introduction to Gel Electrophoresis

- **Definition** and purpose of gel electrophoresis
  - ⇢ **Gel electrophoresis** is a laboratory technique used to separate DNA fragments or other macromolecules based on their size and charge.



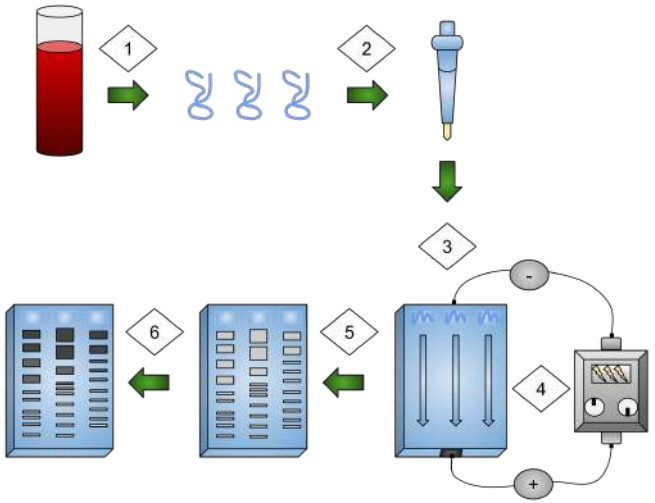


- ⇢ The purpose of gel electrophoresis is to analyze and study DNA samples, identify genetic variations, and determine fragment sizes.

- **Importance and applications in biology research and DNA analysis**
  - ➔ Gel electrophoresis is a fundamental tool in molecular biology research.
  - ➔ It is used in DNA fingerprinting, genetic profiling, paternity testing, and forensic investigations.
  - ➔ It is essential for studying **gene** expression, analyzing protein samples, and characterizing DNA mutations.

Principles of Gel Electrophoresis

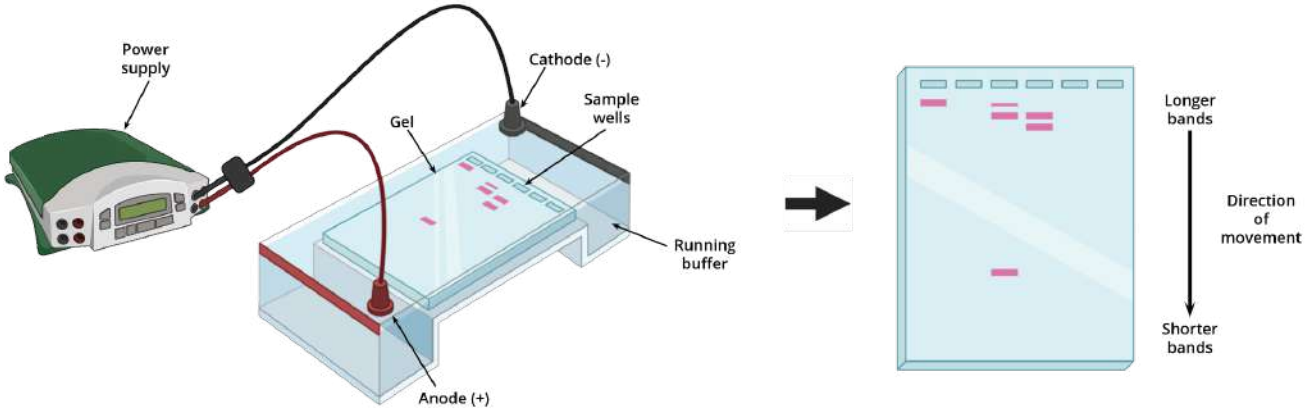
- **Charge and size of DNA molecules**
  - ➔ DNA molecules are **negatively** charged due to the **phosphate** groups in their structure.
  - ➔ **Smaller DNA fragments** migrate **faster** through the gel **than larger** fragments.



- **Agarose gel as the separation matrix**
  - ➔ Agarose gel is a polysaccharide derived from seaweed, used as the medium for gel electrophoresis.
  - ➔ It forms a porous gel matrix that slows down the movement of DNA fragments, allowing for separation based on size.
- **Electrophoretic apparatus and components** (gel box, power supply, electrodes)

➔ The **electrophoretic apparatus** consists of a **gel box** that holds the **gel**, a **power supply** to generate an **electric field**, and electrodes (**anode** and **cathode**).

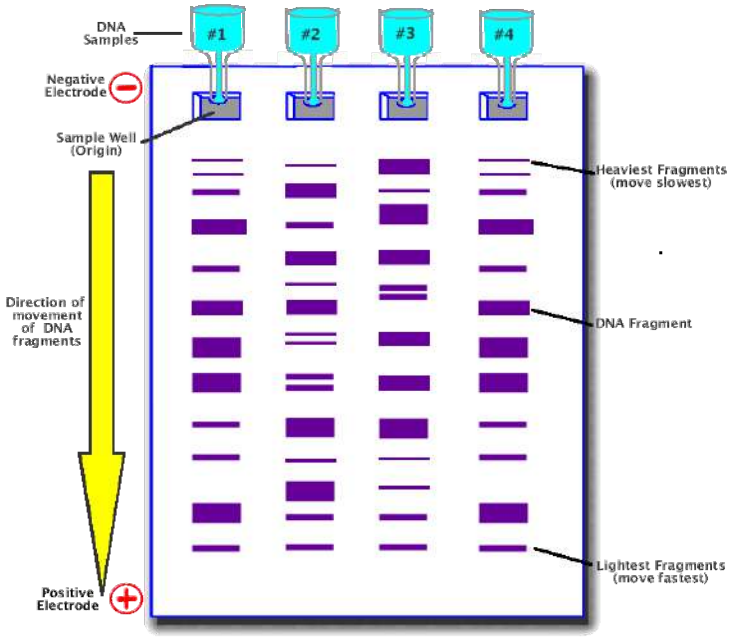
➔ The **anode** attracts the negatively charged DNA fragments, while the **cathode** repels them, causing them to **migrate** through the gel.



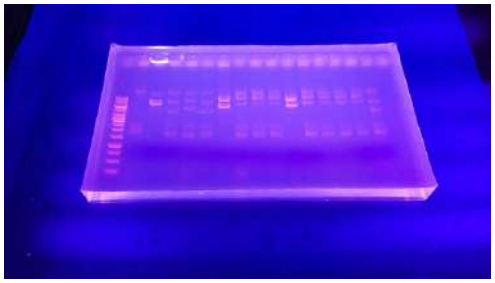
Procedure of Gel Electrophoresis

- **Preparation of the agarose gel**
  - ➔ **Agarose powder** is mixed with a **buffer** solution, **heated**, and poured into a gel tray with comb indentations to create **wells** for sample loading.
  - ➔ The gel is allowed to solidify and form a gel matrix.
- **Loading the DNA samples onto the gel wells**
  - ➔ DNA samples are mixed with a loading buffer that provides density and a tracking dye.
  - ➔ The mixture is carefully loaded into the wells of the gel using a micropipette.

- **Running the electrophoresis**
  - ➔ The gel tray is placed in the electrophoretic apparatus, submerged in a **buffer solution** that conducts electricity.
  - ➔ The **power supply** is turned on to apply an **electric field** across the gel, causing the DNA fragments to migrate.
- **Staining the gel to visualize DNA bands**
  - ➔ After electrophoresis, the gel is stained with a DNA-specific dye, such as **ethidium bromide**.
  - ➔ The dye binds to the DNA fragments, and the gel is **illuminated** with **UV ligh**t to visualize the fluorescent **DNA bands**.



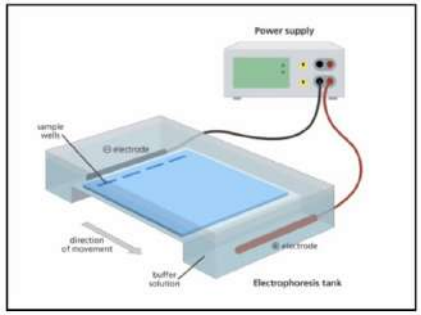
Gel Electrophoresis
(Creating a DNA Profile)

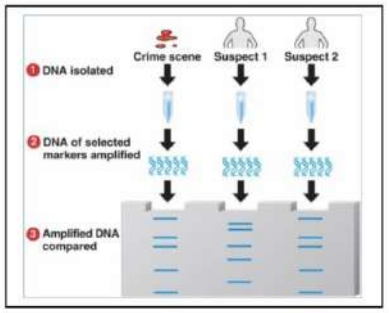


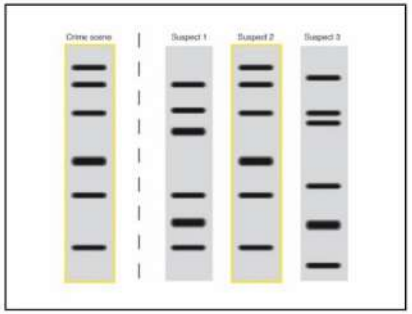
Interpretation of Gel Electrophoresis Results

- **Identifying DNA bands on the gel**
  - ➔ DNA fragments of different sizes appear as distinct bands on the gel.
  - ➔ Each band represents a specific fragment size.

- **Understanding the relationship between DNA fragment size and migration distance**
  - ➔ **Smaller** DNA **fragments** migrate **farther** through the gel, while **larger** fragments stay **closer** to the sample **well**.
- Using DNA markers as a reference for fragment size determination
  - ➔ **DNA markers** are **known fragments** of different sizes that are loaded alongside the samples.
  - ➔ By comparing the **migration** distances of the **DNA bands** with those of the **markers**, the **size** of **unknown** DNA **fragments** can be **estimated**.

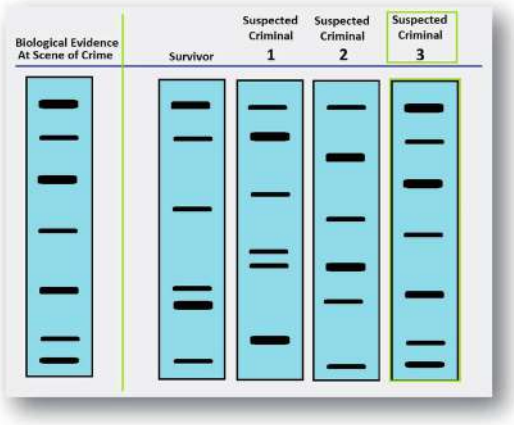






Analysis and Applications of Gel Electrophoresis

- **Comparing DNA samples for genetic variation**
  - ➔ Gel electrophoresis can be used to **compare DNA samples** from **different individuals** or **species** to identify **genetic variations** or **similarities**.
- **DNA fingerprinting and forensic applications**



	➔ Gel electrophoresis is employed in **DNA fingerprinting** to create unique **genetic profiles** for individuals. ➔ It plays a crucial **role** in **forensic** investigations by **comparing** crime scene **DNA** with suspects' DNA. • **Studying gene expression and protein analysis** ➔ Gel electrophoresis is used to **analyze gene expression** by **separating** and **visualizing** RNA **transcripts** (through reverse transcription). ➔ It is also used in **protein analysis** to separate and **identify proteins** based on their **size** and **charge**. **Troubleshooting and Experimental Considerations** • **Common issues during gel electrophoresis and possible solutions** ➔ Issues like smearing, distorted bands, or insufficient separation can occur. ➔ Troubleshooting steps include adjusting gel concentration, running voltage, or buffer composition. • **Factors affecting DNA migration (voltage, gel concentration, buffer composition)** ➔ The **voltage** applied, the concentration of agarose gel, and the buffer composition can influence the rate and resolution of DNA migration. **Ethical Considerations and Safety Measures** • **Ethical considerations in DNA analysis and research** ➔ The responsible use of DNA analysis, considering **privacy**, **consent**, and **potential misuse** of genetic information. ➔ **Privacy**:

- Individuals have the right to keep their genetic information private. Researchers and institutions must ensure that proper safeguards are in place to protect the confidentiality of DNA data.
- Genetic information should be stored securely and only accessible to authorized personnel to prevent unauthorized use or disclosure.
- Anonymization techniques can be employed to remove personal identifiers from genetic data, ensuring that individuals cannot be identified solely based on their DNA information.

➔ **Consent**:

- Informed consent is essential before obtaining and using DNA samples for analysis. Individuals should be fully informed about the purpose, potential risks, and benefits of the study or analysis.
- Researchers must obtain explicit consent from participants, ensuring they understand how their genetic information will be used, who will have access to it, and how long it will be retained.
- Consent should be voluntary, and individuals should have the right to withdraw their consent at any time, with the assurance that their genetic data will be appropriately handled.

➔ **Potential Misuse:**

	◆ Genetic information can reveal sensitive and personal details about individuals, such as susceptibility to certain diseases or inherited traits. It is essential to prevent the misuse of this information. ◆ Genetic discrimination is a concern, where individuals may face discrimination in employment, insurance coverage, or access to certain services based on their genetic information. Safeguards should be in place to protect against such discrimination. ◆ Genetic information should not be used for purposes beyond the scope of the study or analysis without explicit consent. It should not be shared with third parties without proper authorization. ● Proper handling and disposal of chemicals and biohazardous materials ➔ Safety precautions to prevent exposure to hazardous chemicals and proper disposal of biohazardous materials used in gel electrophoresis.
Notes Summary	
