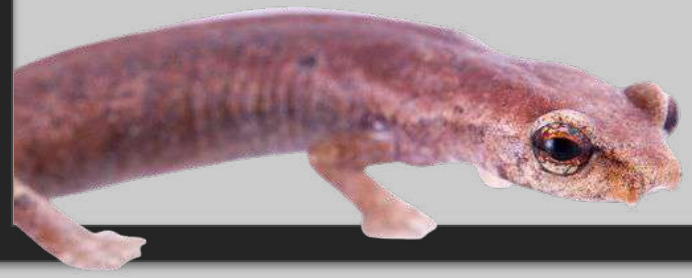
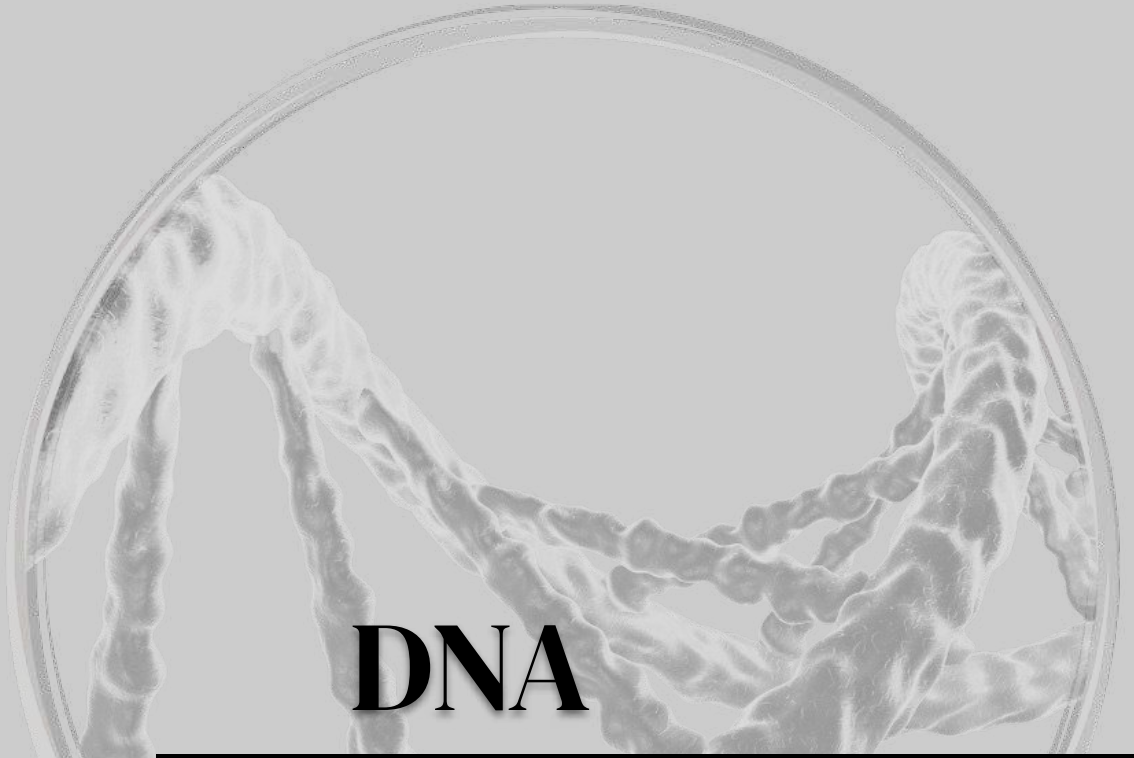
MAMMOTH SCIENCE

EST 2022

PUBLISHING





DNA

- *Identify the components of DNA*
- *Know that traits are determined by proteins that are built according to instructions - coded in DNA*
- *Summarize the process of DNA replication*
- *Understand that enzymes proofread the newly synthesized DNA correcting mistakes*

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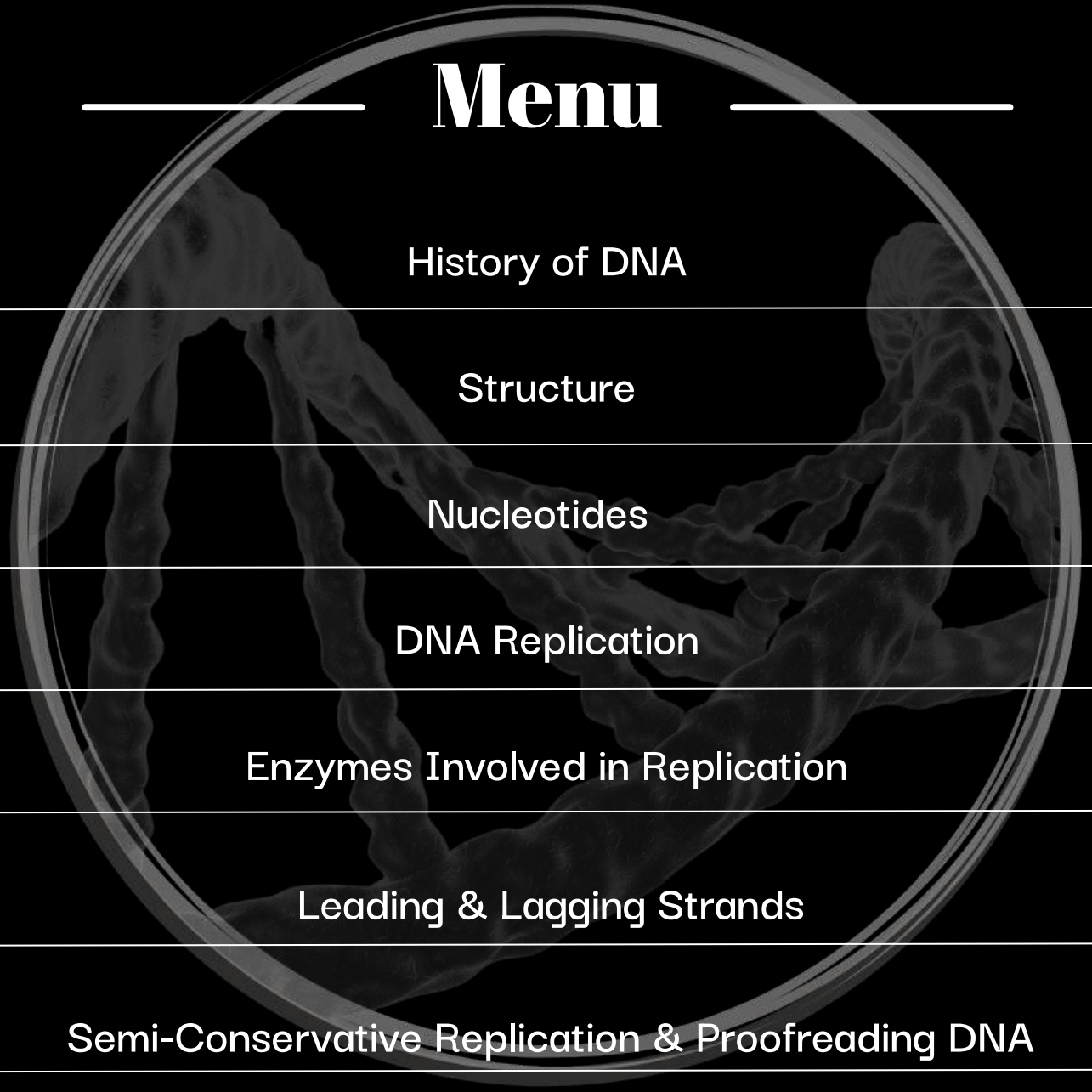
Menu



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Menu

History of DNA

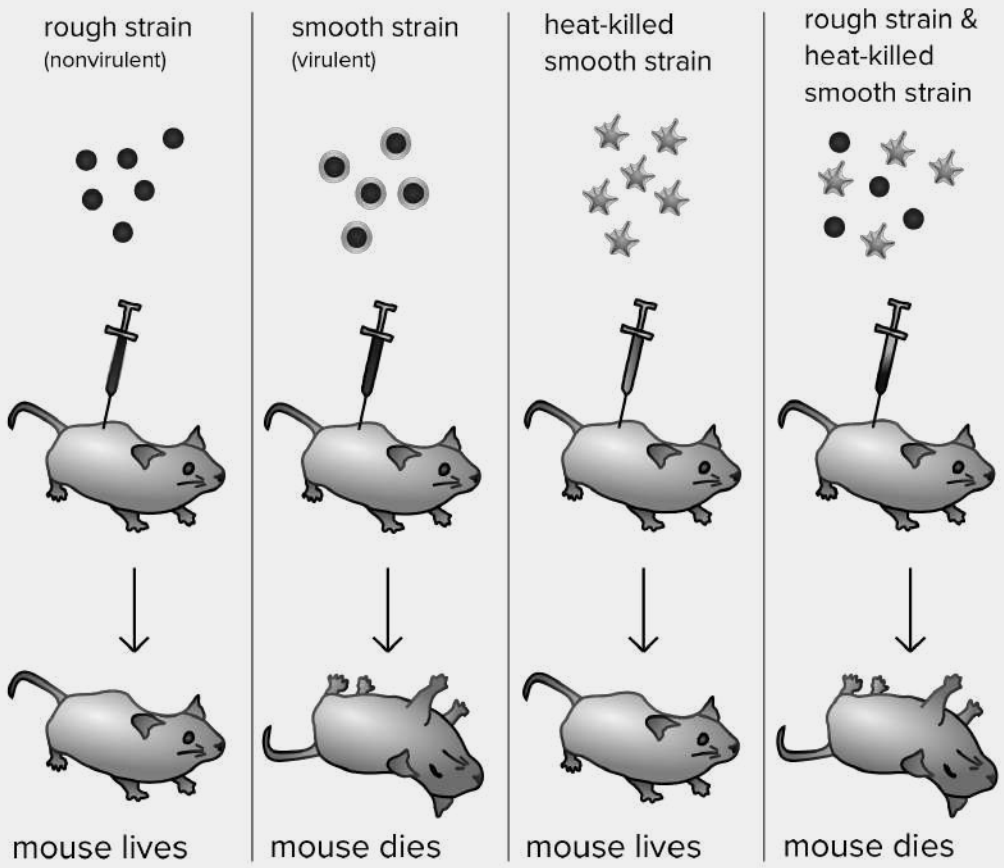
History of DNA

- 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**

Frederick Griffith

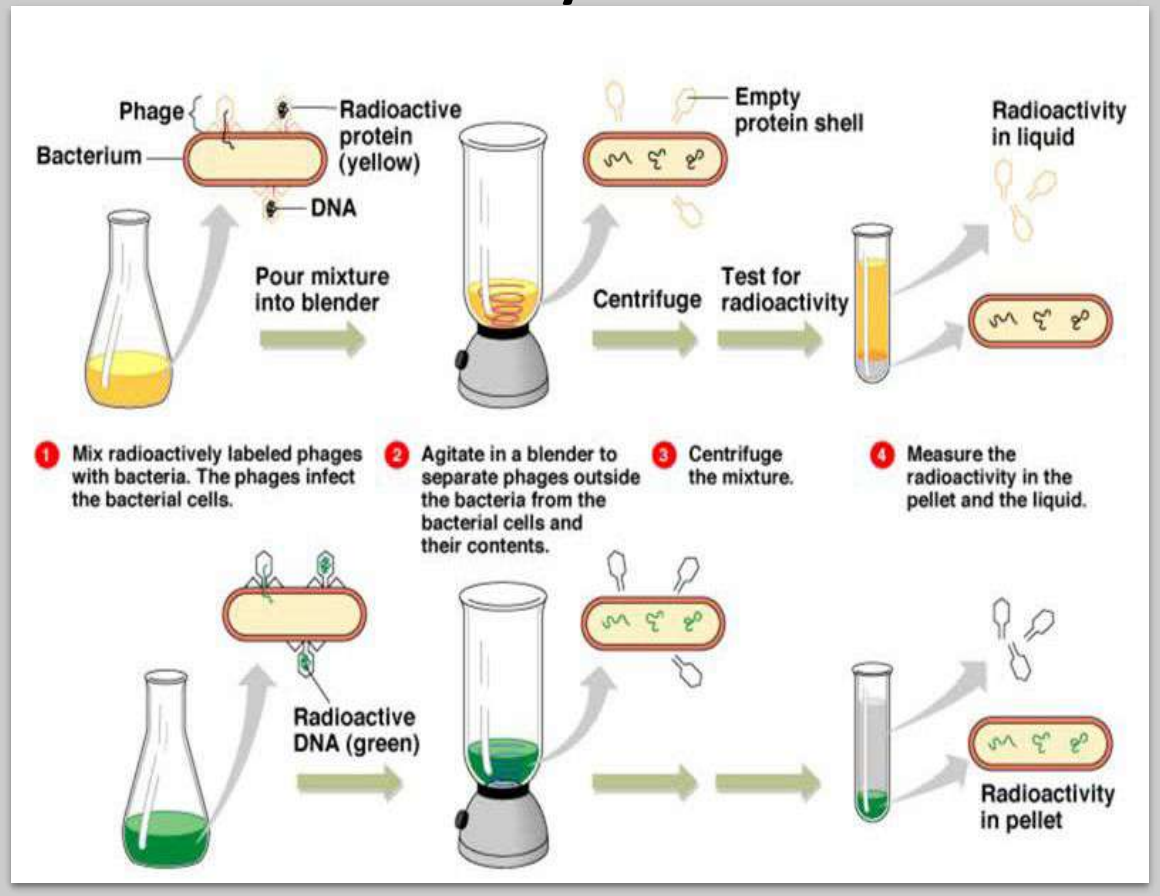
Martha Chase / Alfred Hershey

Experimentation

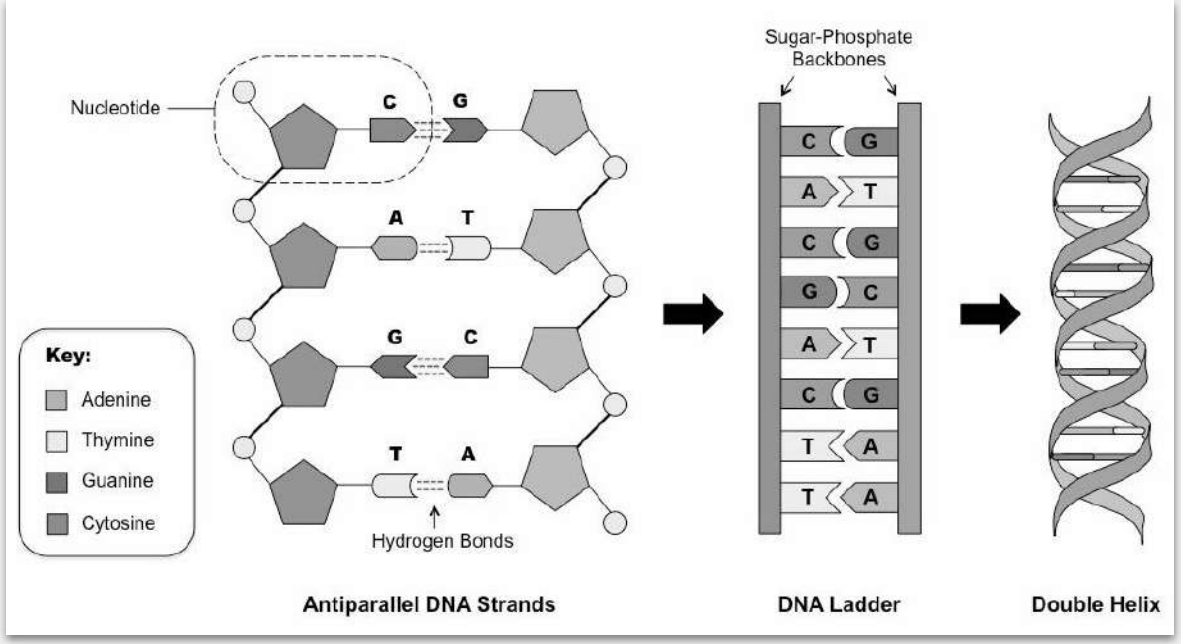


Griffith's Transformation

Hershey - Chase



DNA Structure



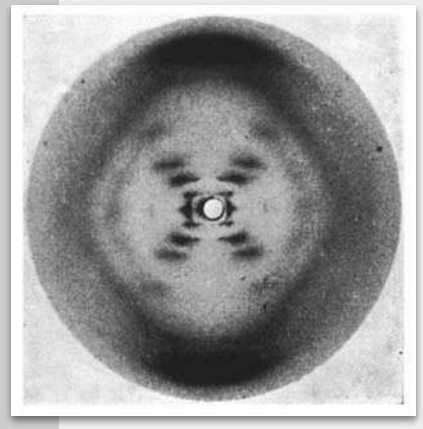
DNA Structure:

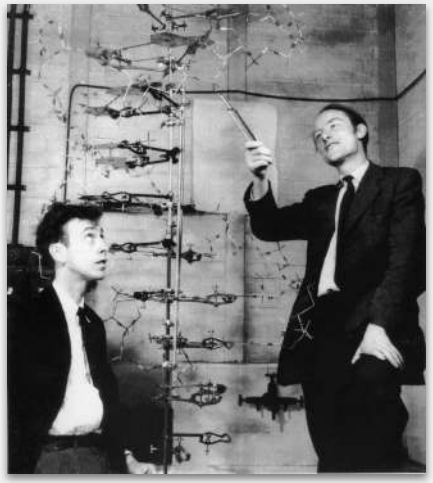
- **Erwin Chargaff** showed the amounts of the four bases on **DNA (A,T,C,G)**
- **In a body or somatic cell:**
  - ➔ **A = 30.3%**
  - ➔ **T = 30.3%**
  - ➔ **G = 19.5%**
  - ➔ **C = 19.9%**
- **Chargaff's Rule:**
  - ➔ **Adenine** must pair with **Thymine**
  - ➔ **Guanine** must pair with **Cytosine**

DNA Structure Continued

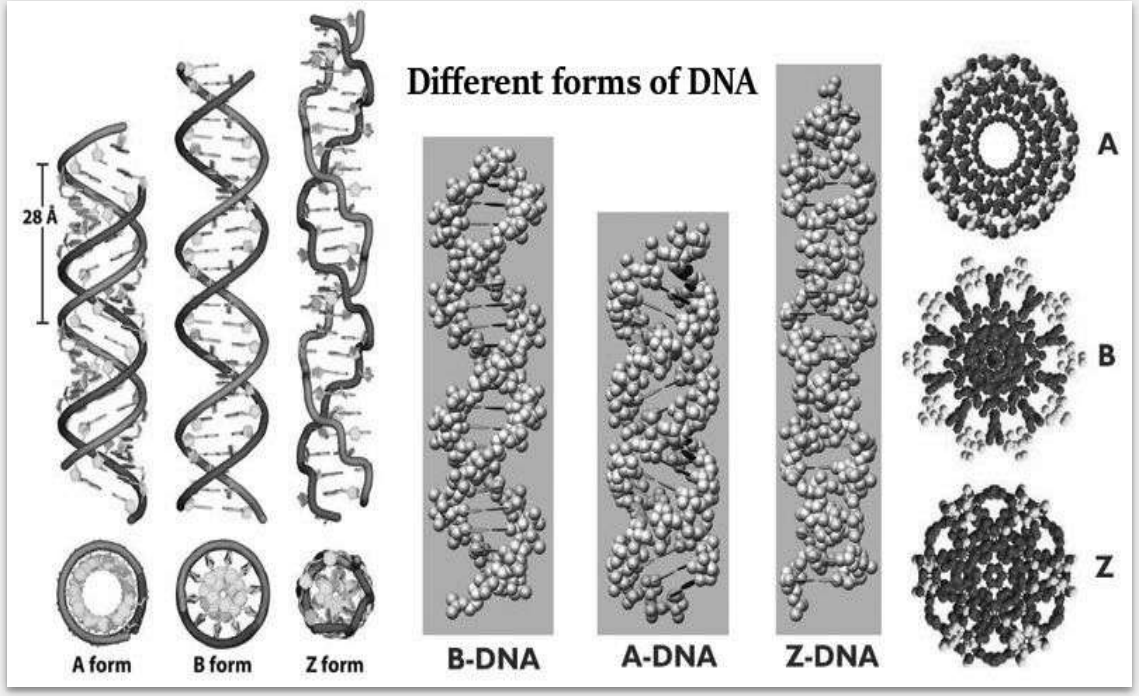
- **The bases are held together by weak hydrogen bonds**
- **DNA's First Photograph**
  - ➔ **Rosalind Franklin** took diffraction x-ray photographs of DNA crystals
  - ➔ In the 1950's, **Watson & Crick** built the first model of DNA using Franklin's X-rays

Francis Crick | James Watson | Rosalind Franklin





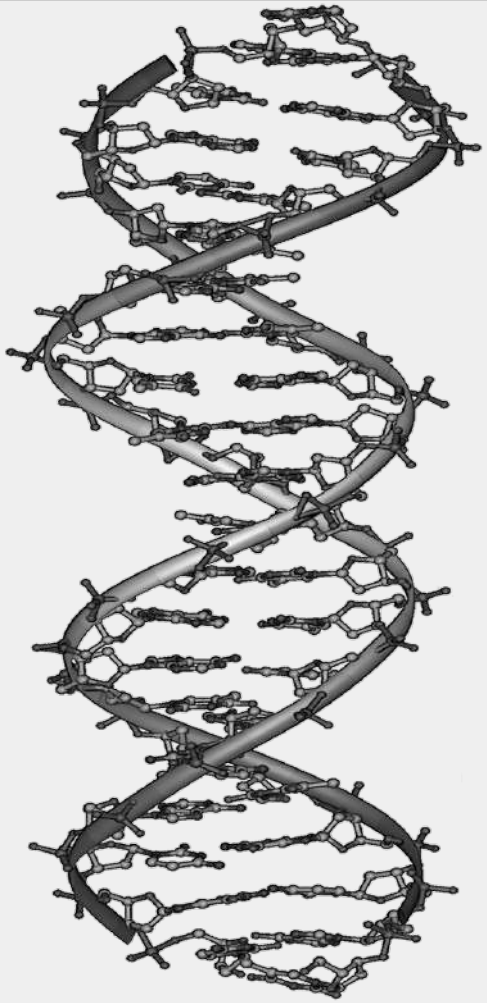
DNA Structure Continued

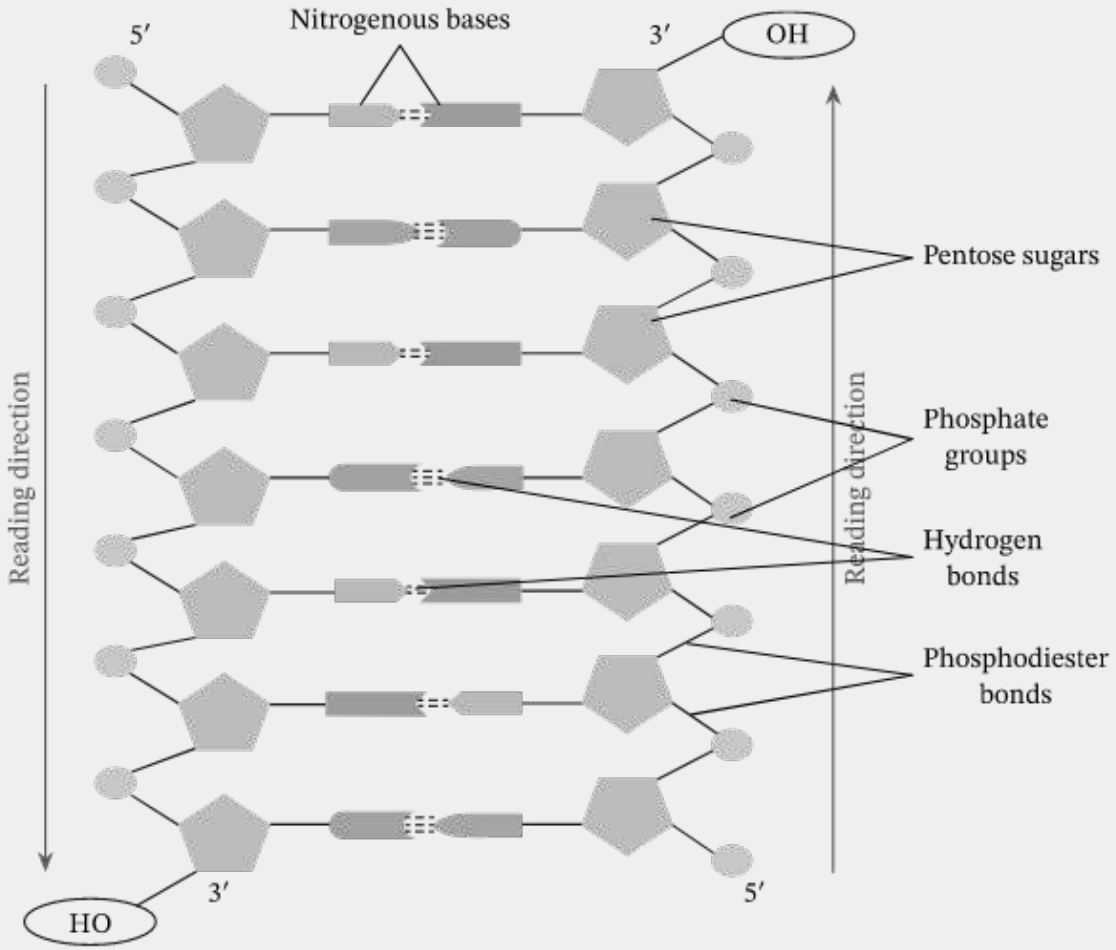


Structure

- 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**
  - ➔ **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

DNA Structure Continued

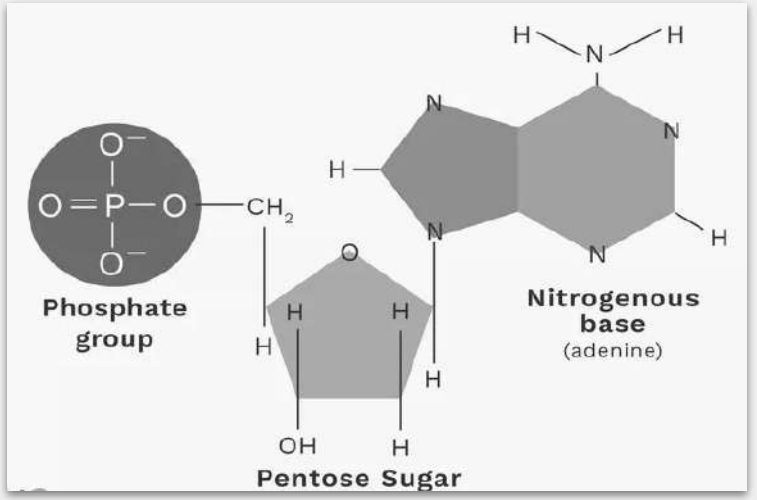


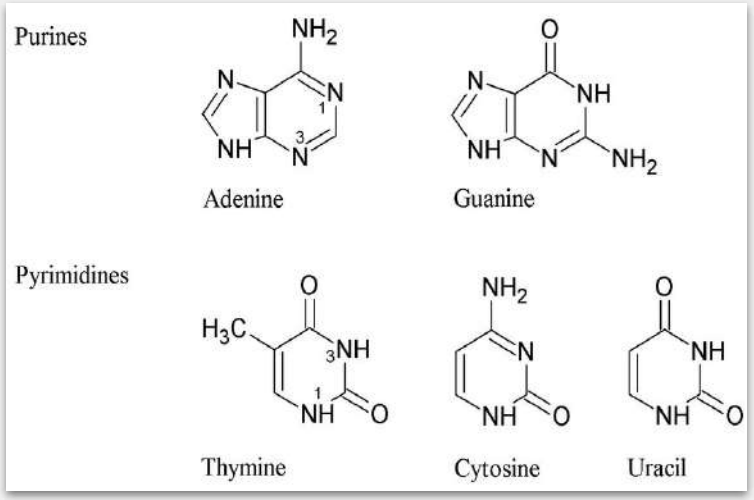


Nucleotides

Nucleotides

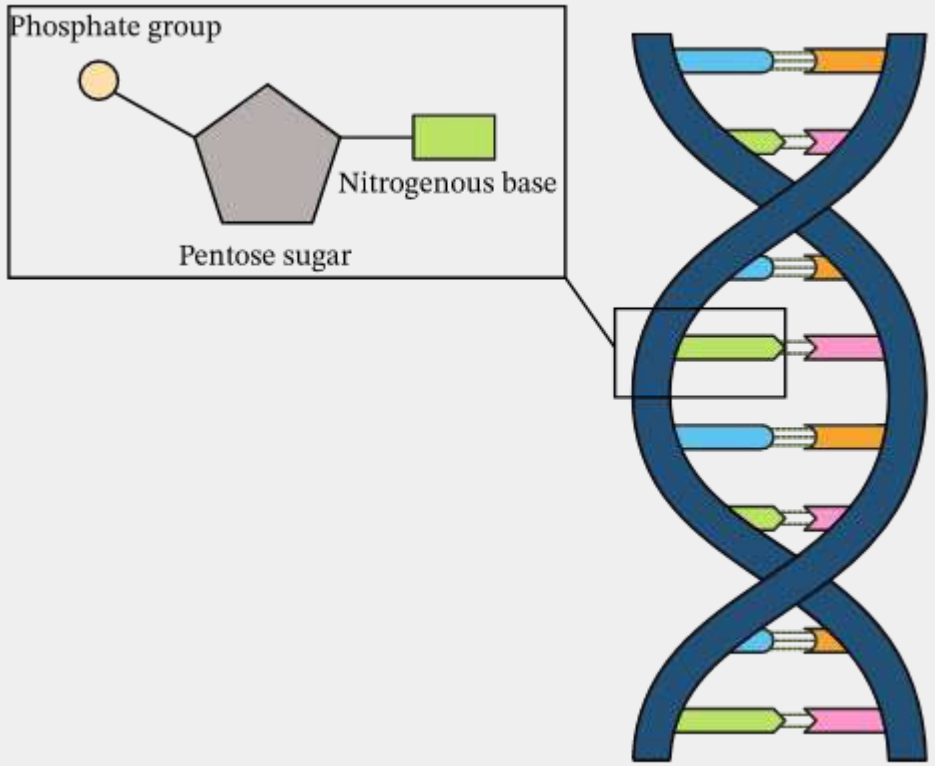
- **DNA** Stands for **Deoxyribonucleic acid**
- Made up of subunits called **nucleotides**
- **Nucleotide** made of:
  - ➔ **Phosphate group**
  - ➔ **5-carbon sugar**
  - ➔ **Nitrogenous base (**genetic code**)**
- **Double** ring **PURINES**
  - ➔ **Adenine (A)**
  - ➔ **Guanine (G)**
- **Single**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



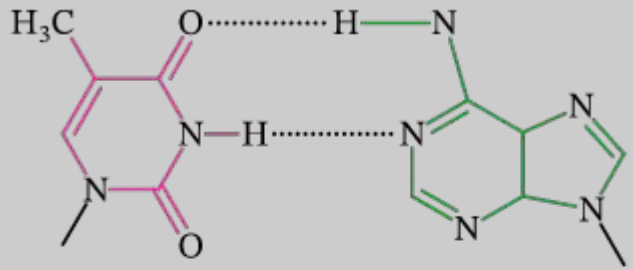


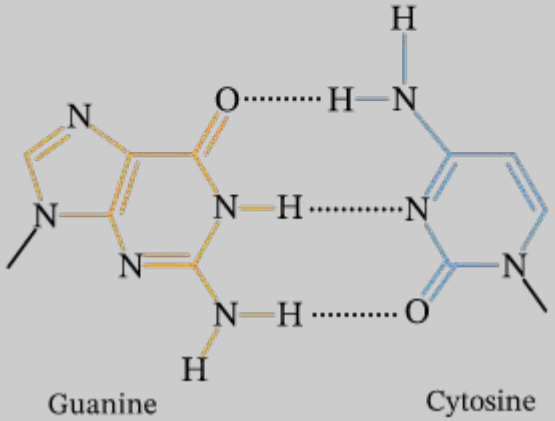
Nucleotides Continued

Nucleotide Structure



Base Pairing





DNA Replication

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)

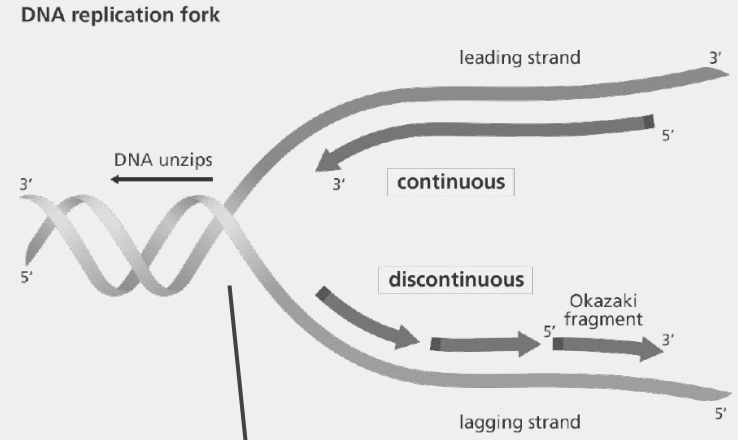
❑ New strands grow at the forks

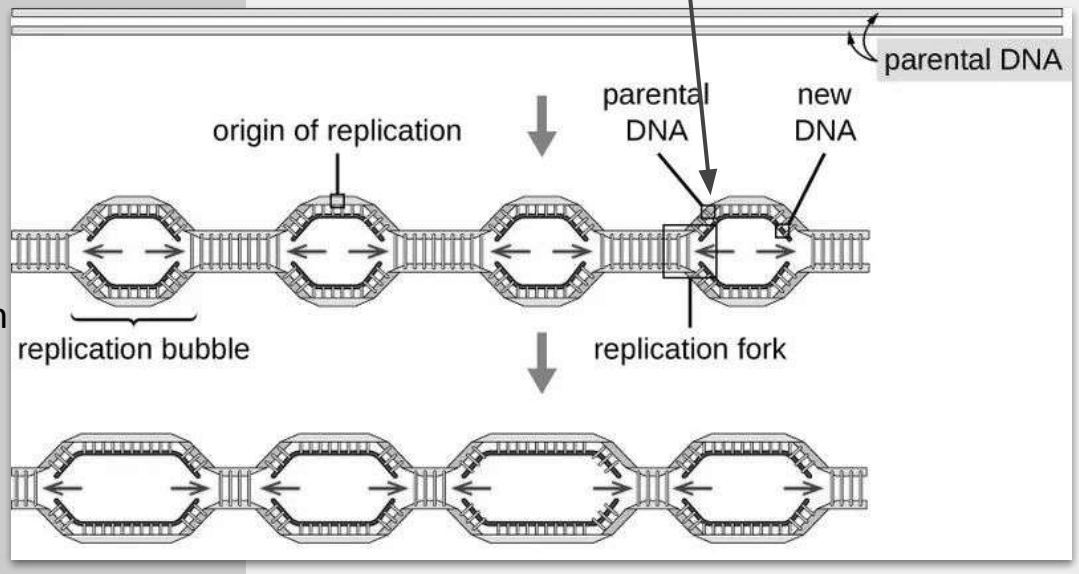
➔ **Replication Bubbles**

❑ As the 2 DNA strands open at the origin, Replication Bubbles form

❑ **Eukaryotic** chromosomes have **MANY** bubbles

❑ **Prokaryotes** (bacteria) have a **single** bubble





Enzymes for Replication

01
Helicase

- Enzyme: **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**

02
Topoisomerase

- **Topoisomerase**
  - ➔ Enzyme: **Topoisomerase attaches** to the 2 forks of the bubble to **relieve stress on the DNA molecule as it separates**

03
RNA Primase

- **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**

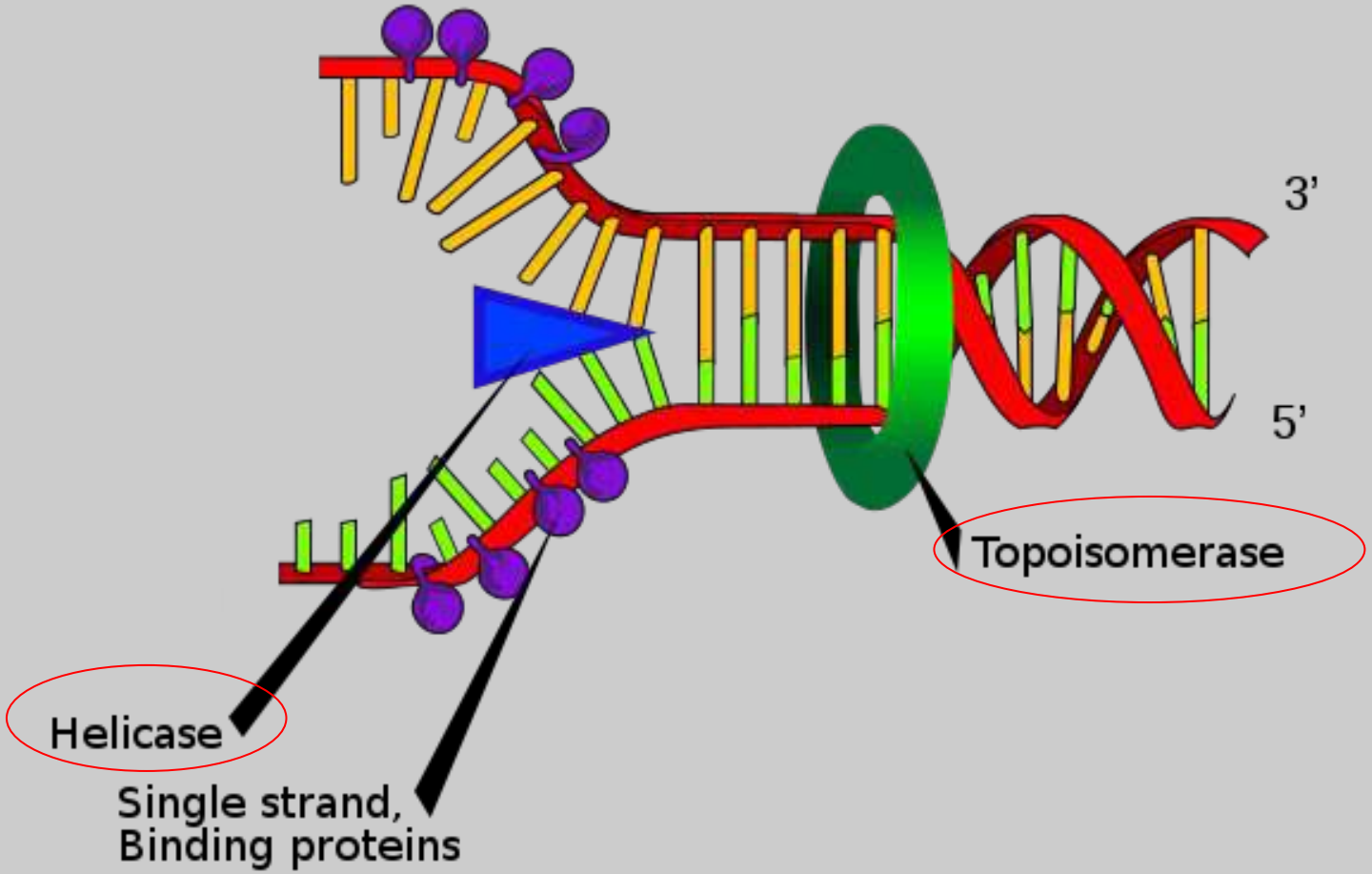
04
DNA Polymerase

- **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

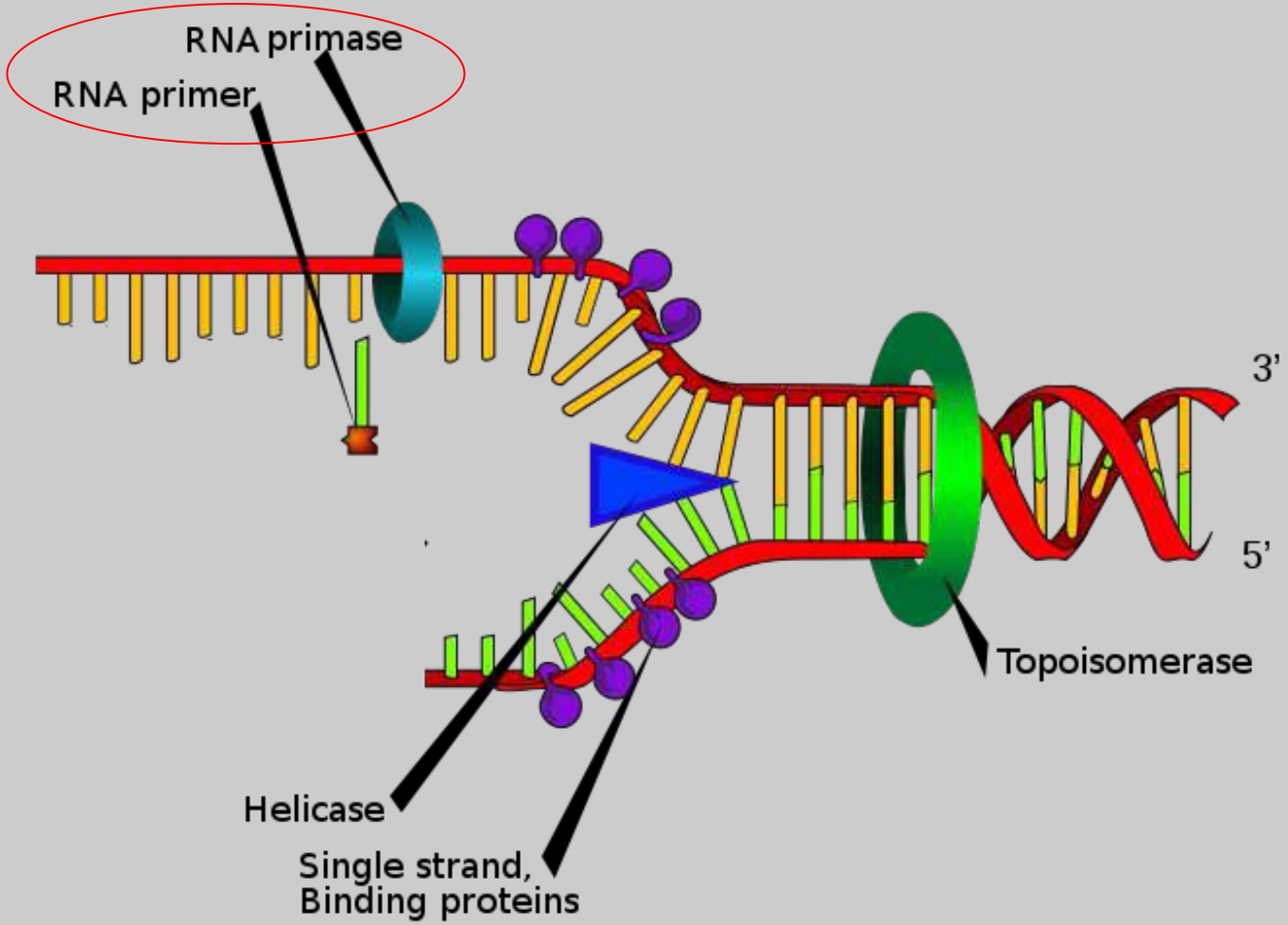
05
Ligase

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

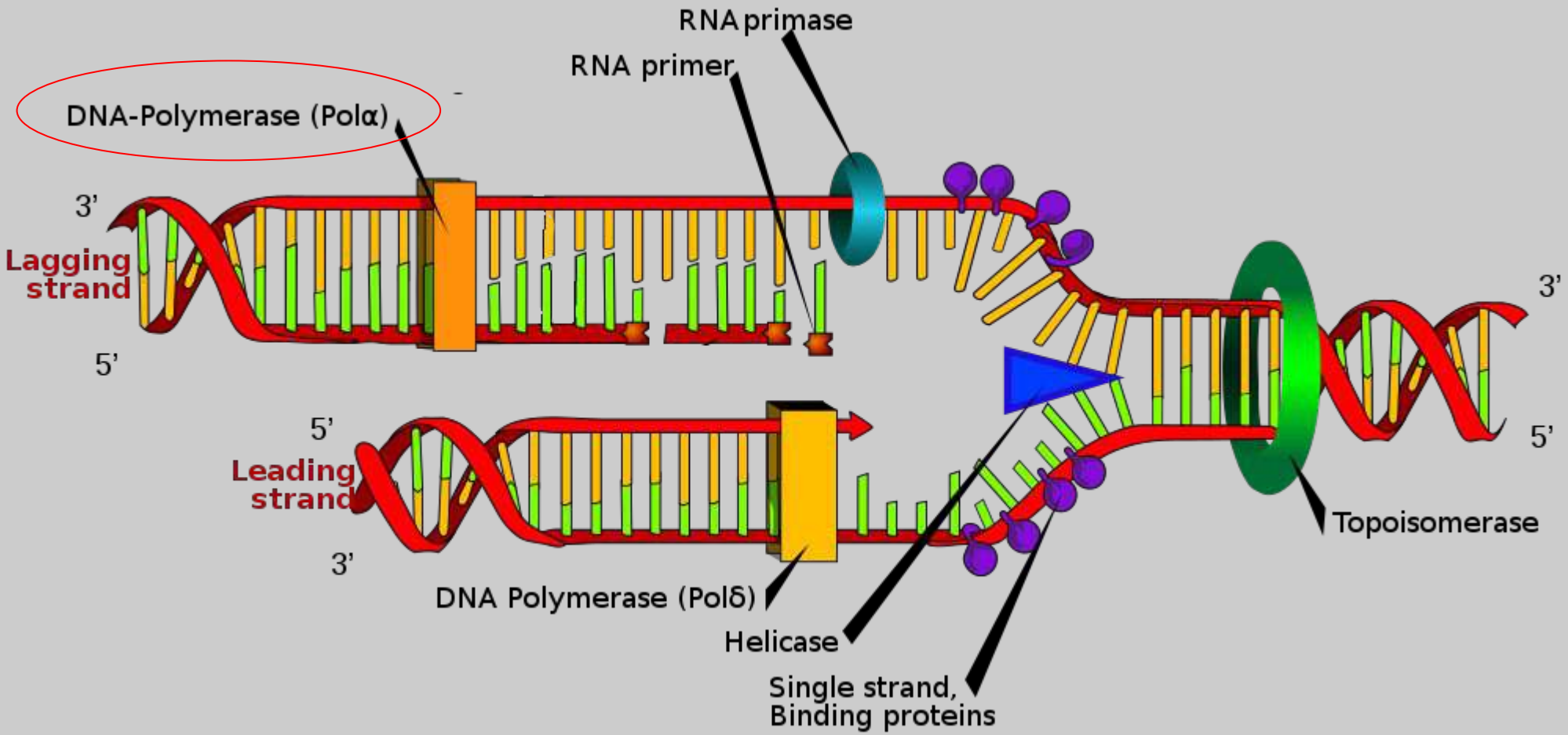
Helicase & Topoisomerase



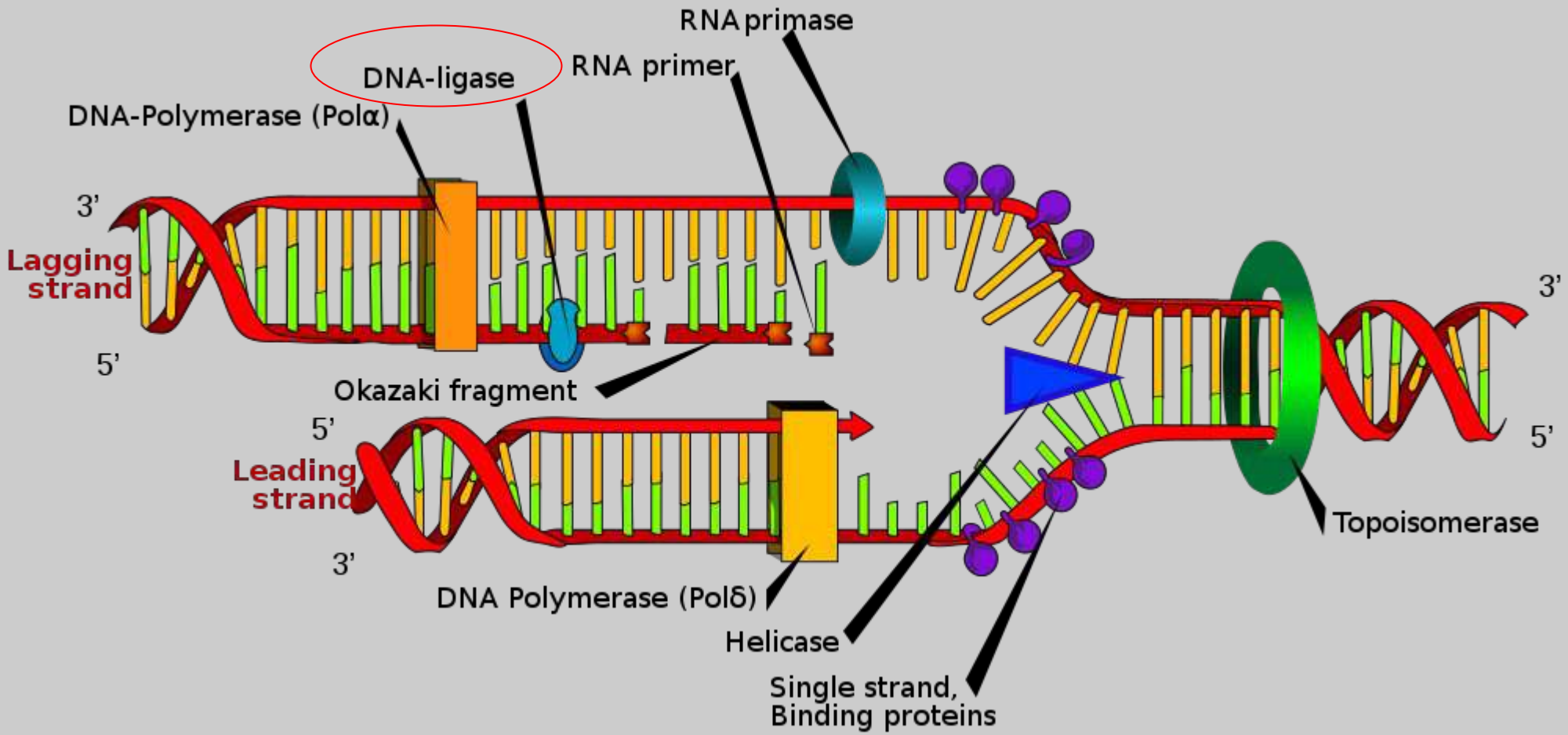
RNA Primase



DNA Polymerase



DNA Ligase



Leading & Lagging Strands

The Leading Strand - (continuous)

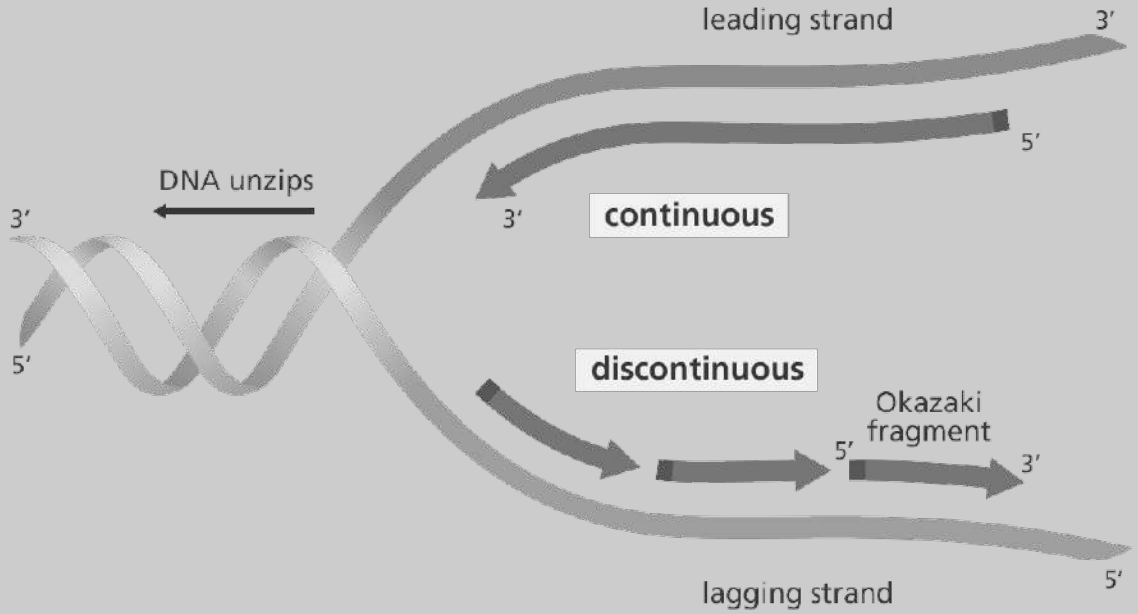
➔ is synthesized as a single strand from the point of **origin toward the opening replication fork**

Lagging Strand: (discontinuous)

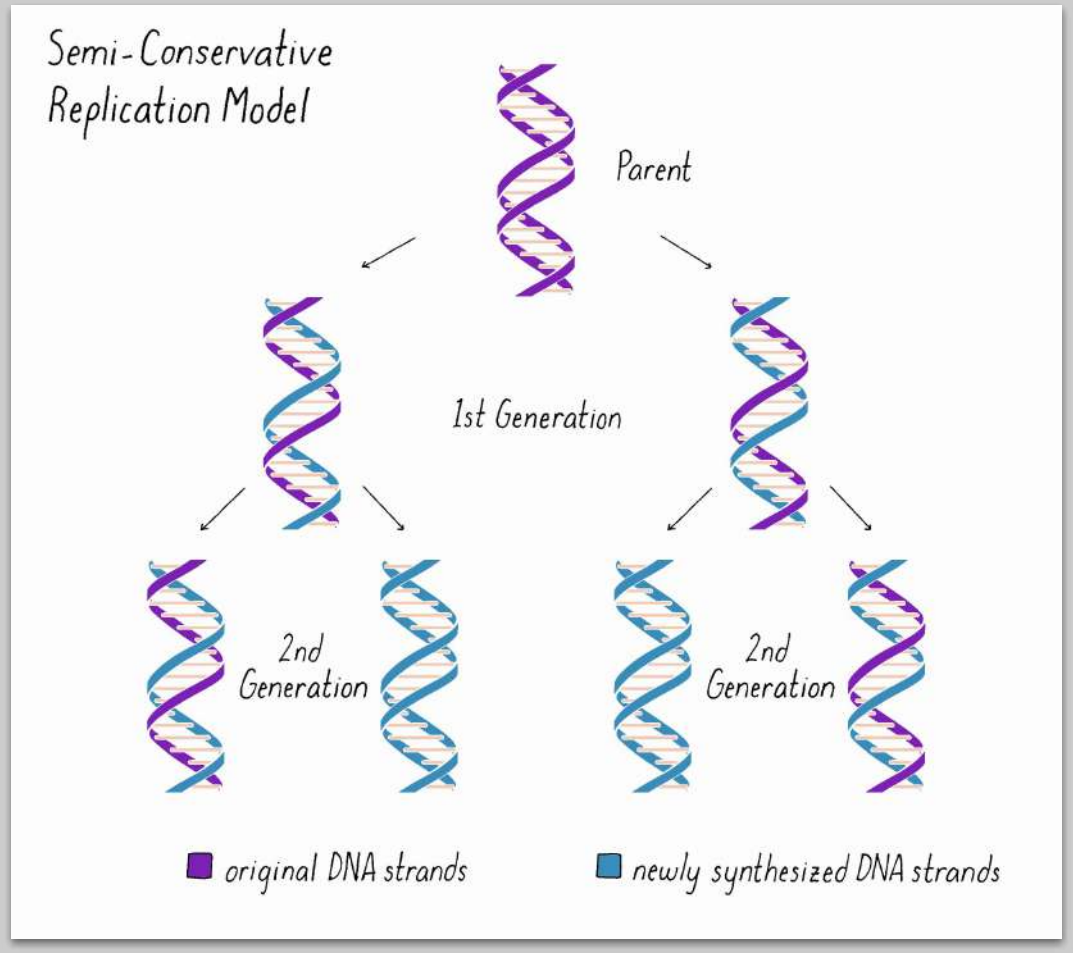
➔ The Lagging Strand is synthesized discontinuously against overall direction of replication

➔ 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



Semi-Conservative Replication & Proofreading



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

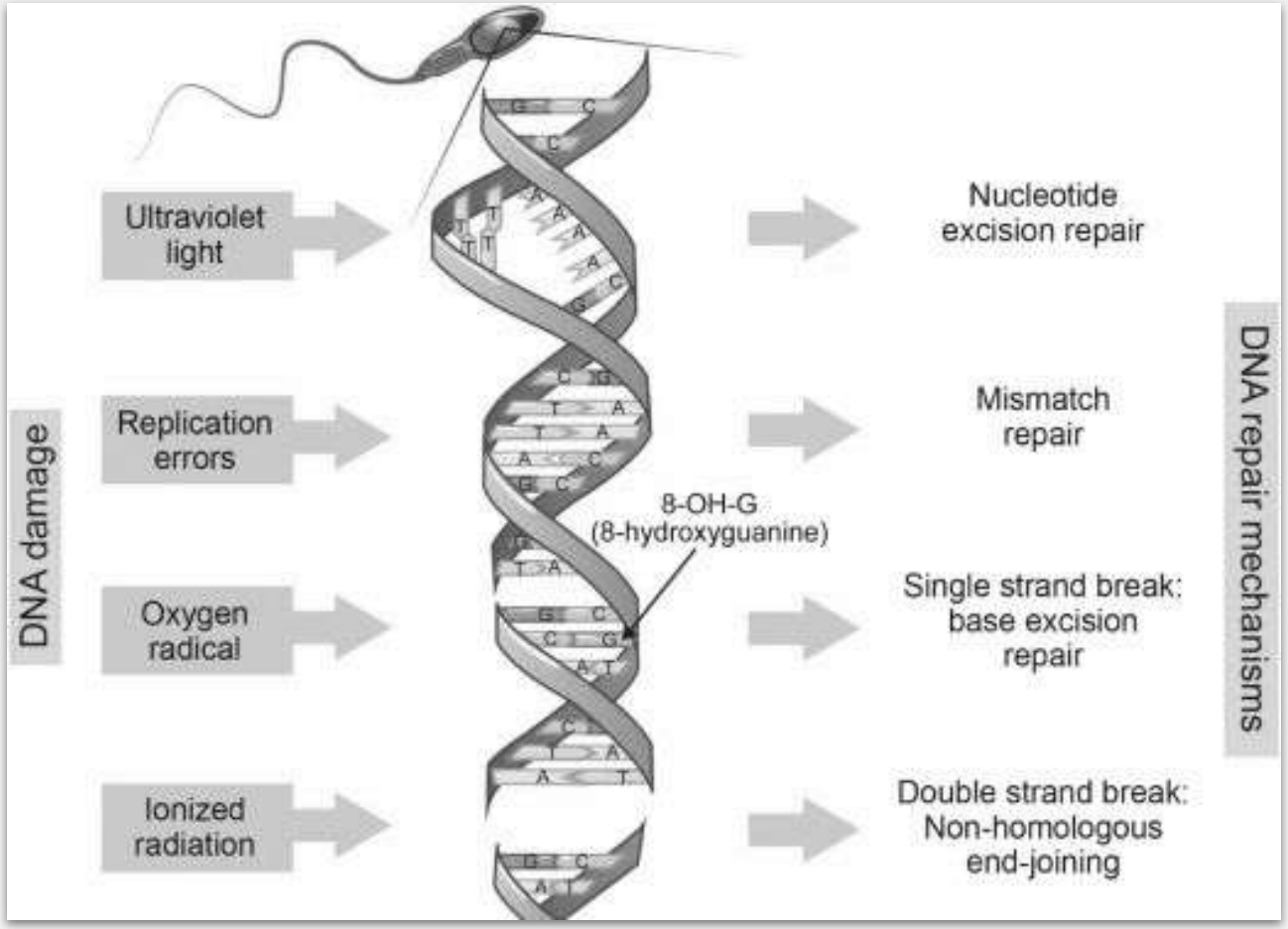
Proofreading DNA

- ➔ 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

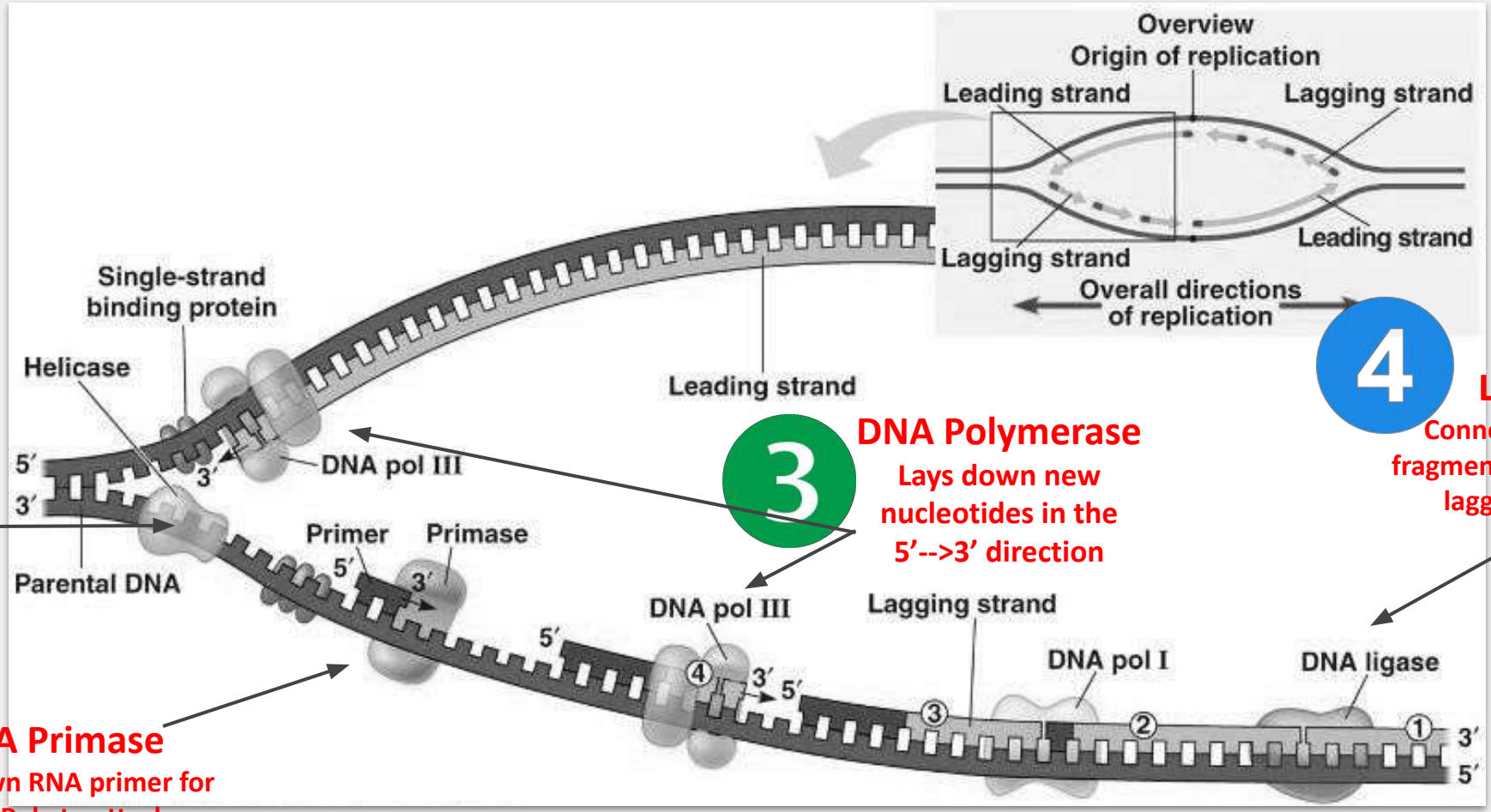
DNA Damage & Repair

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



DNA Replication Summary



1

Helicase & Topoisomerase

Unzips and unwinds DNA

2

RNA Primase

Lays down RNA primer for DNA Poly to attach

3

DNA Polymerase

Lays down new nucleotides in the 5'-->3' direction

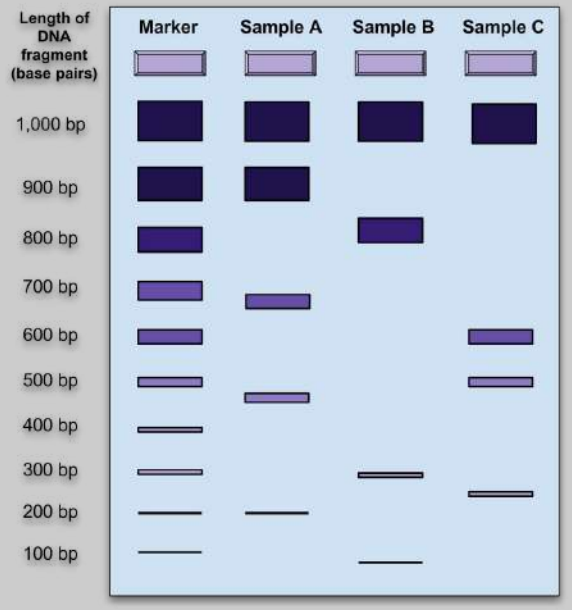
4

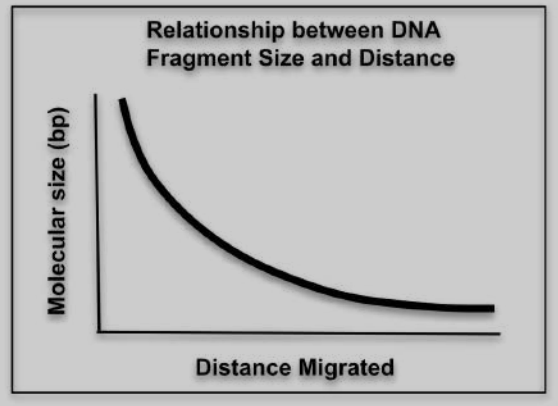
Ligase

Connects okazaki fragments together on lagging strand

Introduction to Gel Electrophoresis

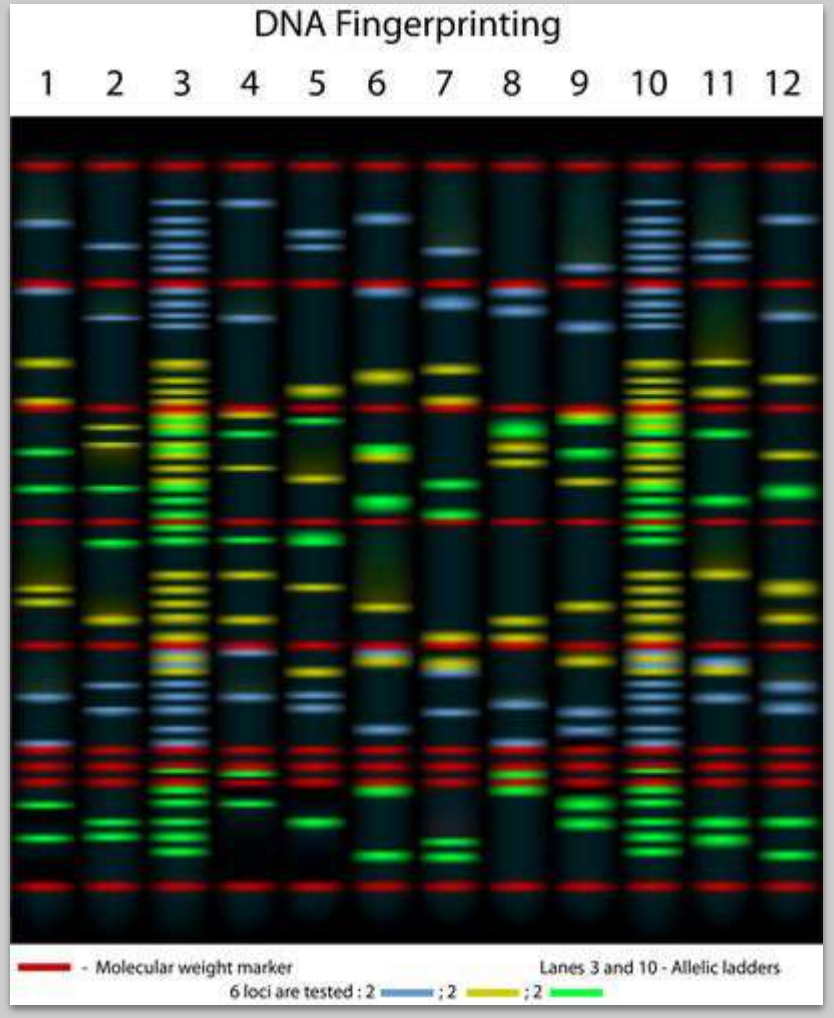
- **Definition** and purpose of gel electrophoresis
  - **Gel electrophoresis** is a laboratory technique used to separate DNA fragments or other macromolecules based on their size and charge.
  - The purpose of gel electrophoresis is to analyze and study DNA samples, identify genetic variations, and determine fragment sizes.





Importance of Gel Electrophoresis

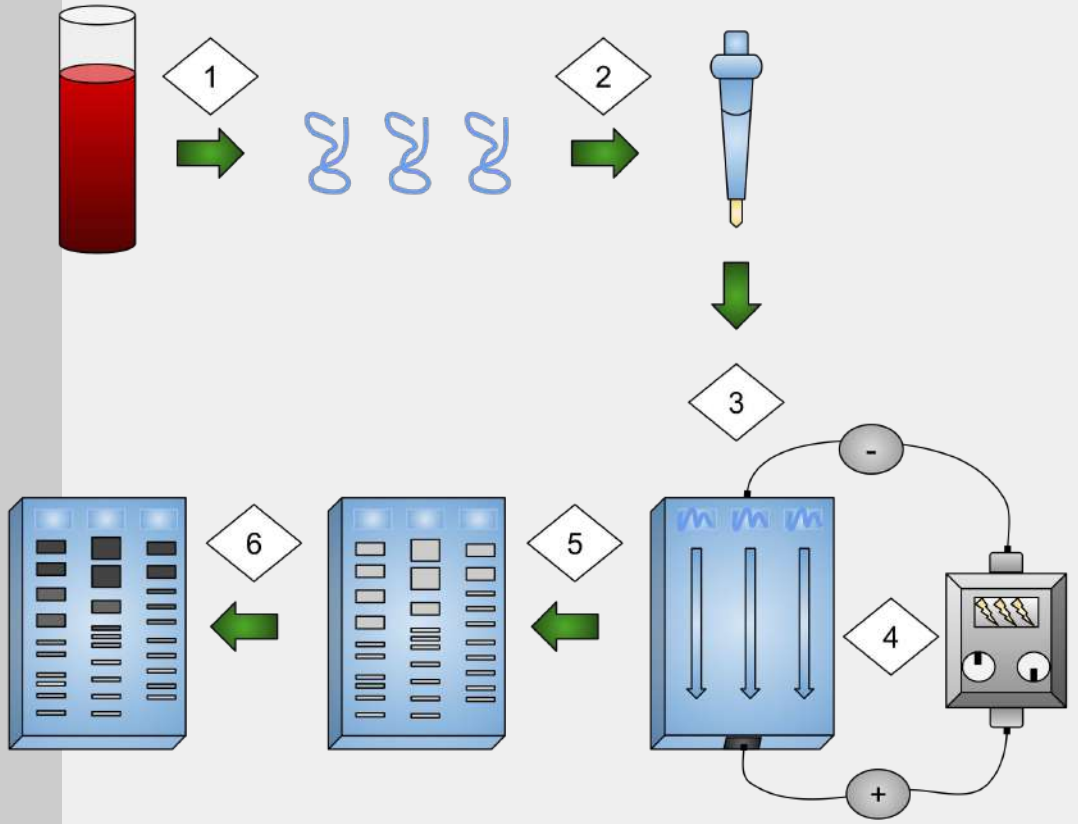
- **Importance and applications in biology research and DNA analysis**
  - ➔ Gel electrophoresis is a fundamental tool in molecular biology research.
  - ➔ It is used in DNA fingerprinting, genetic profiling, paternity testing, and forensic investigations.
  - ➔ It is essential for studying **gene**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



Principles of Gel Electrophoresis

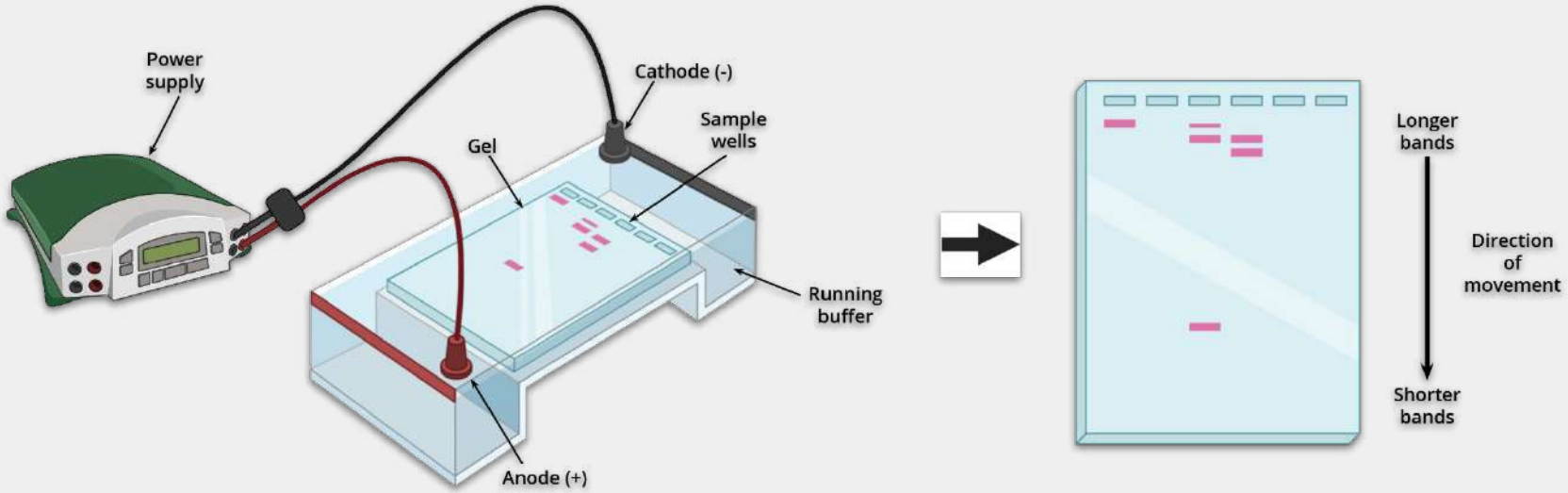
Principles of Gel Electrophoresis

- **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.

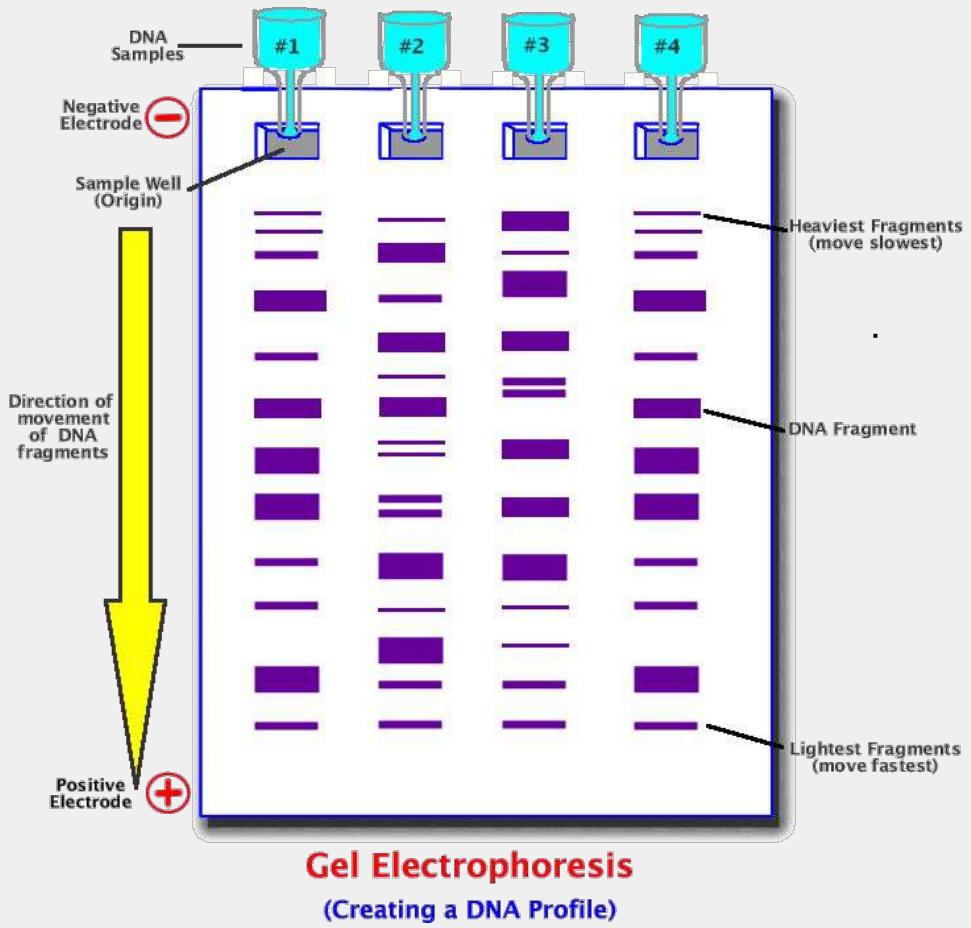


The Setup

- **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.



Step-by-Step

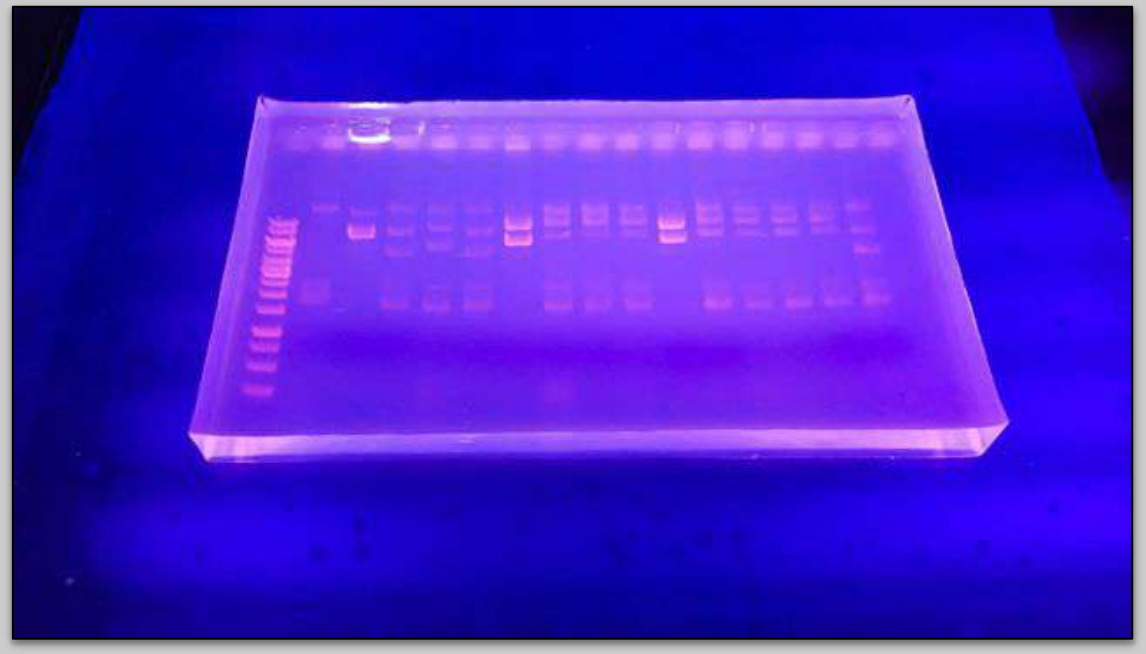


Procedure of Gel Electrophoresis

- **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.

Step-by-Step Cont'

- **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**.

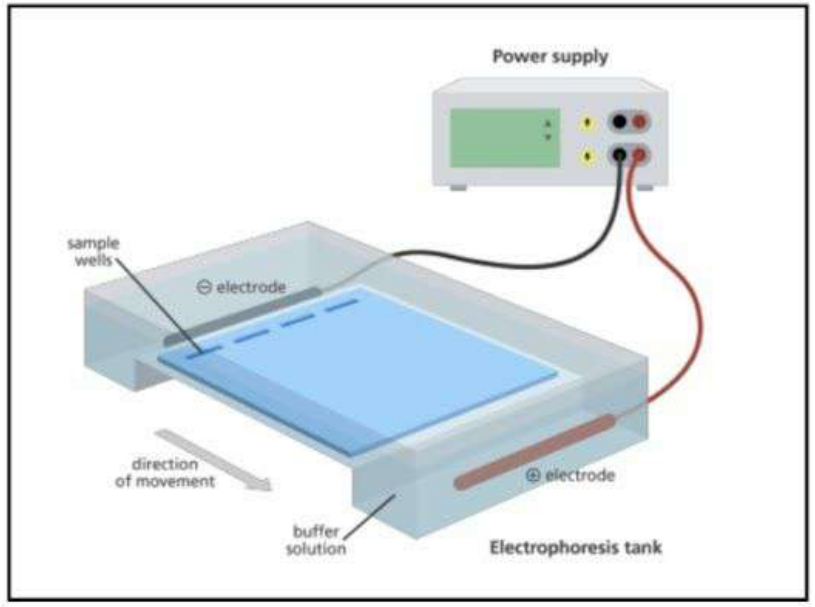


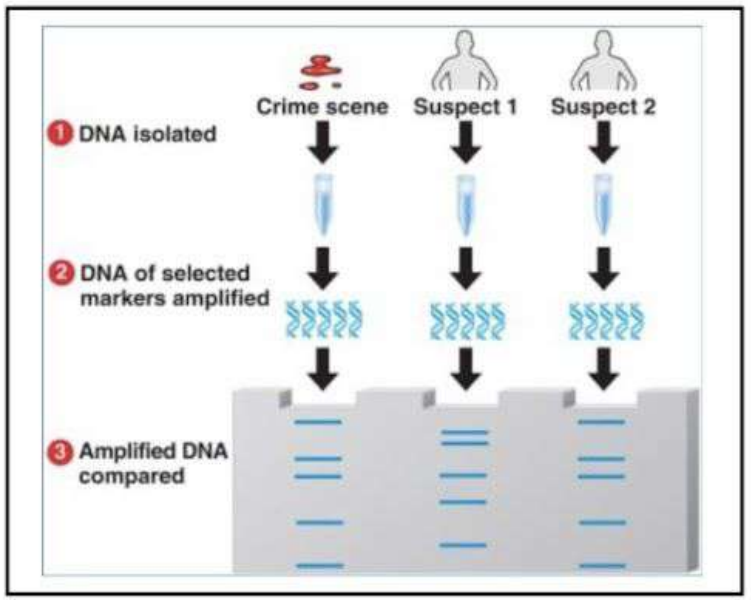
Data Interpretation

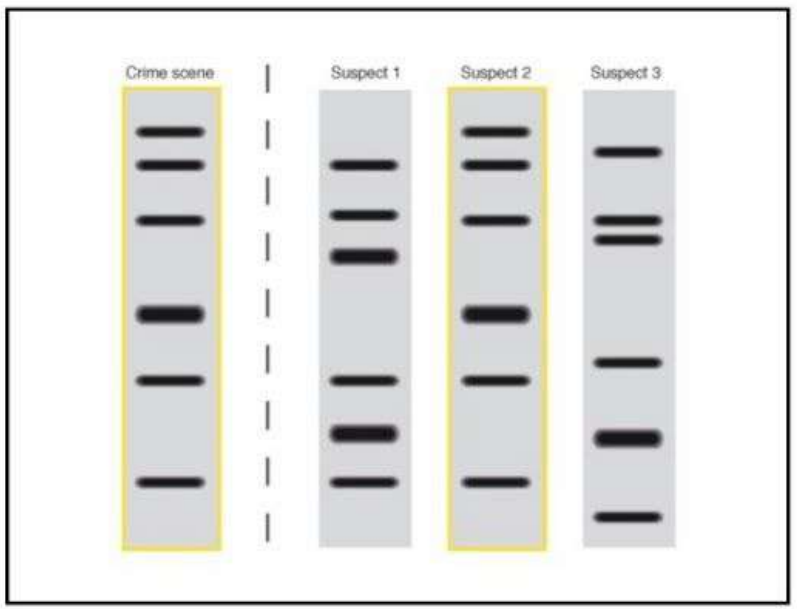
Interpretation of Gel Electrophoresis Results

- **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**.

Data Interpretation - cont’



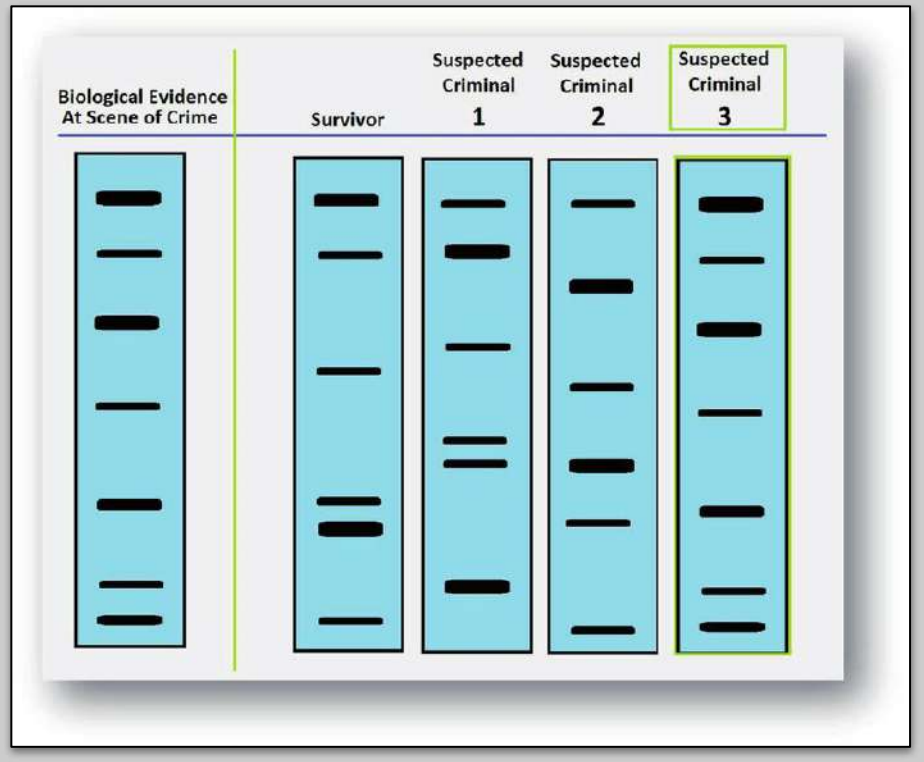




Analysis of Electrophoresis

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

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

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.

Ethical Considerations - cont'

➔ **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.

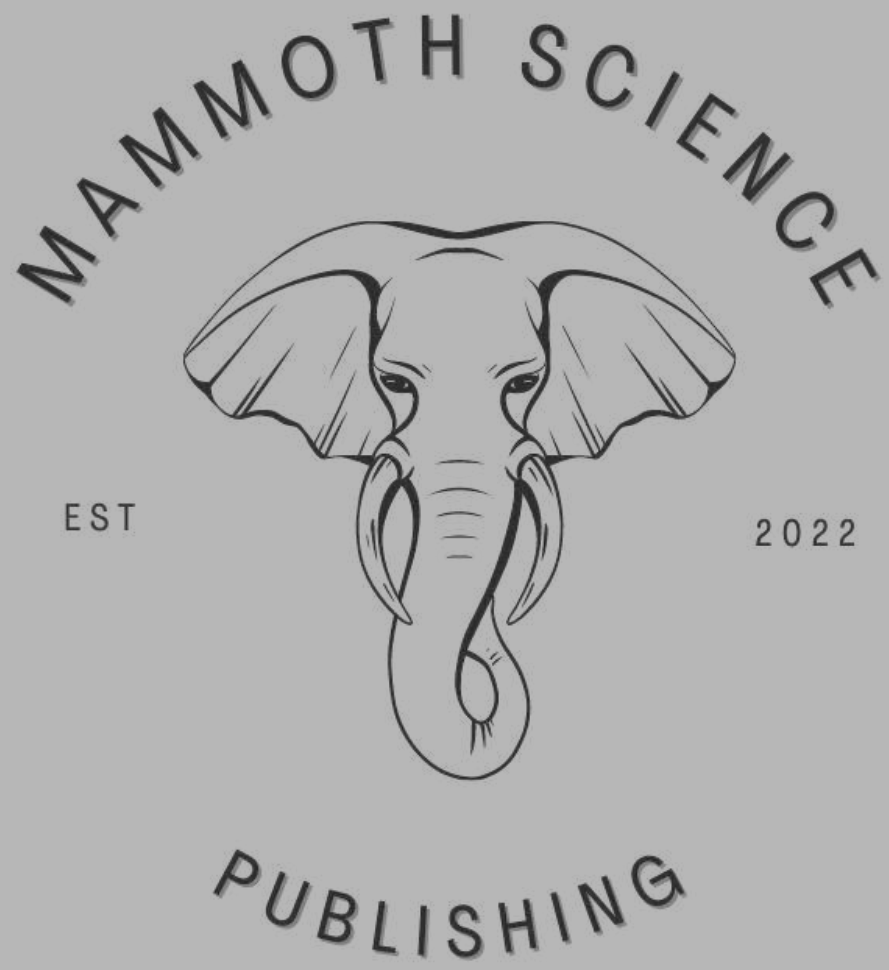
Ethical Considerations - cont’

➔ **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 hazardous materials

➔ Safety precautions to prevent exposure to hazardous chemicals and proper disposal of biohazardous materials used in gel electrophoresis.



Thank you!

Do you have any questions?
instructor@email.com
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