Al-Furat Al-Awsat Technical University

Kufa Techincal Institute

Medical Laboratory Techniques Department

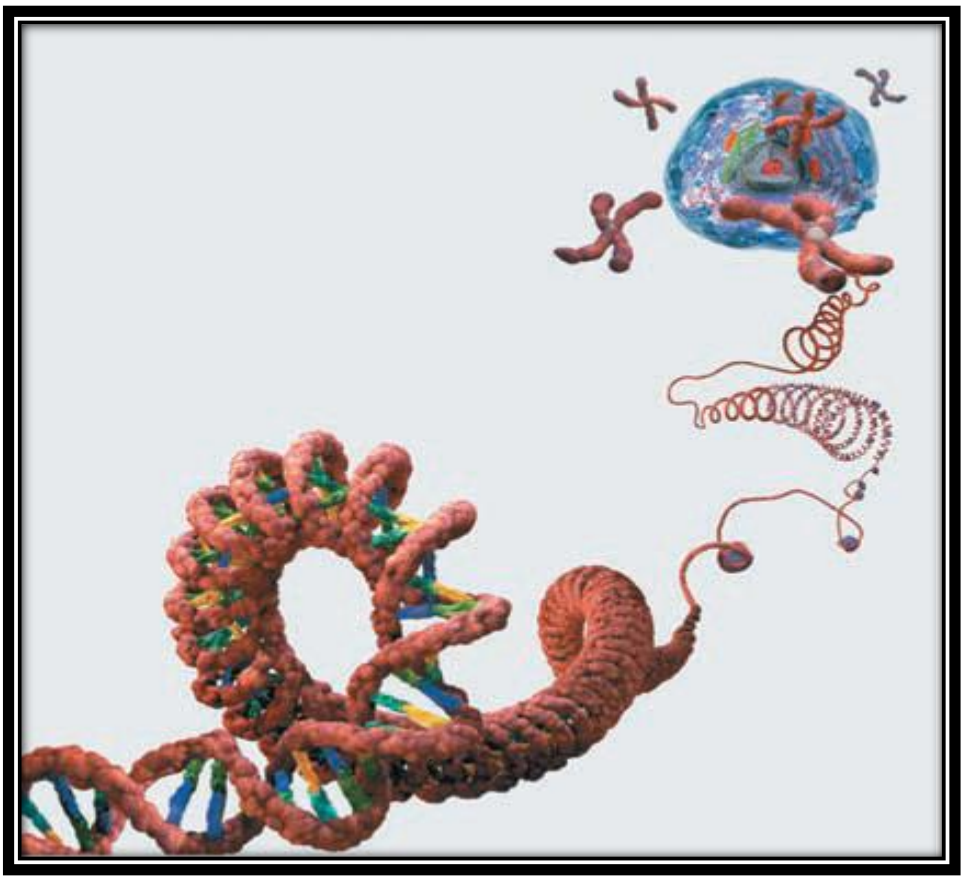
Molecular biology

First class

DR.Taif

[اختر التاريخ]

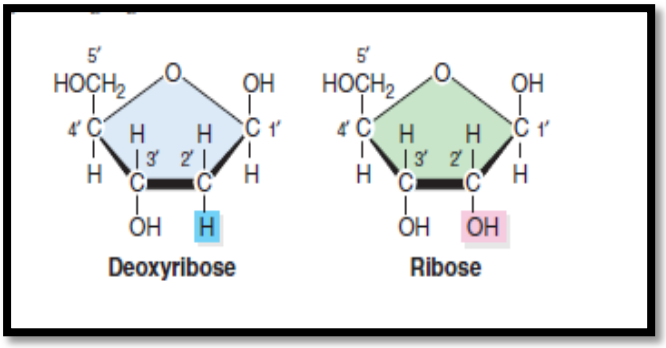
Genetics is the science of heredity**,** concerned primarily with understanding biological properties that are transmitted from parent to offspring .



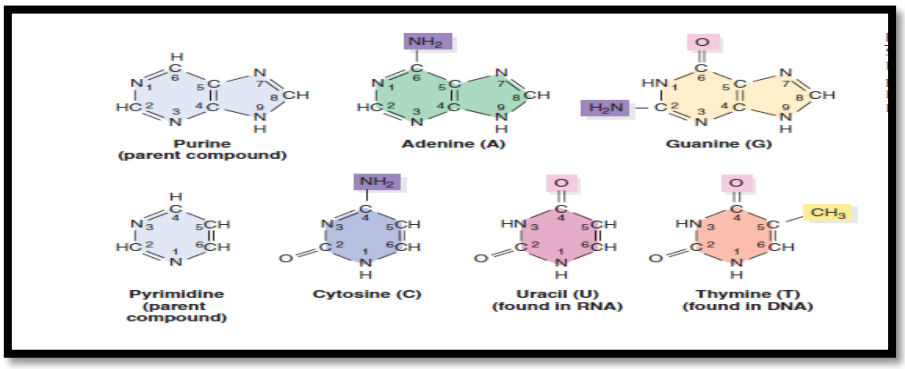
Simple observation shows that a lot of variation exists between individuals of a given species. For example, individual humans vary in eye color, height, skin color, and hair color, even though all humans belong to the species Homo sapiens. The differences between individuals within and among species are mainly the result of differences in the DNA sequences that constitute the genes in their genomes. The genetic information coded in DNA is largely responsible for determining the structure, function, and development of the cell and the organism.

DNA and RNA were carry genetic information. living organisms contain some substance a genetic material—that is responsible for the characteristics that are passed on from parent to child.

The monomers that make up DNA and RNA are nucleotides. Each nucleotide consists of a pentose (five-carbon) sugar، a nitrogenous (nitrogen-containing) base (usually just called a base), and a phosphate group. In DNA, the pentose sugar is deoxyribose, and in RNA it is ribose

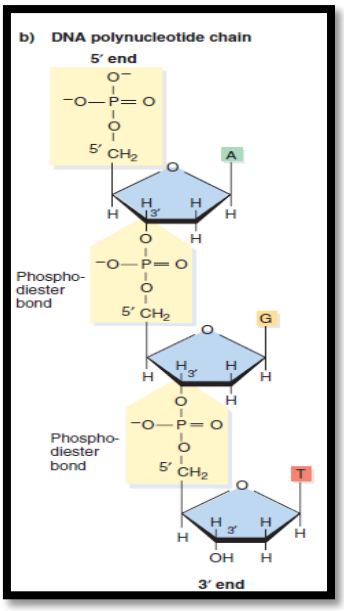


There are two classes of nitrogenous bases: the purines, which are double-ringed structures، and the pyrimidines, which are single-ringed structures. There are two purines—adenine A) and guanine (G)—and three different pyrimidines thymine (T), cytosine (C), and uracil (U) in DNA and RNA.



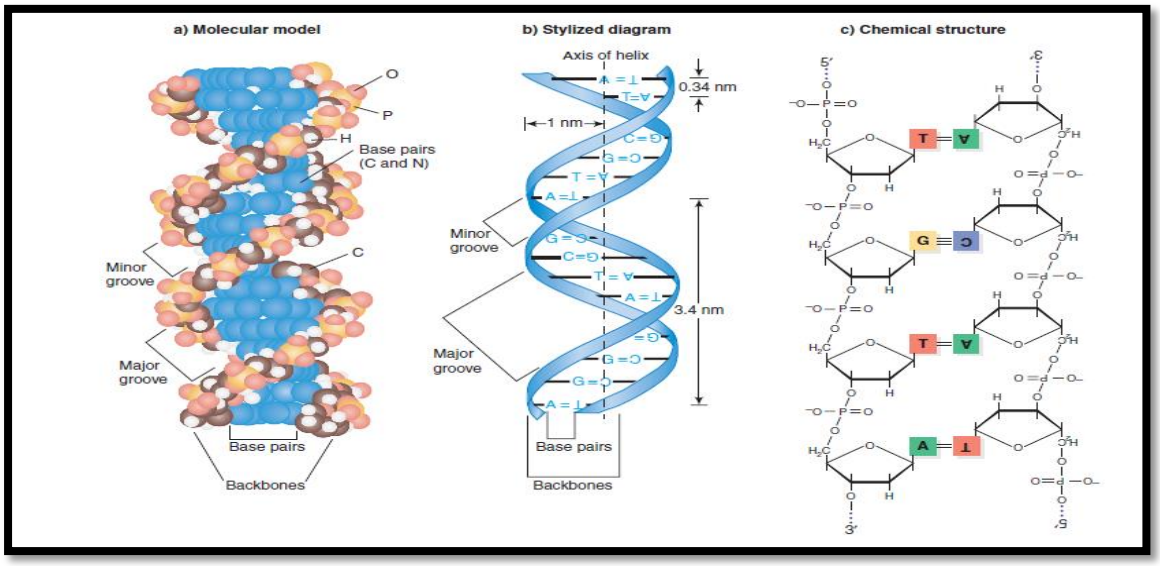
To form polynucleotides of either DNA or RNA, nucleotides are linked together by a covalent bond between the phosphate group of one nucleotide and the carbon of the sugar of another nucleotide called phosphodiester bonds.

Polynucleotide chains have polarity, meaning that the two ends are different: there is a phosphate group at one end and a hydroxyl group at the other end.



Watson and Crick's Model

Watson and Crick's double helix model of DNA has the following main features:
1. The DNA molecule consists of double strand helix.
2. The two strand are antiparallel that is, head of one chain is against the tail of the other chain, and vice versa.
3. The sugar–phosphate backbones are on the outsides of the double helix,
4. The bases in each of the two strands are bonded together by hydrogen bonds, which are relatively weak chemical bonds. The specific pairings observed are A bonded with T (two hydrogen bonds) and G bonded with C (three hydrogen bonds) The hydrogen bonds make it rela tively easy to separate the two strands by heating. The specific A–T and G–C pairs are called complementary base pairs.



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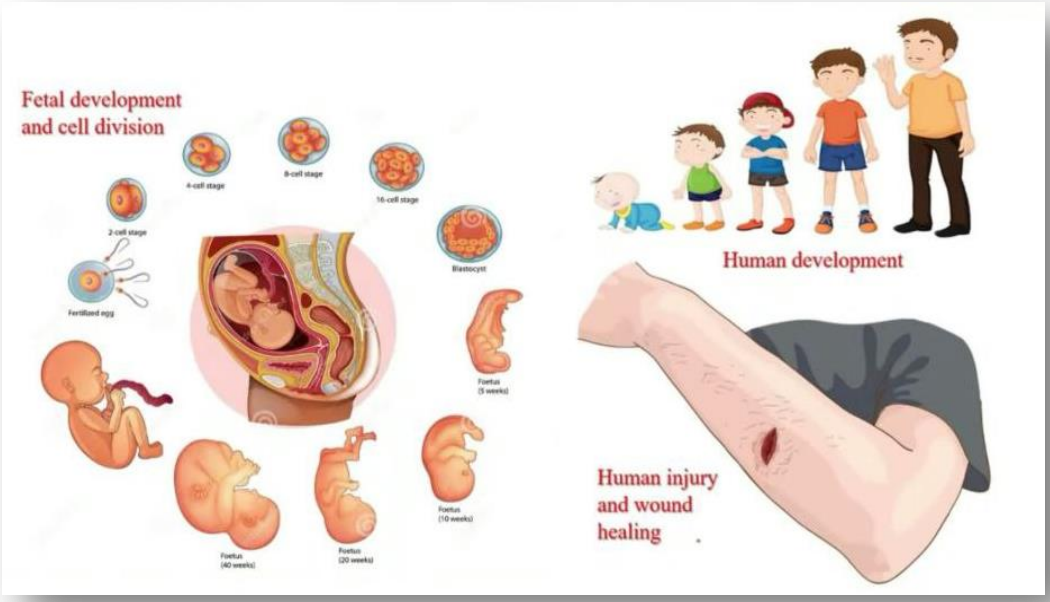
Lac.2

Replication of DNA is vital to the transmission of genomes and the genes they contain from cell generation to cell generation, and from organism generation to organism generation.



Models of DNA Replication

Semiconservative model : If the DNA molecule was untwisted and the two strands separated, each strand could act as a template for the synthesis of a new, complementary strand of DNA.

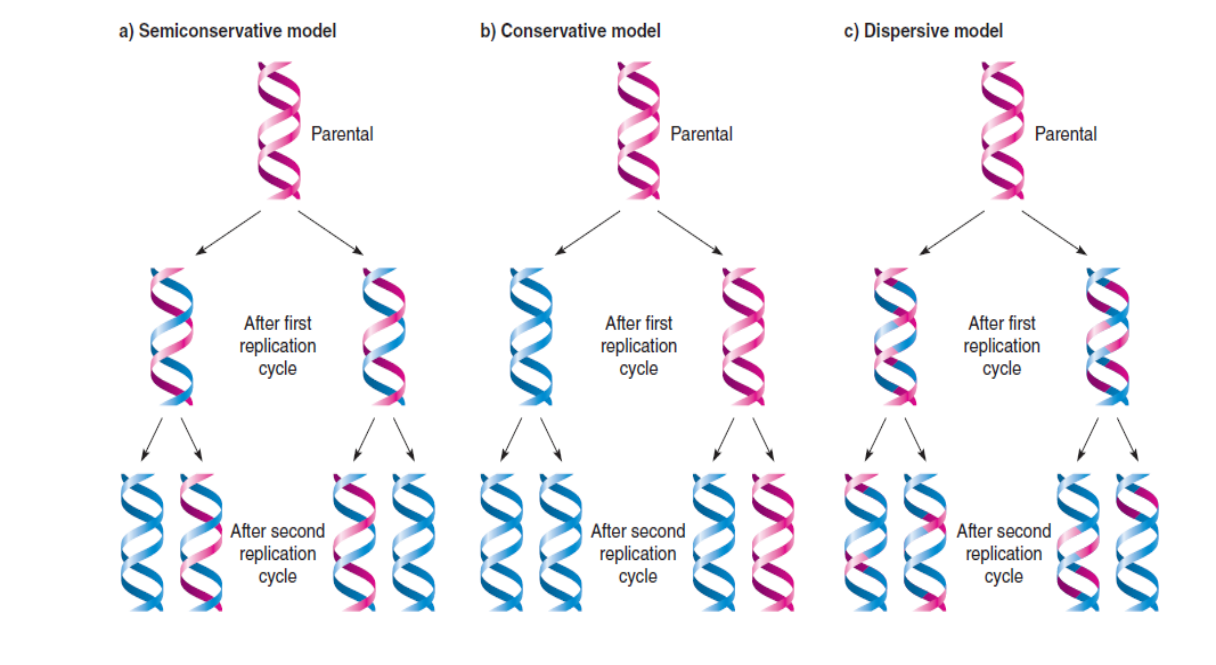
Conservative model : In this model, one of the two progeny DNA molecules is the

parental double-stranded DNA molecule, and the other consists entirely of new material.

Dispersive model : The parental double helix is cleaved into double-stranded DNA segments that act as templates for the synthesis of new double-stranded DNA segments.

Three models for DNA replication. Parental strands are shown in red, and the newly

synthesized strands are shown in blue.



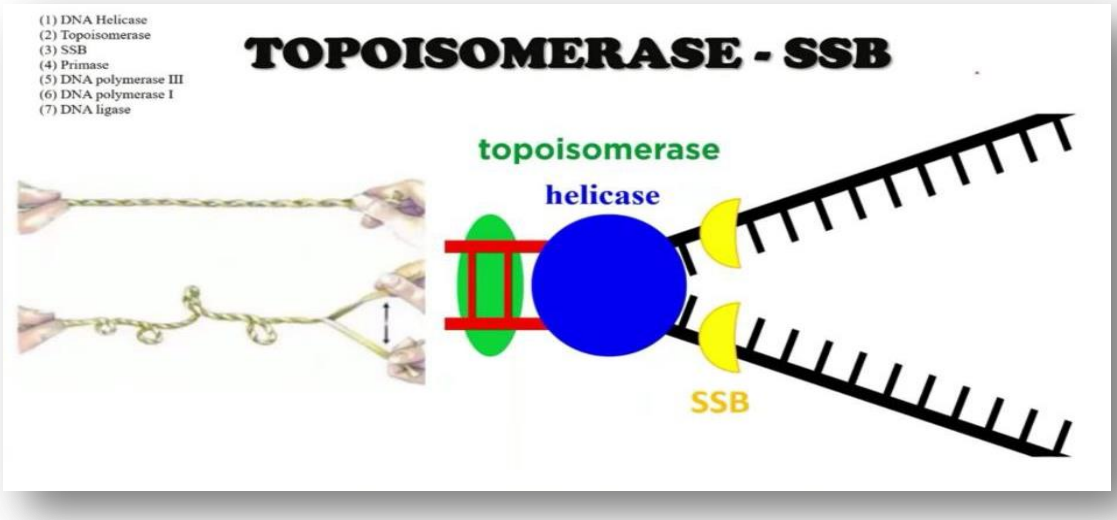
The initiation of replication is directed by a DNA sequence called the **replicator**. The replicator usually includes **the origin of replication**, the specific region where the DNA double helix denatures into single strands. The locally denatured segment of DNA is called a **replication bubble**.

At the molecular level, **several enzymes** and proteins participate in the semiconservative replication of the new DNA strands.

The major events in DNA replication include:

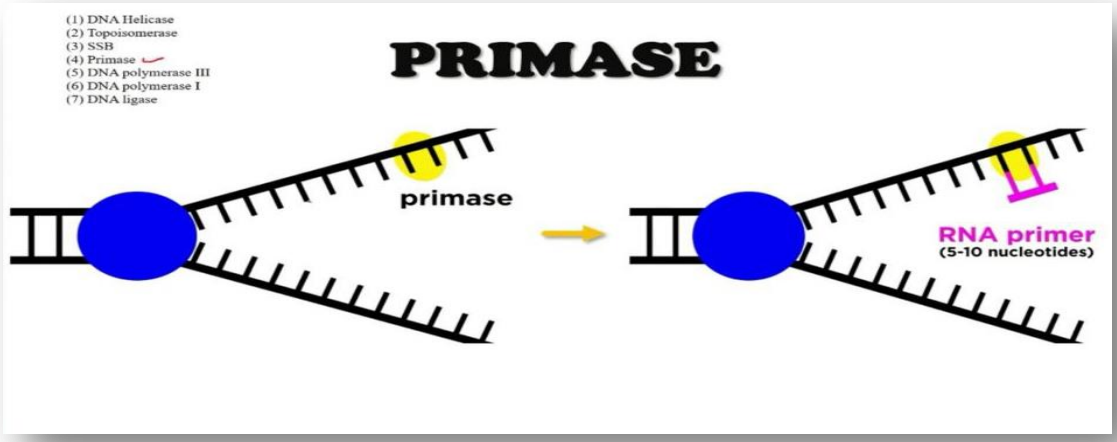
1. The enzyme **DNA helicase** unwinds and "unzips" double stranded DNA by breaking the weak hydrogen bonds between the paired bases.

2. **Single-strand DNA-binding (SSB)** proteins bind to each single-stranded DNA, stabilizing them and preventing them from reforming double-stranded DNA by complementary base pairing (a process called re annealing)



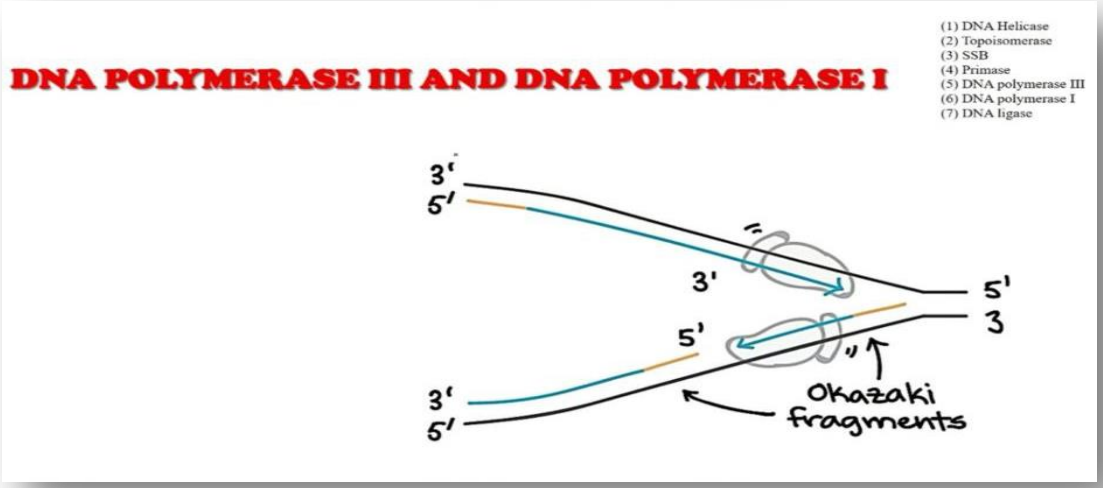
3- The RNA primase (which is a modified RNA polymerase) synthesizes a short RNA primer (about 5–10 nucleotides) to which new nucleotides are added by DNA polymerase. The RNA primer is removed later and replaced with DNA

***A short piece of RNA, called primer RNA because it primes DNA synthesis, is needed before DNA chain formation can begin.**



4- DNA polymerase III: The actual replicating enzyme that synthesizes DNA.Using the RNA primer .DNA polymerase adds deoxyribonucleotides to the primer, thereby elongating the chain. As the enzyme moves along the parent strand, it "reads" each parent nucleotide

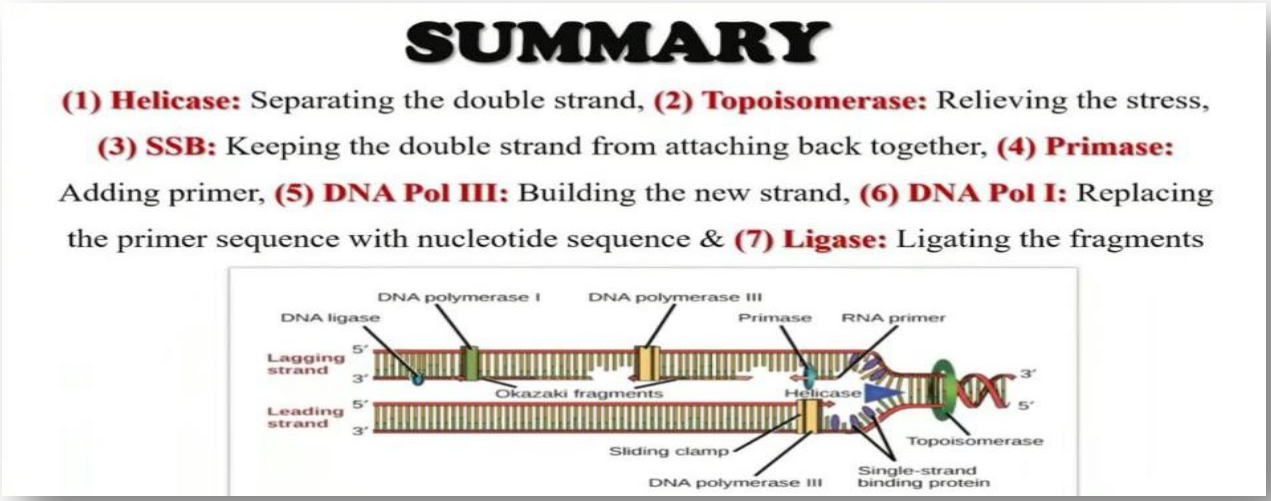
and add new nucleotide to the growing DNA chain. Synthesis is always in the 5' to 3' direction.

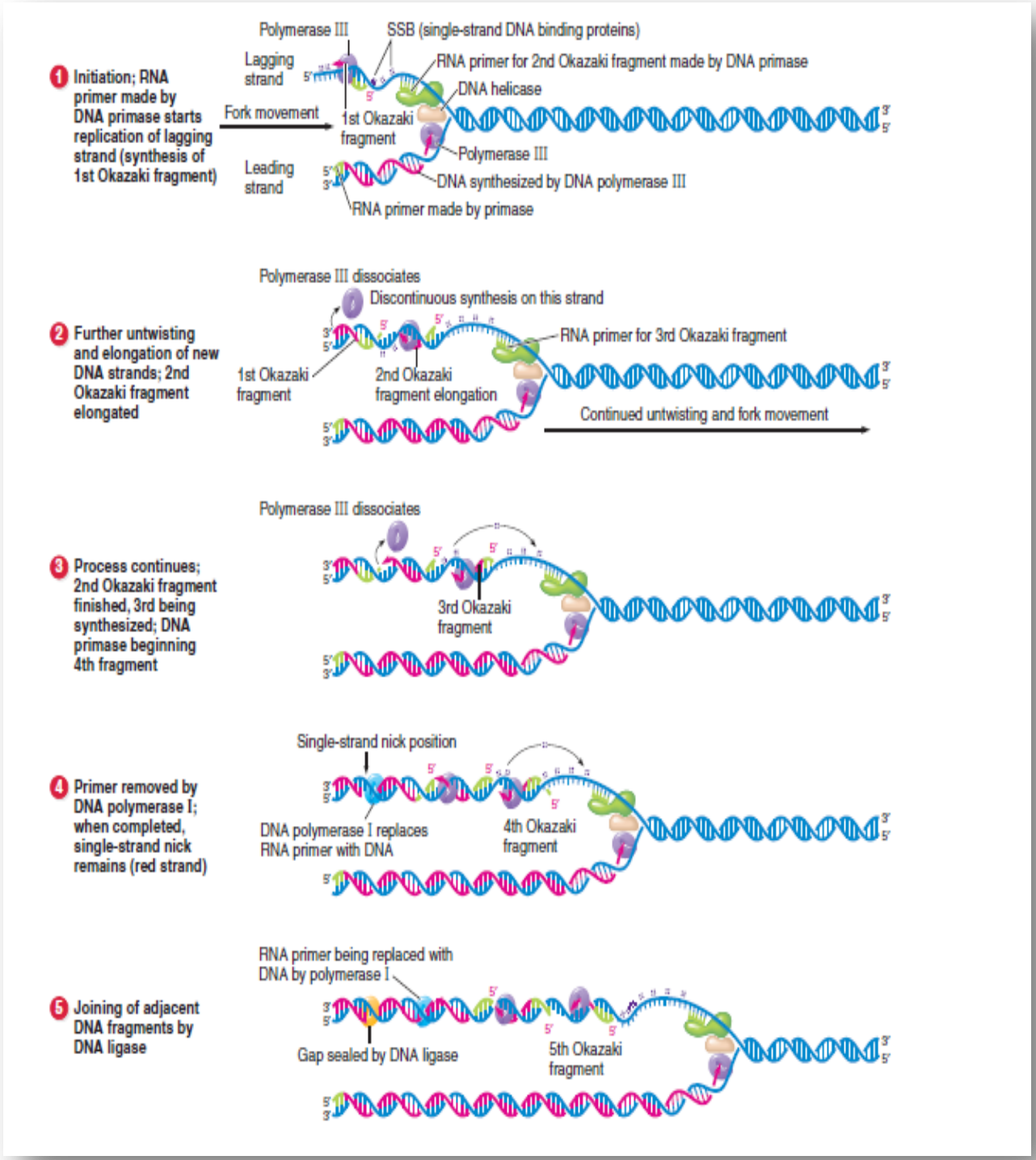


* DNA synthesis process has to do with the way in which each new strand is synthesized. In this process, one strand is elongated by the **continuous** additions of nucleotides to the 3' end, this newly synthesized DNA is called the **leading strand**. while the other new strand is produced by repeated synthesis of primer RNAs and short lengths of DNA (Okazaki fragments). This latter method is called **discontinuous** synthesis of the **lagging strand**

5- DNA polymerase I: Removes the RNA primers and replaces them with DNA.

6- DNA ligase joined the Okazaki fragments into a continuous DNA strand.




1 Initiation; RNA primer made by DNA primase starts replication of lagging strand (synthesis of 1st Okazaki fragment)
Polymerase III
SSB (single-strand DNA binding proteins)
Lagging strand
RNA primer for 2nd Okazaki fragment made by DNA primase
DNA helicase
Fork movement
1st Okazaki fragment
Polymerase III
Leading strand
DNA synthesized by DNA polymerase III
RNA primer made by primase
2 Further untwisting and elongation of new DNA strands; 2nd Okazaki fragment elongated
Polymerase III dissociates
Discontinuous synthesis on this strand
RNA primer for 3rd Okazaki fragment
1st Okazaki fragment
2nd Okazaki fragment elongation
Continued untwisting and fork movement
3 Process continues; 2nd Okazaki fragment finished, 3rd being synthesized; DNA primase beginning 4th fragment
Polymerase III dissociates
3rd Okazaki fragment
4 Primer removed by DNA polymerase I; when completed, single-strand nick remains (red strand)
Single-strand nick position
DNA polymerase I replaces RNA primer with DNA
4th Okazaki fragment
5 Joining of adjacent DNA fragments by DNA ligase
RNA primer being replaced with DNA by polymerase I
Gap sealed by DNA ligase
5th Okazaki fragment

Molecular biology

Dr. TAYF RAZAQ

The structure, function, development, and reproduction of an organism depend on the properties of the proteins present in each cell and tissue. When a protein is needed in the cell, the genetic code for the amino acid sequence of that protein must be read from the DNA and the protein made by Gene Expression.

Gene Expression: The first step in gene expression is called *transcription*, and the second step is called *translation*

RNA (Ribonucleic Acid) RNA is a polymer composed of nucleotides. Each nucleotide within the RNA structure includes a nitrogenous base, a ribose sugar and a phosphate. RNA is involved in many important biological processes.

General Features of RNA:

In nature, there are essentially two kinds of nucleic acid including DNA and RNA. RNA is different in many respects from DNA:

1. RNA has a single-stranded nucleotide chain structure. It is not like the double-stranded structure of DNA. This allows more flexibility of RNA and formation of complexes in a much greater variety, in three-dimensions.

2. RNA comprises ribose sugar in its nucleotides instead of deoxyribose sugar present in DNA.

3. RNA nucleotides (ribonucleotides) comprises adenine, guanine, cytosine bases and uracil base instead of thymine base in DNA.

4. RNA can catalyze important biological reactions similar to proteins but DNA cannot. RNA molecules functioning such as protein enzymes is called ribozyme.

There are four main types of RNA molecules, each encoded by its own type of gene, but only one of them is translated:

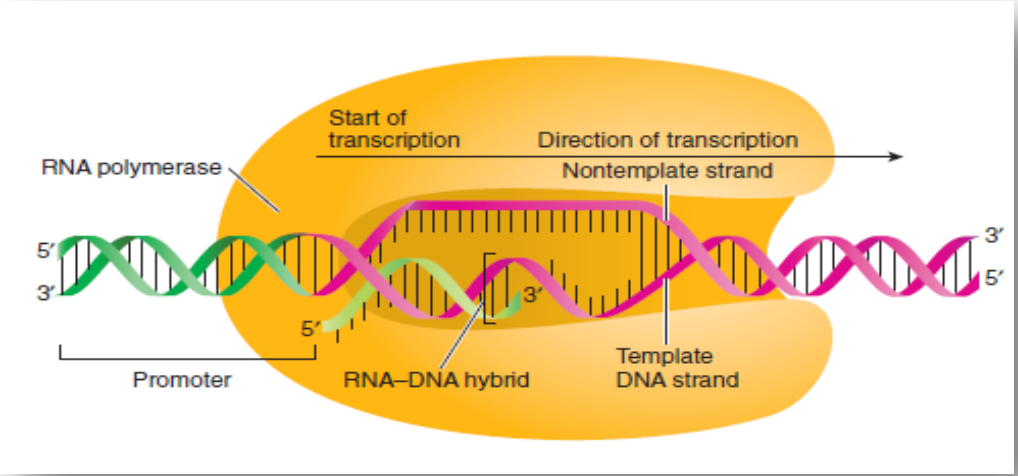
1. mRNA (messenger RNA) encodes the amino acid sequence of a polypeptide. mRNAs are the transcripts of *protein-coding genes*. Translation of an mRNA produces a polypeptide.

2. rRNA (ribosomal RNA), with ribosomal proteins, makes up the ribosomes—the structures on which mRNA is translated.

3. tRNA (transfer RNA) brings amino acids to ribosomes during translation.

4. snRNA (small nuclear RNA), with proteins, forms complexes that are used in eukaryotic RNA processing to produce functional mRNAs.

Transcription: It is the process by which, the nucleotide sequence of one strand of DNA is transcribed into a complementary molecule of RNA (messenger RNA, mRNA). The DNA helix is opened by a complex set of proteins. The DNA strand in the 3' to 5' direction (noncoding, template, antisense or minus strand) serves as the template for the transcription of DNA into RNA by RNA polymerase. RNA is synthesized in the 5' to 3' direction . The transcription occurs in three phases - initiation, elongation and termination



1- **Initiation**: RNA polymerase binds to specific DNA sequences called promoters to initiate RNA synthesis to the 3' side of the promoter. The RNA polymerase binds to the dsDNA at a promoter sequence, resulting in local DNA unwinding. The position of the first synthesized base of the RNA is called the start site .

2- **Elongation**: RNA polymerase moves along the DNA and sequentially synthesizes the RNA chain. DNA is unwound ahead of the moving polymerase, and the helix is reformed behind it.

3- **Termination**: RNA polymerase recognizes the terminator which causes no further ribonucleotides to be incorporated.

Translation: The Process of Protein Synthesis Polypeptide synthesis takes place on ribosomes, where the genetic message encoded in mRNA is translated. The mRNA is translated in the 5-to-؟ direction, and the polypeptide is made

The mechanism of translation can be split into three steps – initiation, elongation and termination

Initiation: involving the assembly of the ribosome subunits and the binding of the mRNA.

Elongation: where specific amino acids are used to form polypeptides, this being directed by the codon sequence in the mRNA.

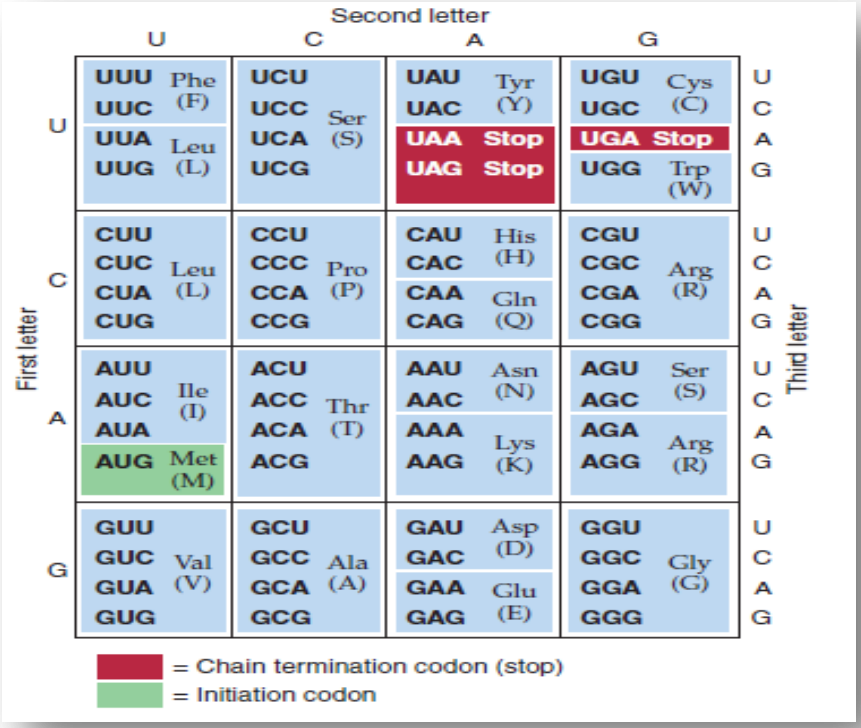
Termination: which involves the disassembly of the components of translation following the production of a polypeptide.

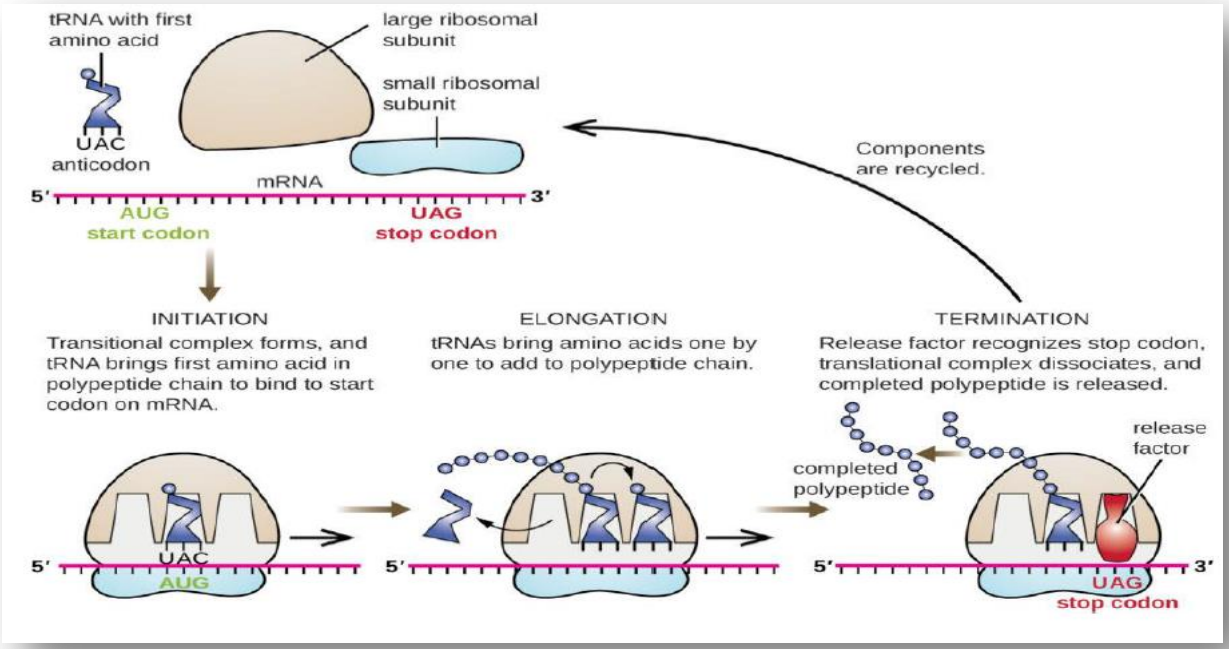
Structure and Function of Proteins

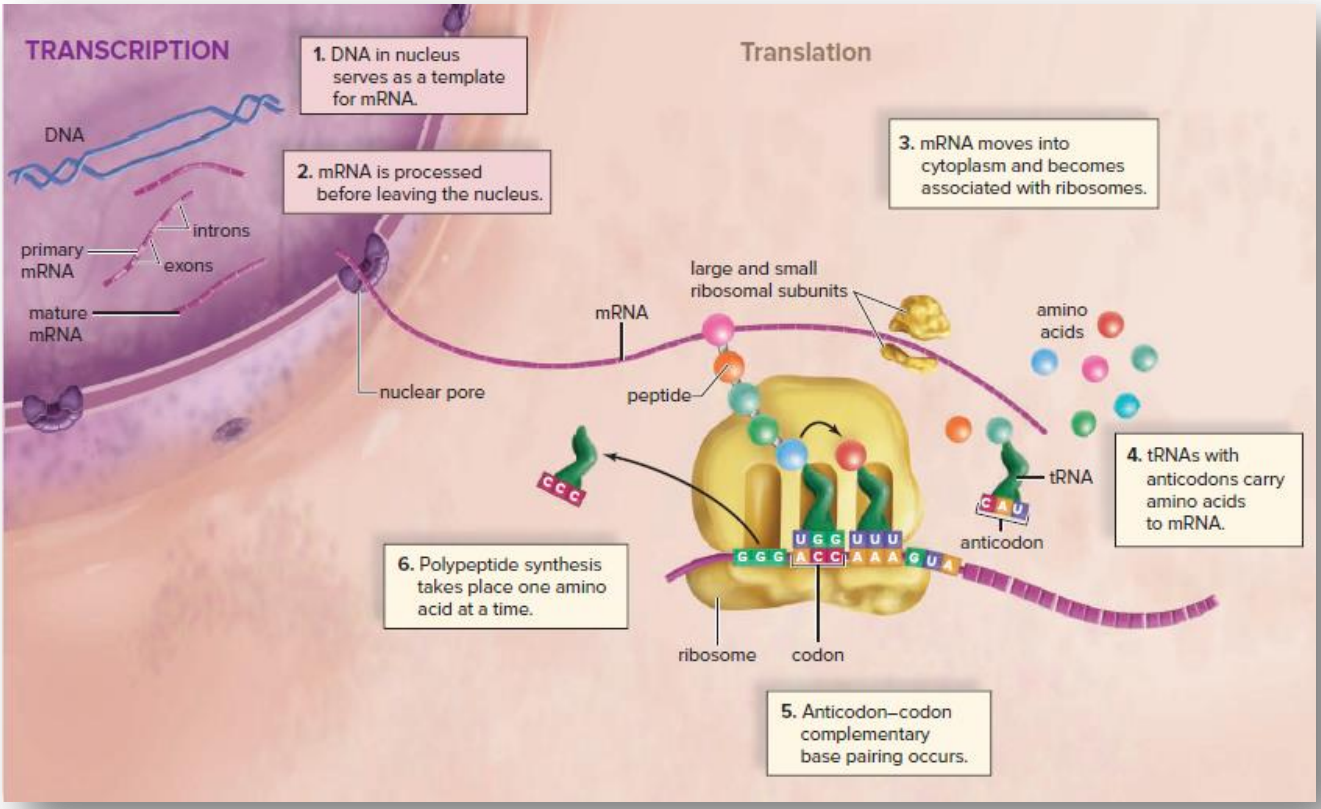
A protein is a high-molecular-weight, nitrogen-containing organic compound of complex shape and composition. A protein consists of one or more macromolecular subunits called polypeptides, which are composed of smaller building blocks: the amino acids. There are 20 different amino acids that may be found in most proteins. Proteins differ because the number and order of their amino acids differ. The sequence of amino acids in a protein leads to its particular shape .Proteins have many different functions in the body, as they determine the structure and function of various cells in the body. Proteins are used as structural and regulatory components of cells. They are used as enzymes to catalyze chemical reactions, antibodies for the immune system, and hormones to change activities of certain cells.

Transfer RNA During translation of mRNA, each transfer RNA (tRNA) brings a specific amino acid to the ribosome to be added to a growing polypeptide chain. The correct amino acid sequence of a polypeptide is achieved as a result of: (1) the binding of each amino acid to a specific tRNA; and (2) the binding between the codon of the mRNA and the complementary anticodon in the tRNA.

The genetic code corresponds to a three-base sequence in the mRNA molecule called a codon. Each codon represents a specific amino acid. However, the use of three bases allows for 64 possible codons, more than enough to code for the 20 amino acids.




tRNA with first amino acid
large ribosomal subunit
small ribosomal subunit
UAC
anticodon
mRNA
5′
3′
AUG
start codon
UAG
stop codon
Components are recycled.
INITIATION
Transitional complex forms, and tRNA brings first amino acid in polypeptide chain to bind to start codon on mRNA.
ELONGATION
tRNAs bring amino acids one by one to add to polypeptide chain.
TERMINATION
Release factor recognizes stop codon, translational complex dissociates, and completed polypeptide is released.
release factor
completed polypeptide
UAC
AUG
UAG
stop codon


TRANSCRIPTION
1. DNA in nucleus serves as a template for mRNA.
DNA
2. mRNA is processed before leaving the nucleus.
introns
primary mRNA
exons
mature mRNA
nuclear pore
Translation
3. mRNA moves into cytoplasm and becomes associated with ribosomes.
large and small ribosomal subunits
mRNA
amino acids
peptide
tRNA
anticodon
4. tRNAs with anticodons carry amino acids to mRNA.
6. Polypeptide synthesis takes place one amino acid at a time.
ribosome
codon
5. Anticodon–codon complementary base pairing occurs.

Mutations

Objectives:

1- To describe the Mutations.

2- To mention the Types of Mutations.

Mutations

A gene mutation is a permanent change in the DNA sequence that makes up a gene. Mutations range in size from a single DNA building block (DNA base) to a large segment of a chromosome.

The agents (physicals or chemicals) which can induce mutations are collectively known as mutagens. The changes that occur in DNA on mutation are reflected in replication, transcription and translation

Mutations are mainly of two major types: point mutations and frameshift mutations (Figure 9.1).

1. **Point mutations**: the replacement of one base pair by another results in point mutation. They are of two sub-types:

Transitions: in this case, a purine (or a pyrimidine) is replaced by another.

Transversions: these are characterized by replacement of a purine by a pyrimidine or vice versa.

. . . . .

2. **Frameshift mutations**: these occur when one or more base pairs are inserted in or deleted from the DNA, respectively, causing insertion or deletion mutations.

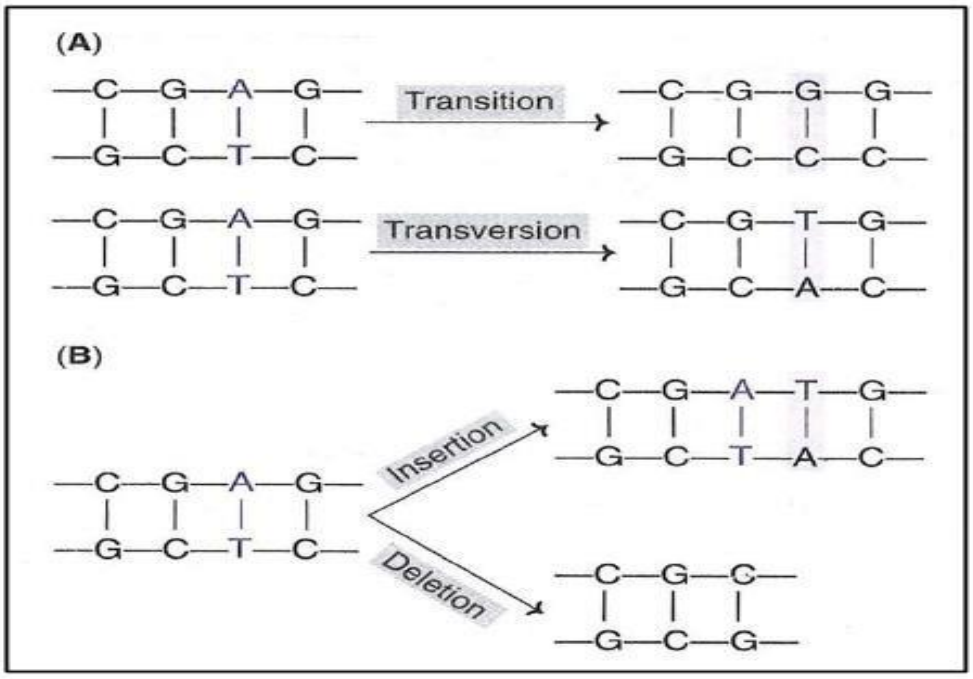


Figure 9.1: An illustration of mutations (A)-point mutations; (B)-Frameshift mutations

❖ **Consequences of point mutations**: the change in a single base sequence in point mutation may cause one of the following (Figure 9.2).

1. **Silent mutation**: The codon (of mRNA) containing the changed base may code for the same amino acid. For instance, UCA codes for serine and change in the third

base (UCU) still codes for serine. This is due to degeneracy of the genetic code. Therefore, there are no detectable effects in silent mutation.

2. **Missense mutation**: In this case, the changed base may code for a different amino acid. For example, UCA codes for serine while ACA codes for threonine. The mistaken (or missense) amino acid may be acceptable, partially acceptable or unacceptable with regard to the function of protein molecule. Sickle-cell anemia is classical example of a missense mutation.

For example: The point mutation that causes sickle cell disease is a missense mutation. The DNA sequence CTC encodes the mRNA codon GAG, which specifies glutamic acid. In sickle cell disease, the mutation changes the DNA sequence to CAC, which encodes GUG in the mRNA, specifying valine. This mutation changes the protein's shape, altering its function.

3. **Nonsense mutation**: Sometimes, the codon with the altered base may become a termination (or nonsense) codon. For instance, change in the second base of serine codon (UCA) may result in UAA. The altered codon acts as a stop signal and causes termination of protein synthesis, at that point.

For example, in factor XI deficiency, which is a blood clotting disorder, a GAA codon specifying glutamic acid is changed to UAA, signifying "stop." The shortened clotting factor cannot halt the profuse bleeding that occurs during surgery or from injury.

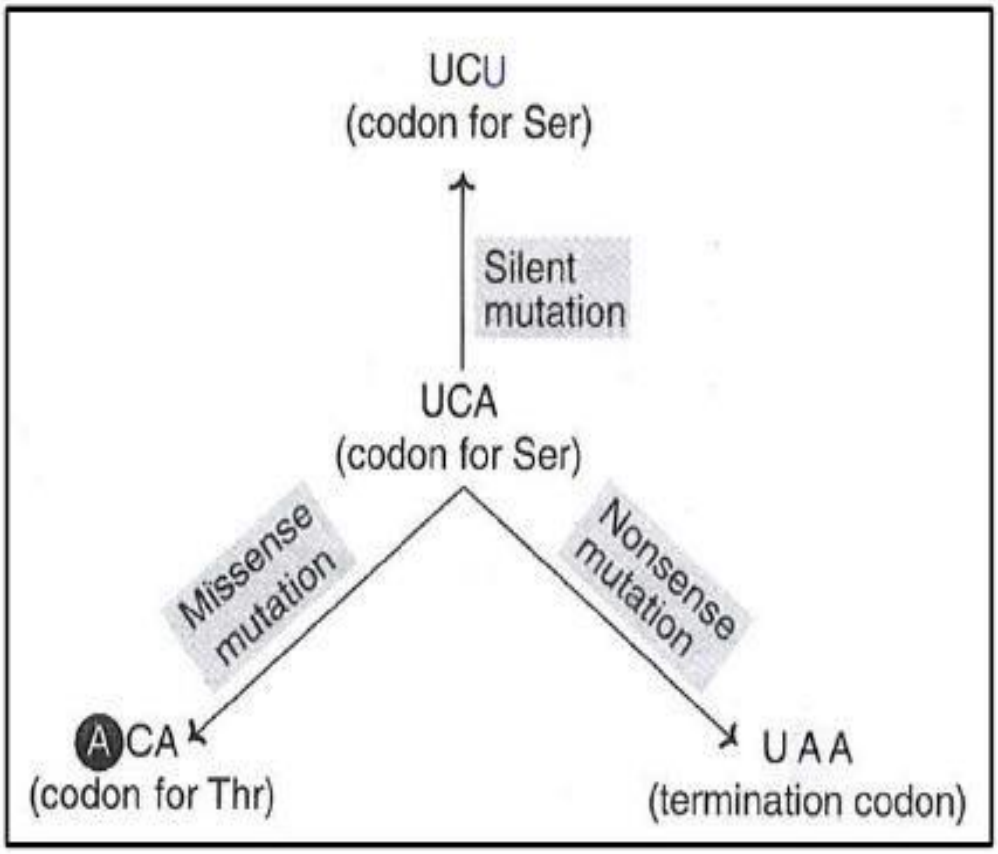


Figure 9.2: An illustration of point mutations (represent by a codon of mRNA)

Consequences of frameshift mutations: the insertion or deletion of a base in a gene results in an altered reading frame of the mRNA (hence the name frameshift) (figure 9.3). The machinery of mRNA (containing codon) does not recognize that a base was missing or a new base was added. Since there are no punctuations in the reading of codons, translation continues. The result is that the protein synthesized will have several altered amino acids and/or prematurely terminated protein.

•

1. Deletion Mutations

A **deletion mutation:** meaning removes DNA. Deletions range from a single DNA nucleotide to thousands of bases to large parts of chromosomes.

For example: Many common inherited disorders result from deletions, including male infertility caused by tiny deletions in the Y chromosome.

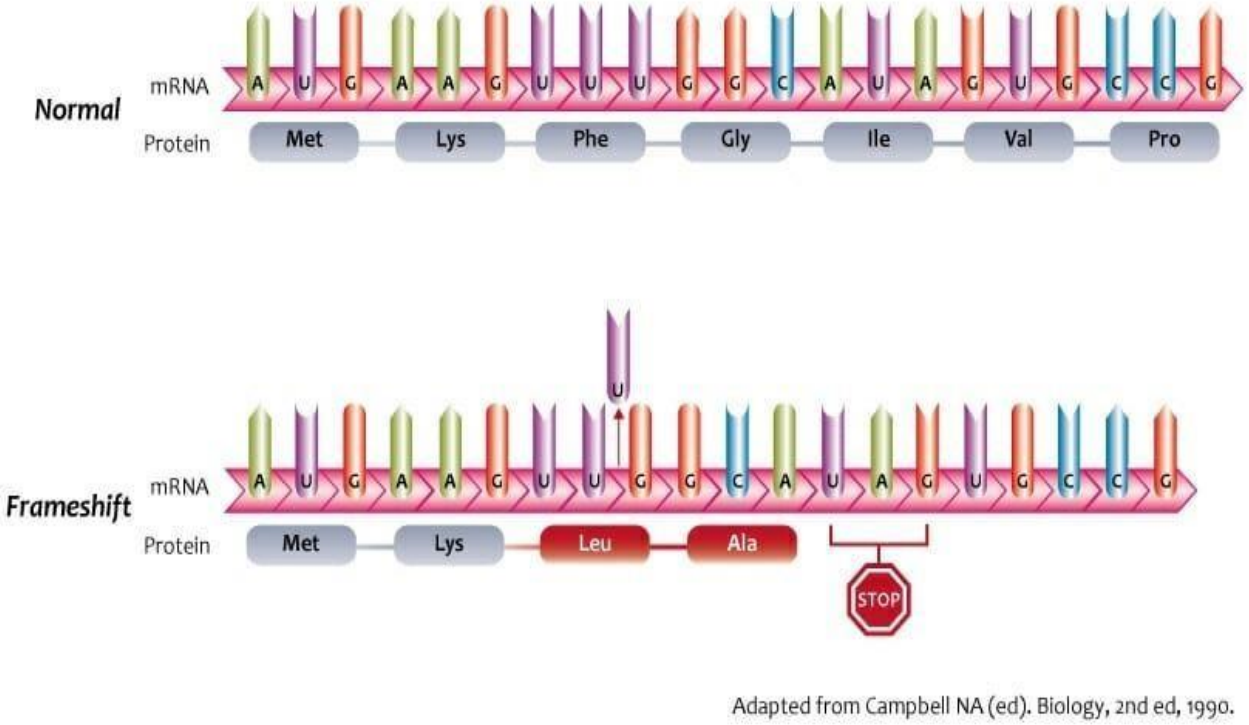
2. Insertion Mutations insertion

mutation: meaning **adds** DNA.

For example: In one form of Gaucher disease, an inserted single DNA base prevents production of an enzyme that normally breaks down glycolipids in lysosomes. The resulting buildup of glycolipid enlarges the liver and spleen and causes easily fractured bones and neurological impairment.

Adding or deleting a number of bases devastates a gene's function because it disrupts the gene's reading frame.

- **Mutations and Cancer**: mutations are permanent alterations in DNA structure, which have been implicated in the etiopathogenesis of cancer.



Adapted from Campbell NA (ed). Biology, 2nd ed, 1990.

Figure 9.3: Frameshift mutations

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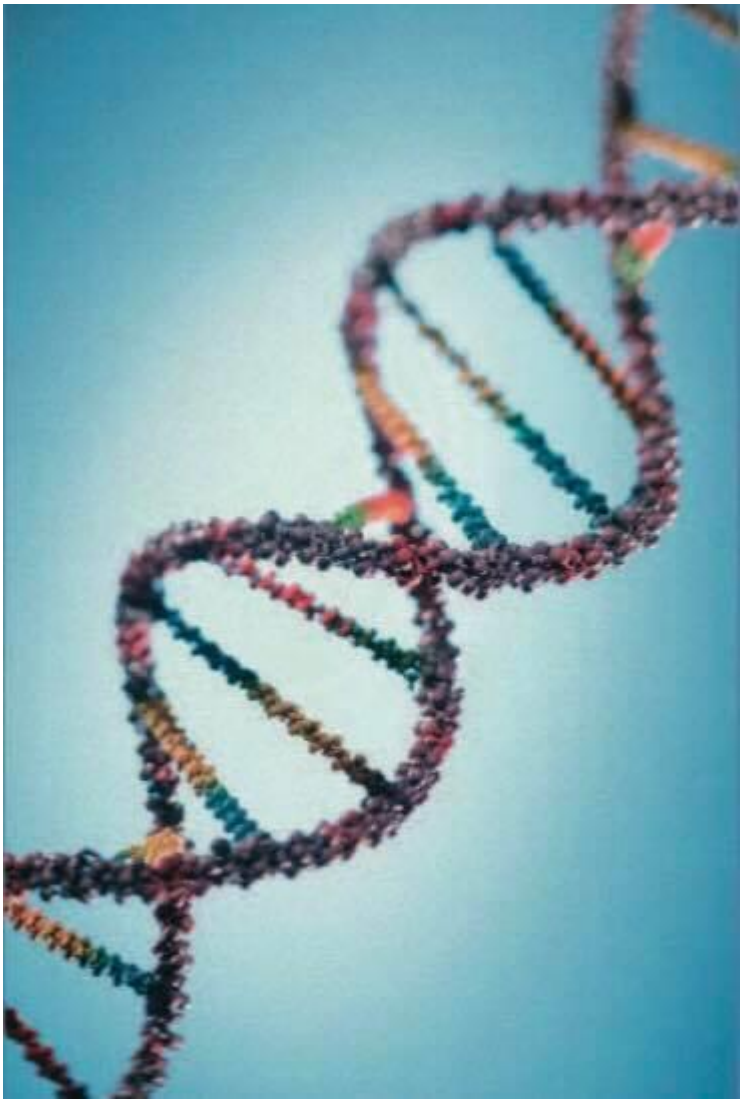
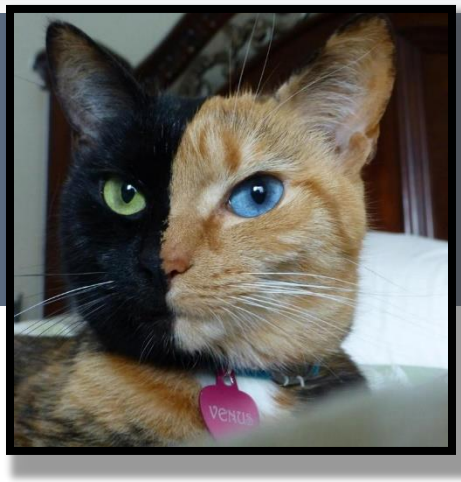


MOLECULAR BIOLOGY

SECOND CLASS

Haider

PART 1 Molecular Biology



Color Mutations in Animals

LECTURE 12

Gene Mutation

Lecture Contents

12.1 The Nature of Mutations

A **mutation** is a change in a DNA sequence that is rare in a population and typically affects the phenotype. “Mutate” refers to the process of altering a DNA sequence.

Mutations range from substitution of a single DNA base; to deletion or duplication of tens, hundreds, thousands, or even millions of bases; to missing or extra entire chromosomes.

Mutation can affect any part of the genome:

- Sequences that encode proteins
- control transcription
- introns; repeats

The effects of mutation vary.

- Mutations may impair a function
- have no effect
- beneficial

12.2 Causes of Mutation

A mutation can occur spontaneously or be induced by exposure to a chemical or radiation. An agent that causes mutation is called **a mutagen.**

- **Chemicals (Alkylating and Intercalating agents)**
- **ultraviolet Light**
- **Ionizing radiation (X rays, gamma rays, and cosmic rays)**

12.3 Types of Mutations

Mutations are classified by whether they remove, alter, or add a function, or by how they structurally alter DNA. The same single-gene disorder can result from different types of mutations.

Point Mutations:

A **point mutation** is a change in a single DNA base.

- It is a **transition** if a purine replaces a purine (A to G or G to A) or a pyrimidine replaces a pyrimidine (C to T or T to C).
- It is a **transversion** if a purine replaces a pyrimidine or vice versa (A or G to T or C).

Addition or deletion of a single DNA base is also considered to be a point mutation. A point mutation can have any of several consequences—or it may have no obvious effect at all on the phenotype, acting as a silent mutation.

A. Missense Mutations

A point mutation that changes a codon that normally specifies a particular amino acid into one that codes for a different amino acid is called a **missense mutation**. About a third of missense mutations harm health.

For example: The point mutation that causes sickle cell disease **(Figure 12.1)** is a missense mutation. The DNA sequence CTC encodes the mRNA codon GAG, which specifies glutamic acid. In sickle cell disease, the mutation changes the DNA sequence to CAC, which encodes GUG in the mRNA, specifying valine. This mutation changes the protein's shape, altering its function.

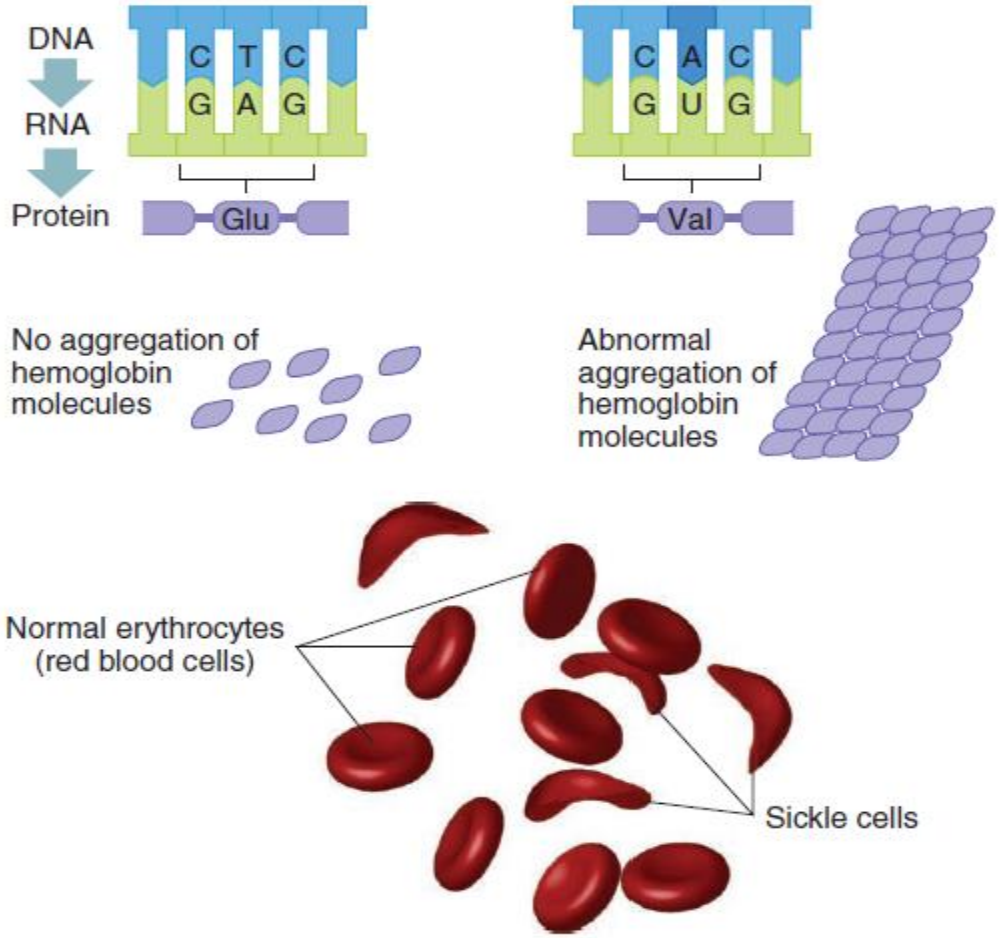


Figure 12.1 Sickle cell disease results from a single DNA base change that substitutes one amino acid in the protein (valine replaces glutamic acid). This changes the surfaces of the molecules, and they aggregate into long, curved rods that deform the red blood cell. The illustration

B. Nonsense Mutation

A point mutation that changes a codon specifying an amino acid into a "stop" codon—UAA, UAG, or UGA in mRNA—is a **nonsense mutation**. It shortens the protein product, which can greatly influence the phenotype.

For example, in factor XI deficiency, which is a blood clotting disorder, a GAA codon specifying glutamic acid is changed to

UAA, signifying "stop." The shortened clotting factor cannot halt the profuse bleeding that occurs during surgery or from injury.

However, what happen in the case of the opposite of a nonsense mutation; a normal stop codon mutates into a codon that specifies an amino acid?

C. Silent Mutation: is a point mutation alters a codon but does not result in a change in the amino acid at that position in the protein (due to degeneracy of the genetic code).

D. Deletion Mutations

A **deletion mutation:** meaning removes DNA. Deletions range from a single DNA nucleotide to thousands of bases to large parts of chromosomes.

For example: Many common inherited disorders result from deletions, including male infertility caused by tiny deletions in the Y chromosome.

E. Insertion Mutations

insertion mutation: meaning **adds** DNA.

For example: In one form of Gaucher disease, an inserted single DNA base prevents production of an enzyme that normally breaks down glycolipids in lysosomes. The resulting buildup of glycolipid enlarges the liver and spleen and causes easily fractured bones and neurological impairment.

Adding or deleting a number of bases devastates a gene's function because it disrupts the gene's reading frame.

- **reading frame:** which refers to the nucleotide position where the DNA begins to encode protein. Most exons are "readable" (have no stop codons) in only one of the three possible reading frames.
- A reading frame that is readable in that it is translatable into protein is called an **open reading frame**.
- A change that alters the reading frame is called a **frameshift mutation.**

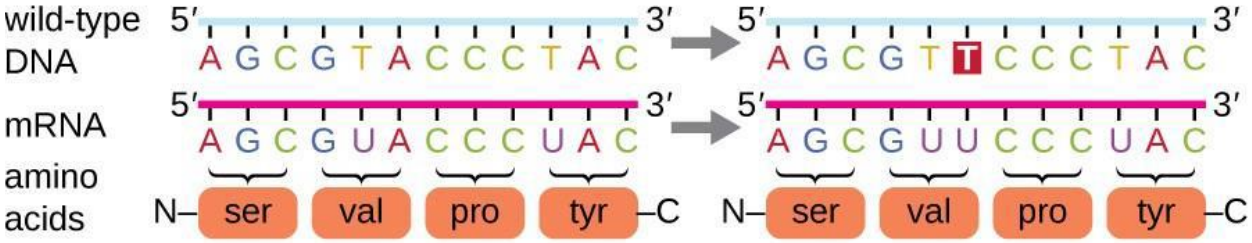
Table 12.1 Types of Mutations

A sentence comprised of three-letter words is analogous to the effect of mutations on a gene's DNA sequence:

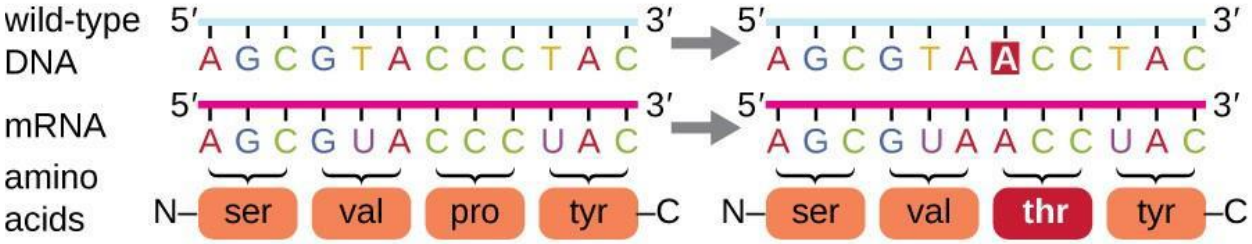
Normal	THE ONE BIG FLY HAD ONE RED EYE
Missense	THQ ONE BIG FLY HAD ONE RED EYE
Nonsense	THE ONE BIG
Frameshift	THE ONE QBI GFL YHA DON ERE DEY
Deletion	THE ONE BIG HAD ONE RED EYE
Insertion	THE ONE BIG WET FLY HAD ONE RED EYE
Duplication	THE ONE BIG FLY FLY HAD ONE RED EYE

point mutation: substitution of a single base

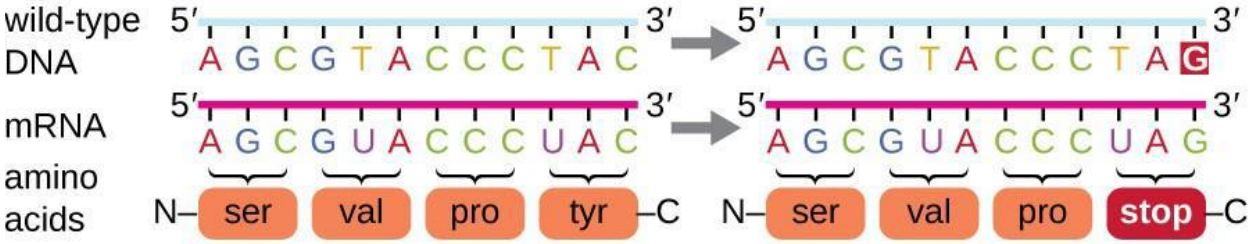
silent: has no effect on the protein sequence



missense: results in an amino acid substitution

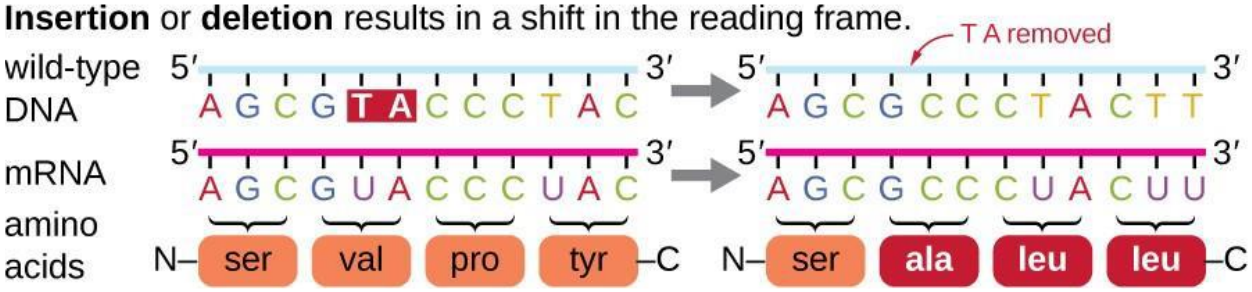


nonsense: substitutes a stop codon for an amino acid



frameshift mutation: insertion or deletion of one or more bases

Insertion or **deletion** results in a shift in the reading frame.



12.4 Summary

1. A **mutation** is a change in a gene's nucleotide base sequence that is rare in a population and can cause a **mutant** phenotype. A polymorphism is a more common and typically less harmful genetic change.

2. A mutation disrupts the function or abundance of a protein or introduces a new function. Most loss-of-function mutations are recessive, and most gain-of-function mutations are dominant.

3. A spontaneous mutation arises due to chemical phenomena or to an error in DNA replication.

4. Mutagens are chemicals or radiation that delete, substitute, or add bases. An organism may be exposed to a mutagen intentionally, accidentally, or naturally.

5. A **point mutation** alters a single DNA base. It may be a **transition** (purine to purine or pyrimidine to pyrimidine) or a **transversion** (purine to pyrimidine or vice versa). A **missense mutation** substitutes one amino acid for another, while a **nonsense mutation** substitutes a "stop" codon for a codon that specifies an amino acid, shortening the protein product. Splice-site mutations add or delete amino acids.

6. A **deletion mutation** removes genetic material and an **insertion mutation** adds it. A **frameshift mutation** alters the sequence of amino acids (**reading frame**). A **tandem duplication** is a copy of a gene next to the original.

12.5 Study Questions

1. Explain how a mutation causes sickle cell disease.
2. Distinguish between
   a. a transition and transversion.
   b. a missense mutation and nonsense mutation.
3. List two types of mutations that can alter the reading frame.
4. List ways that DNA can mutate without affecting the phenotype.

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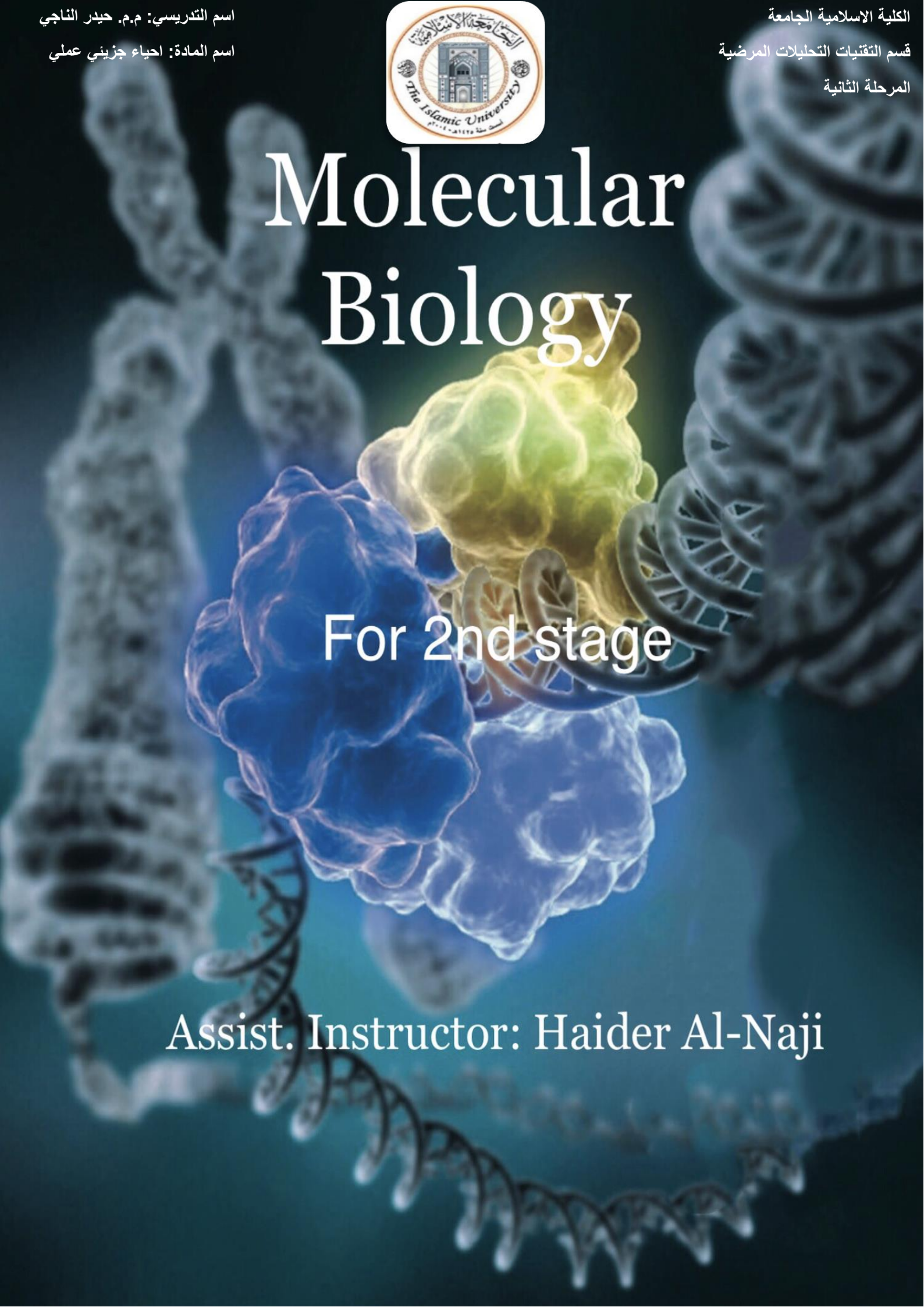
قسم التقنيات التحليلات المرضية
المرحلة الثانية

اسم التدريسي: م.م. حيدر الناجي
اسم المادة: احياء جزيئي عملي

Molecular Biology



For 2nd stage

Assist. Instructor: Haider Al-Naji

الكلية الاسلامية الجامعة
قسم التقنيات التحليلات المرضية
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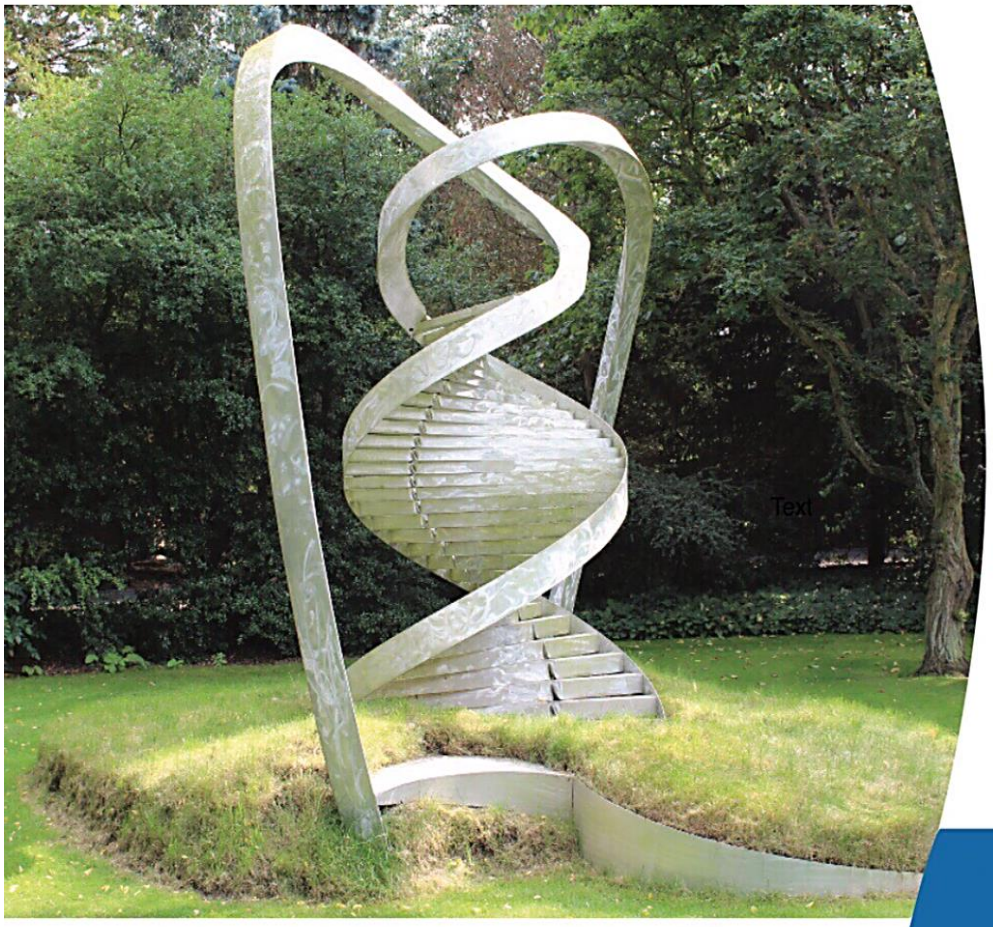
عنوان المحاضرة: مقدمة للاحياء الجزيئي

1

Introduction to Molecular Biology



This sculpture of DNA stands in the garden of Clare College Memorial Court at the University of Cambridge, England. It was erected to honor the discovery of DNA structure by Francis Crick and James Watson working at the University of Cambridge (Watson lived in Clare College Memorial Court during his time in Cambridge), as well as to honor the contributions of Rosalind Franklin and Maurice Wilkins working at Kings College, London.

LECTURE OUTLINE

1.1 Introduction

On April 25,1953, in British journal *Nature*, a paper, two columns in length, appeared. It was entitled "Molecular Structure of Nucleic acids: A Structure for Deoxyribose Nucleic Acid" and was authored by the American Watson and Crick. Watson and Crick's paper accurately described the molecular structure of DNA as a double helix composed of two strands of DNA with an invariant sugar-phosphate backbone on the the outside and nucleotide bases—adenine, thymine, guanine, and cytosine—arrayed in complementary base pairs that orient themselves toward the center of the molecule. This scientific paper paved the way to generated new discipline called *Molecular Biology*.

Molecular Biology: the branch of biology that deals with the nature of biological phenomena at the molecular level through the study of DNA and RNA, proteins, and other macromolecules involved in genetic information and cell function.

1.2 Chemical Composition of DNA and RNA

Two types of nucleic acids are important to the cell. The first is deoxyribonucleic acid (DNA), which carries all of the genetic information for an organism. The second type is ribonucleic acid (RNA), which is responsible for interpreting the genetic information into proteins that will carry out the essential cellular functions.

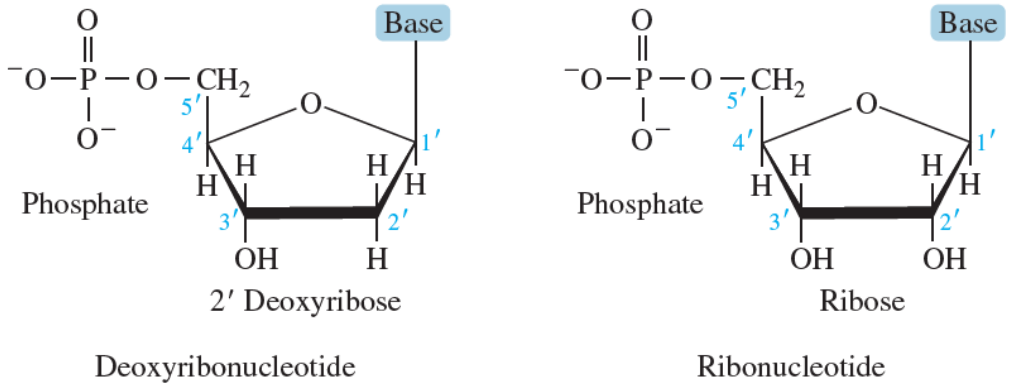
The components of these Nucleic Acids include

1. a five-carbon sugar,
   The sugar in DNA is 2-deoxyribose and the sugar in RNA is ribose. These differ only in the absence of a hydroxyl group at the carbon-2 position of 2-deoxyribose
2. phosphate
3. one of five heterocyclic amines called nitrogenous bases.
   The nitrogenous bases are divided into two families known as **pyrimidines** and **purines**

The pyrimidine bases in DNA are cytosine (C) and thymine (T), whereas in RNA are cytosine (C) and uracil (U)

The major purines of both DNA and RNA are adenine (A) and guanine (G).

These chemical components (either ribose or deoxyribose, one of the five nitrogenous bases, and one or more phosphoryl groups) constitute the Nucleotide, is the building Blocks of Nucleic Acids



1.3 DNA Structure: The Double Helix

A single strand of DNA is a polymer of nucleotides bonded to one another by 3′ –5′ phosphodiester bonds.

Hydrogen bonds, noncovalent bonds, form between complementary base pairs to join the two DNA strands into a double helix, at the basis of Chargaff's rule (C-G) and (A-T).

Complementary strands of DNA are **antiparallel**, meaning that the polarities of the complementary strands run in opposite directions—one strand is oriented 5′ to 3′ and the complementary strand is oriented 3′ to 5′

1.4 RNA Structure

The sugar-phosphate backbone of RNA consists of ribonucleotides, also linked by 3′ – 5′ phosphodiester bonds. These phosphodiester bonds are identical to those found in DNA. However, RNA molecules differ from DNA molecules in three basic properties.

- RNA molecules are usually *single-stranded.*
- The sugar-phosphate backbone of RNA consists of *ribonucleotides* linked by 3′ – 5′ phosphodiester bonds. Thus, the sugar *ribose* is found in place of 2-deoxyribose.

- The nitrogenous base *uracil* (U) replaces thymine (T).

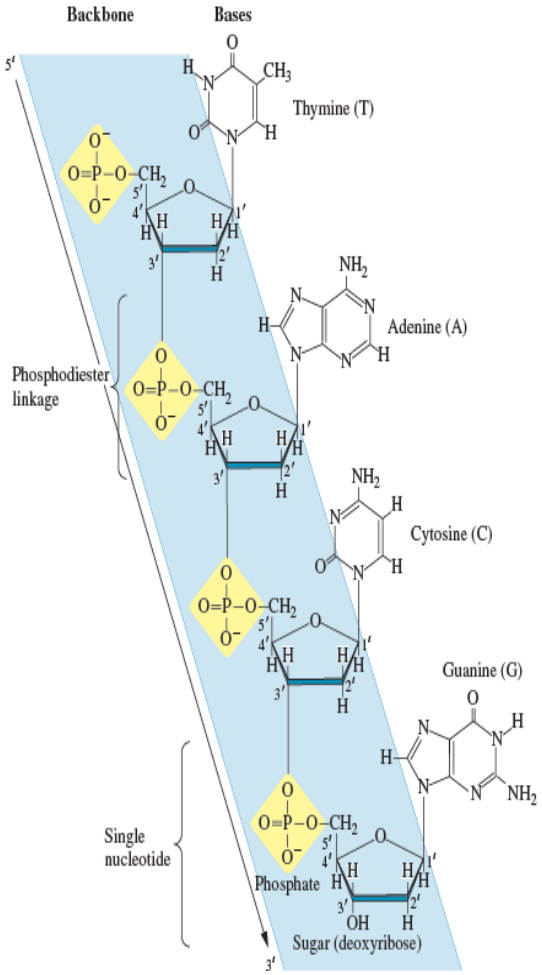
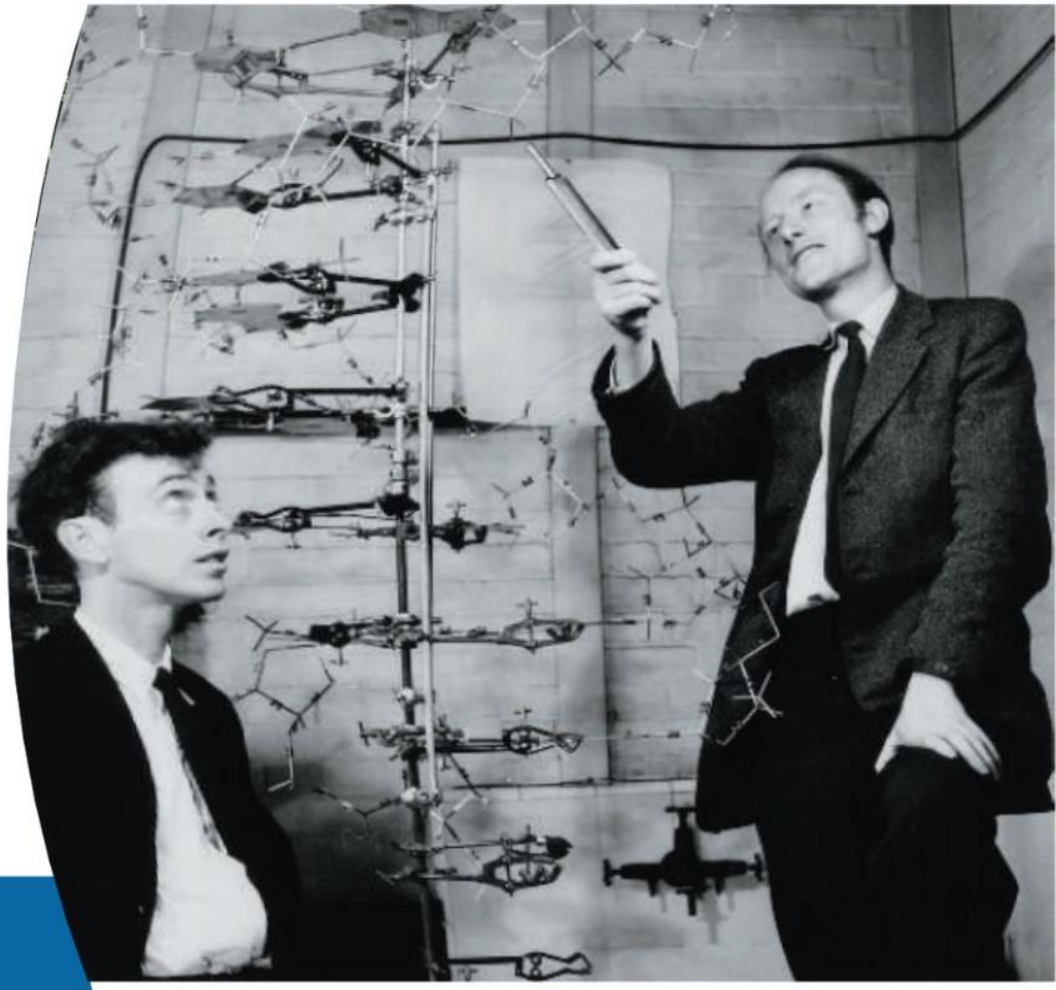


Figure 20.4 The covalent, primary structure of DNA.

2 DNA Is the Hereditary Molecule of Life

LECTURE OUTLINE



James Watson (left) and Francis Crick (right) in 1953 with their cardboard-and-wire model of DNA.

2.1 DNA Is the Hereditary Molecule of Life

When scientists speak of the "hereditary molecule" of a species, they mean the molecular substance that carries and conveys the species' genetic information.

2.2 Experimental Evidence for DNA as the Genetic Material

At the beginning of the twentieth century, geneticists did not know that DNA was the genetic material.

2.2.1 Chemical studies locate DNA in chromosomes

In 1869, Friedrich Miescher extracted a weakly acidic, phosphorus-rich material from the nuclei of human white blood cells and named it "nuclein."

A procedure first reported in 1923 made it possible to discover where in the cell DNA resides, The reaction shows that DNA is localized almost exclusively within chromosomes.

2.2.2 Bacterial transformation implicates DNA as the genetic material

The phenomenon of transformation In 1928, Griffith published the astonishing finding that genetic information from dead bacterial cells could somehow be transmitted to live cells. He was working with two types of the *S. pneumoniae* bacteria

S strain: formed colonies that were rounded and smooth because they synthesize a polysaccharide capsule that surrounds pairs of cells the polysaccharide capsule helps protect the bacteria from an animal's immune response, the S bacteria are virulent and kill most laboratory animals exposed to them.

R strain: When grown in a petri dish, the R bacteria formed colonies rough appearance (which arise spontaneously as mutants of S, are unable

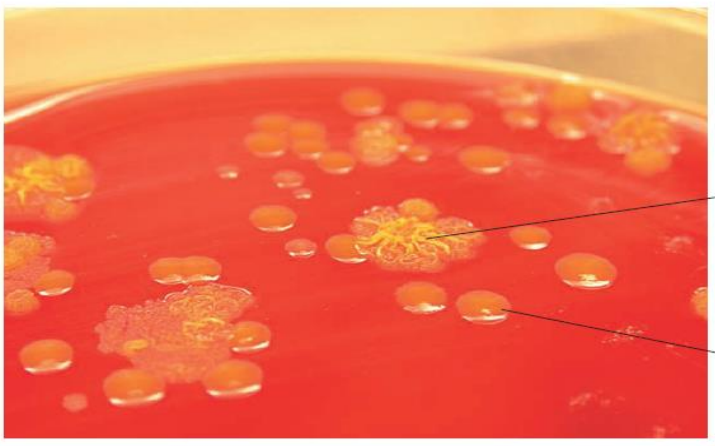
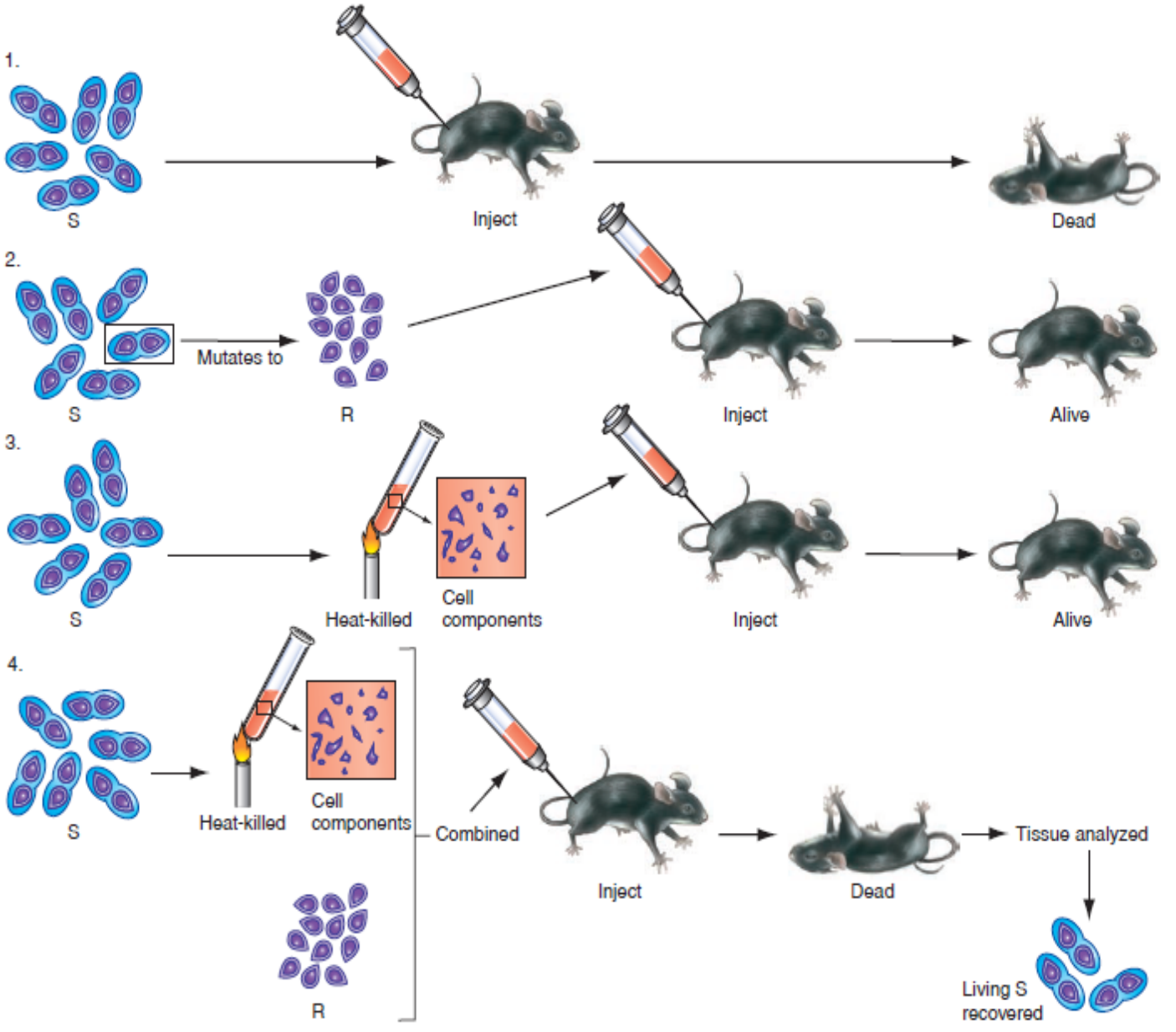


Figure 6.3 Griffith's demonstration of bacterial transformation. Smooth (S) and rough (R) colonies of *S. pneumoniae*.

to make the capsular polysaccharide, and as a result, their colonies appear to have a rough surface) thus, R bacteria were nonvirulent.

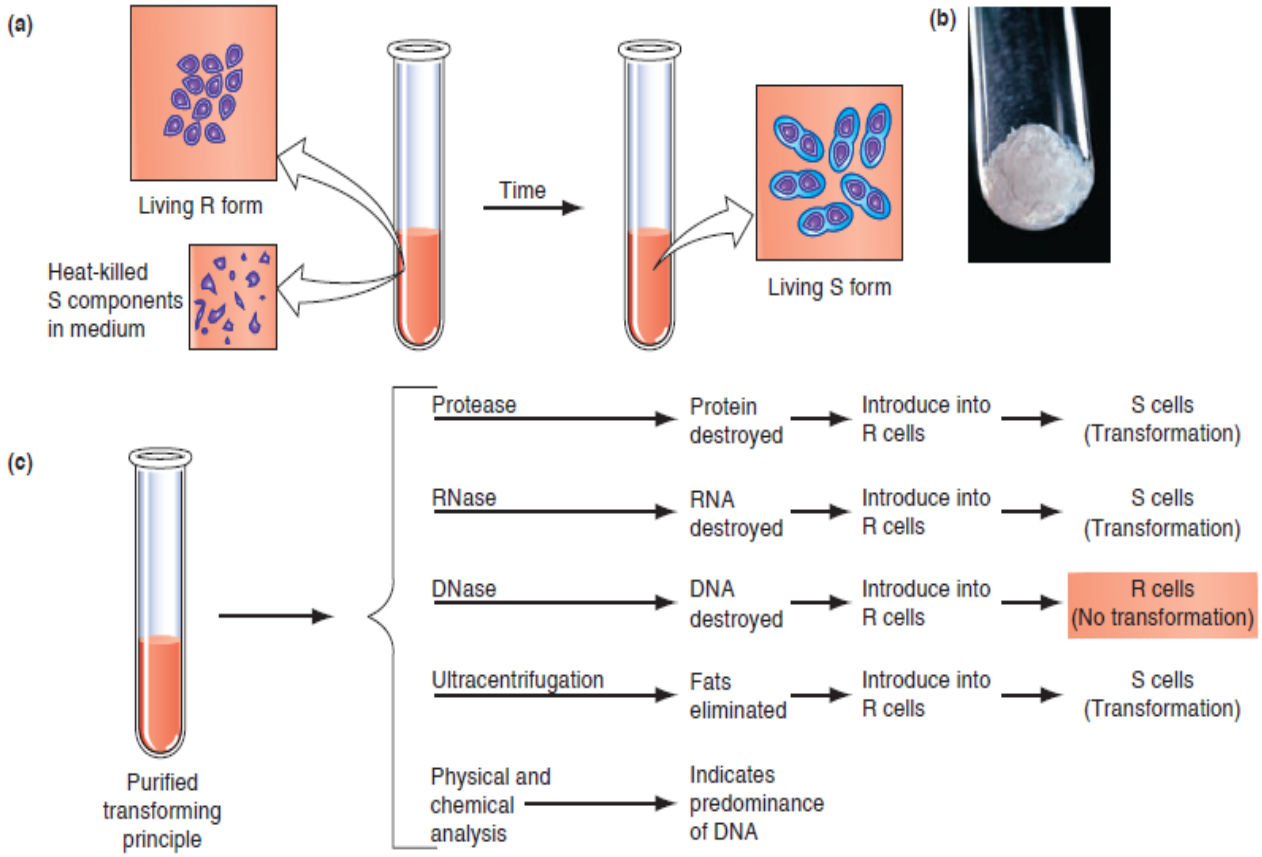
Griffith's experiment: live R forms and heat-killed S forms. Neither the heat-killed S forms nor the live R forms produced infection when injected into laboratory mice (Fig. 6.4.2 and 3); but a mixture of the two killed the animals (Fig. 6.4.4). Furthermore, bacteria recovered from the blood of the dead animals were living S forms (Fig. 6.4.4). The ability of a substance to change the genetic characteristics of an organism is known as **transformation.** Something from the heat-killed S bacteria must have transformed the living R bacteria into S. This transformation was permanent and most likely genetic, because all future generations of the bacteria grown in culture were the S form.

Figure 6.4 Griffith's experiment: (1) S bacteria are virulent and can cause lethal infections when injected into mice. (2) Injections of R mutants by themselves do not cause infections that kill mice. (3) Similarly, injections of heat-killed S bacteria do not cause lethal infections. (4) Lethal infection does result, however, from injections of live R bacteria mixed with heat-killed S strains; the blood of the dead host mouse contains living S-type bacteria.



2.2.3 DNA AS THE ACTIVE AGENT OF TRANSFORMATION

Figure 6.5 The transforming principle is DNA: Experimental confirmation. **(a)** Bacterial transformation occurs in culture medium containing the remnants of heat-killed S bacteria. Some "transforming principle" from the heat-killed S bacteria is taken up by the live R bacteria, converting (transforming) them into virulent S strains. **(b)** A solution of purified DNA extracted from white blood cells. **(c)** Chemical fractionation of the transforming principle. Treatment of purified DNA with a DNA-degrading enzyme destroys its ability to cause bacterial transformation, while treatment with enzymes that destroy other macromolecules has no effect on the transforming principle.



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2.2.4 Viral studies point to DNA, not protein, in replication

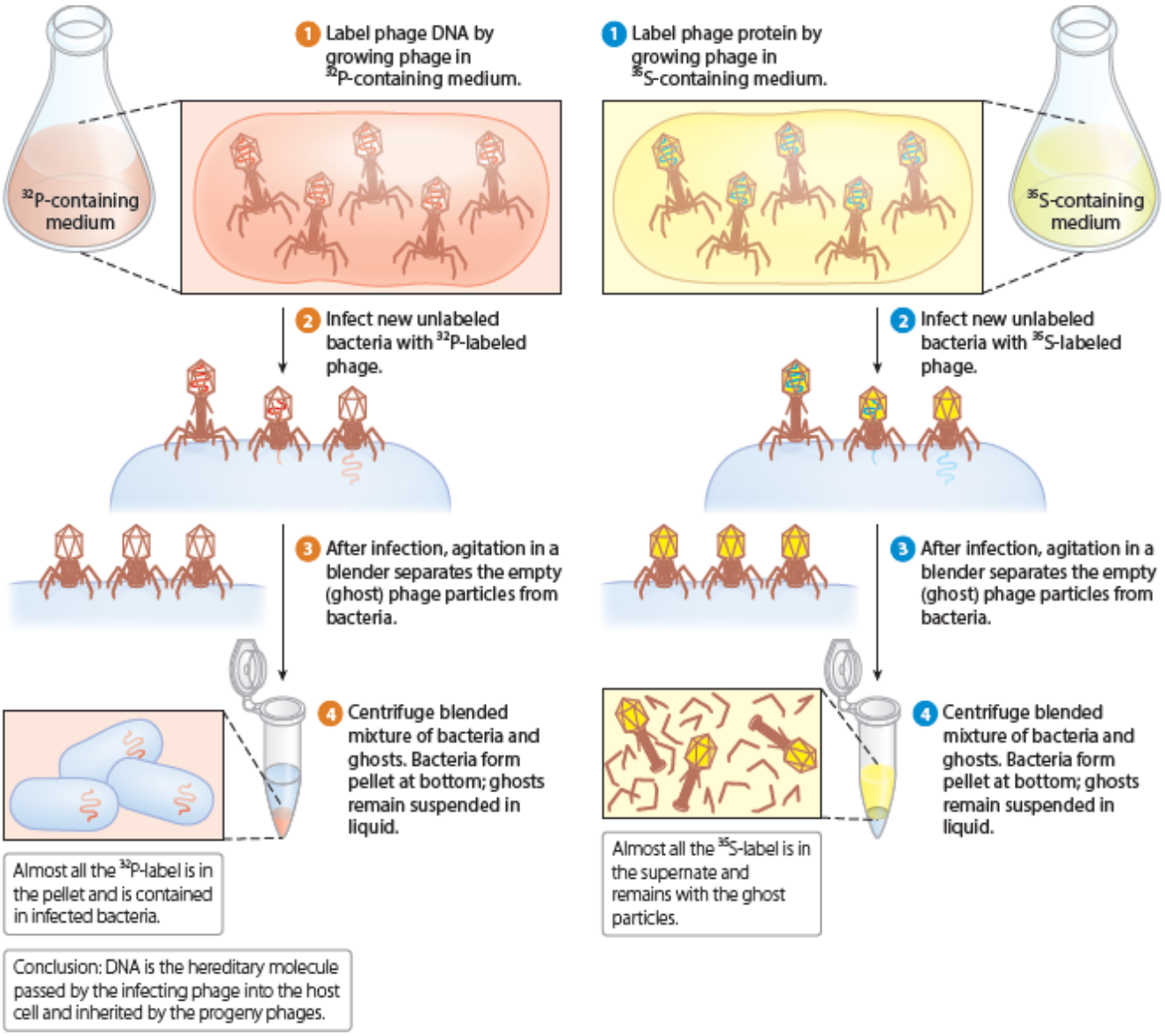
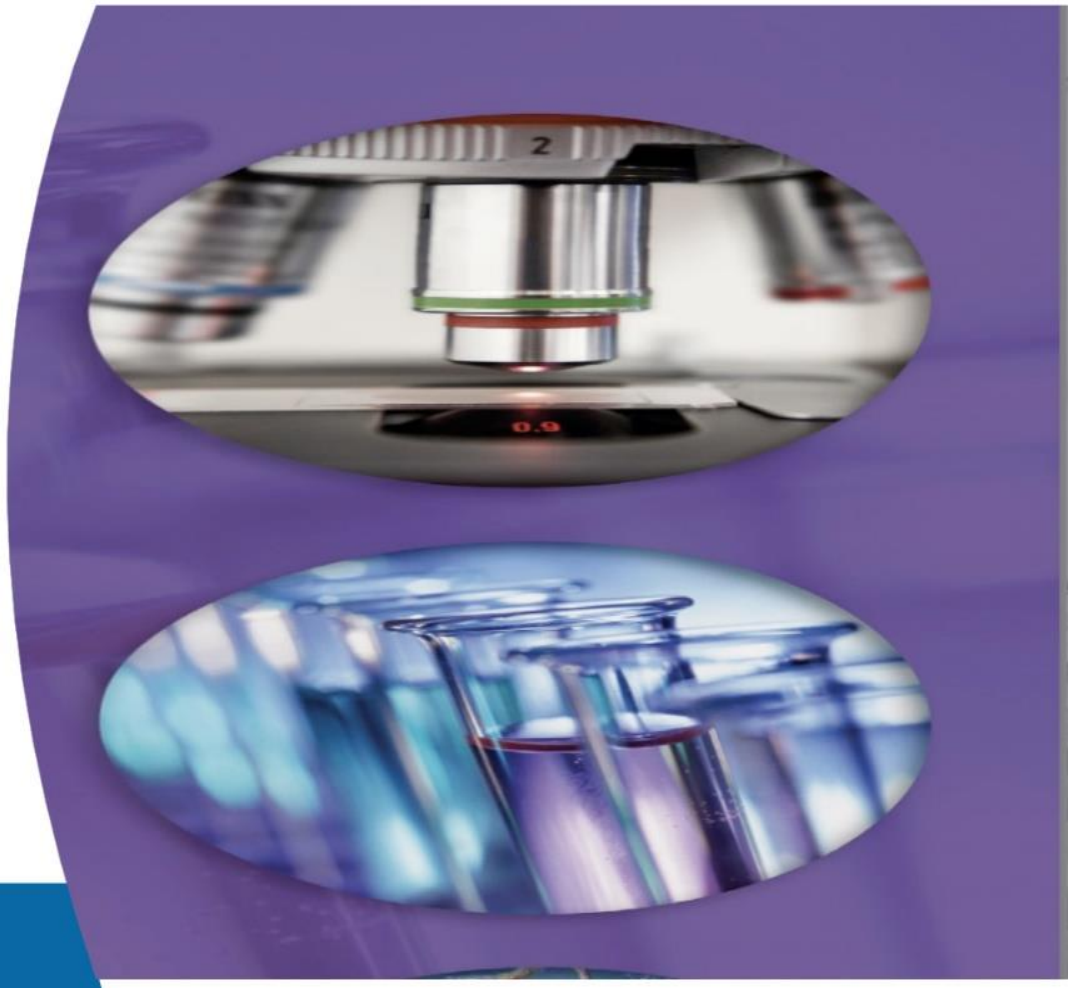


Figure 7.4 Hershey-Chase experiment showing DNA to be the molecule in bacteriophages that causes lysis of infected bacterial cells.

3 Common Laboratory Tools and Equipment Used in Molecular Biology



3.1 Biological samples and chemical substances in the laboratory

Tissue or cell samples from a living organism, different cell cultures grown in a laboratory incubator under controlled conditions, homogenates or extracts of cells and tissues, solutions of isolated and purified components (e.g. proteins, nucleic acids) can all be referred to as "biological samples". As the medium of life is water, the majority of biological samples can be defined as aqueous solutions with one or more components, colloidal systems, or water-based suspensions (e.g. bacterial cells dispersed in a liquid medium). Consequently, most biochemical experiments also take place in aqueous environments. Therefore, laboratory vessels used to store liquids and laboratory tools required for the manipulation, transfer and accurate volume measurements of liquids will be introduced in this Lecture. Different solids (e.g. chemical substances obtained from different companies, synthetic oligonucleotides or peptides) are also often necessary for biochemical research. In most cases, solids are dissolved in water (or sometimes in other solvents) prior to the experiments. Therefore, the methods of preparing solutions and measuring accurately the weight of the required solids will also be discussed below.

3.2 Plastic and glass tubes used for the storage of liquids

Vessels made of different transparent plastics are widely used in laboratory practice for the storage of liquids.

- Plastics are cheap and flexible.
- plastic containers are ideal because they retain their flexibility in a wide range of temperatures, while glass can be more sensitive to temperature changes or can be broken easily.
- inertness against the majority of chemical substances used in most experiments.

For the storage of liquids, probably the most important criterion is the air-tightness of the vessels. Why?

3.2.1 Falcon tubes:

Falcon tubes are manufactured with different nominal volumes (most typically, 15 mL and 50 mL) and are supplied with screw-caps. The conical bottom of the tube is particularly advantageous when there is only a small

amount of liquid left in the tube. In this case all drops can be collected readily by centrifugation.

3.2.2 Test tubes and Wassermann tubes:

Usually made of glass and provided without caps. They have a U-shaped bottom. They are mainly used for temporary purposes The main advantage of glass is its high resistance against most chemical substances and solvents used in typical biochemical experiments

3.2.3 Eppendorf tubes:

These tubes are available in different nominal volumes (e.g. 0.5 mL,1.5 mL, 2 mL and 5 mL). The most common size is 1.5 mL. Eppendorf tubes have a conically-shaped bottom with an attached plastic snap-lid.

3.2.4 PCR tubes:

Named after their main purpose of usage(PCR), They look like Eppendorf tubes, but volumes of PCR tubes typically range up to 200 µl.

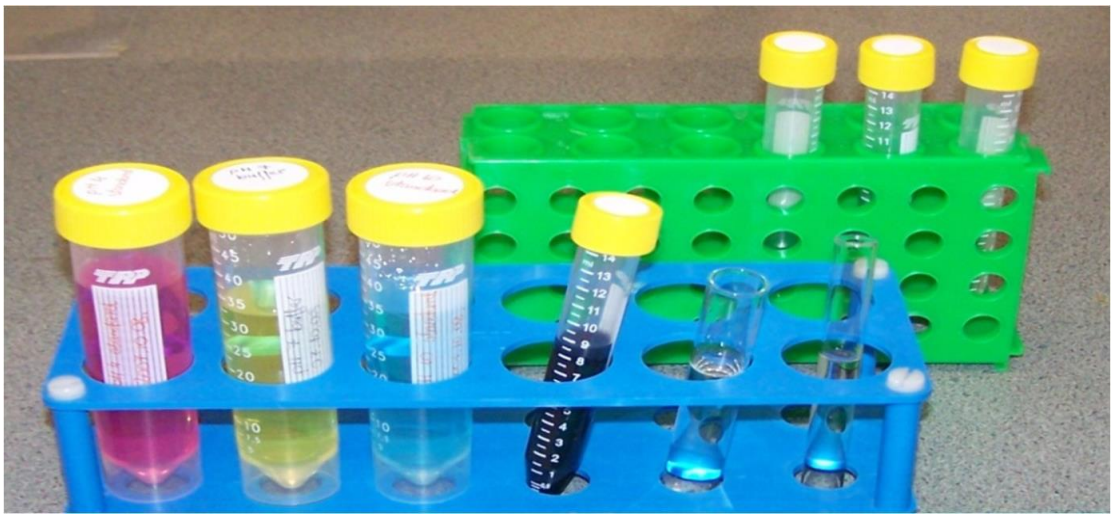


Figure (3.1) 50-mL and 15-mL Falcon tubes, glass test tube and Wasserman tube (left to right in blue rack) in plastic racks.

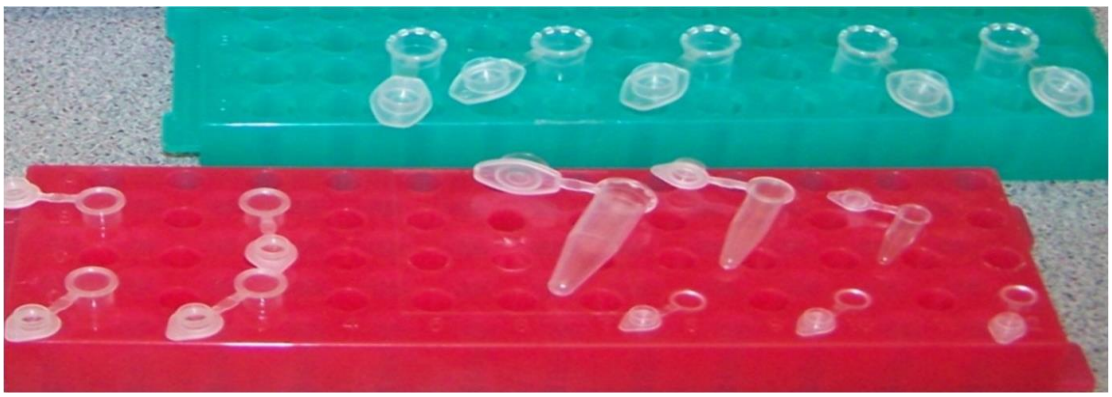


Figure 3.2 Eppendorf tubes with nominal volumes of 0.5 mL (left) and 1.5 mL (top green rack) and PCR tubes with a nominal volume of 200 µl (bottom right corner).

3.3 Precise volumetric measurements with graduated cylinders and micropipettes

3.3.1 Graduated cylinders:

used to measure the volume of liquids precisely, ranging from a few millilitres to several litres.

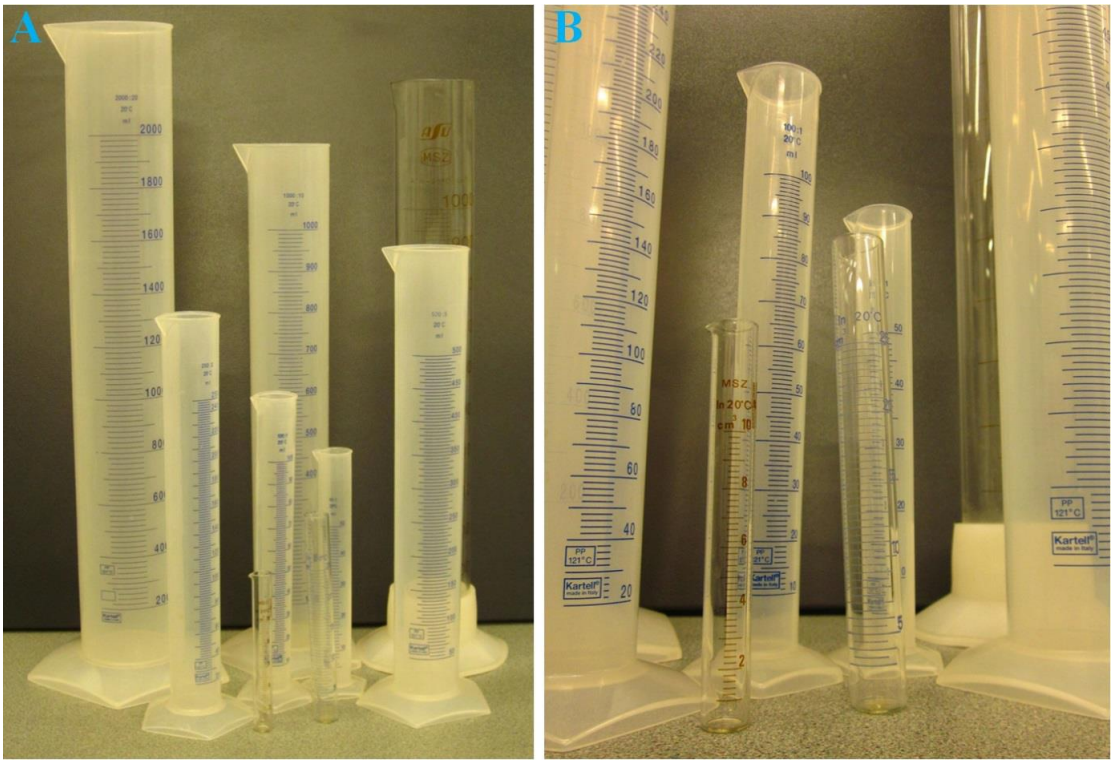


Figure 3.3 Glass and plastic graduated cylinders of various sizes.

3.3.2 Micropipettes:

The most common laboratory tools applicable in this volume range, depend on generates vacuum after a button on the top of the device. The vacuum is used to draw up liquid into a removable transparent plastic tip. Volume ranges: 200 µL - 1000 µL, 20 µL - 200 µL, 10 µL - 100 µL, 5 µL - 50 µL, 2 µL - 20 µL and 0.5 µL - 10 µL.

3.3.3 Tips: Different tips can be obtained from suppliers according to the volume range of the pipette.

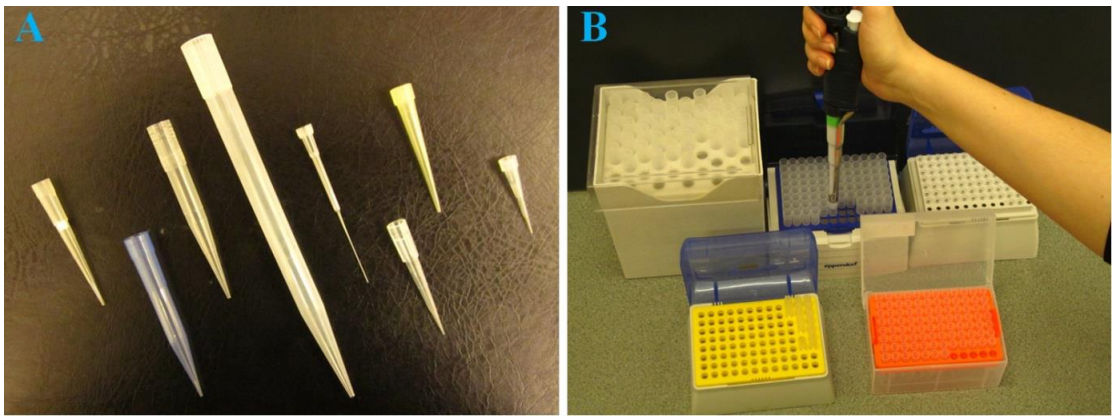


Figure 3.4 A, Pipette tips suitable for different pipettes and purposes (e.g. tips with sterile filters or elongated tips). B, Fastening of a tip onto the pipette.

Figure 3.5 A-C, Three positions of the button of the pipette. D-H, Liquid handling with pipettes. D, Adjusting the volume. E, Fastening an appropriate tip. F, Drawing of the liquid. G, Transferring the liquid into an Eppendorf tube. H, Dispensing the liquid.

3.4 Mixing of liquids

3.4.1 Pipetting:

Pipettes are perfect tools for mixing Submerging the tip into the solution and subsequent pushing and releasing the button several times will ensure extensive mixing.

3.4.2 Vortex mixer:

3.4.3 Magnetic stirrers

Used for the mixing of large volumes. Magnetic stirrers contain a strong permanent magnet. A stirring bar (a rod-like strong magnet with coating made of an inert plastic) is dropped into the liquid.

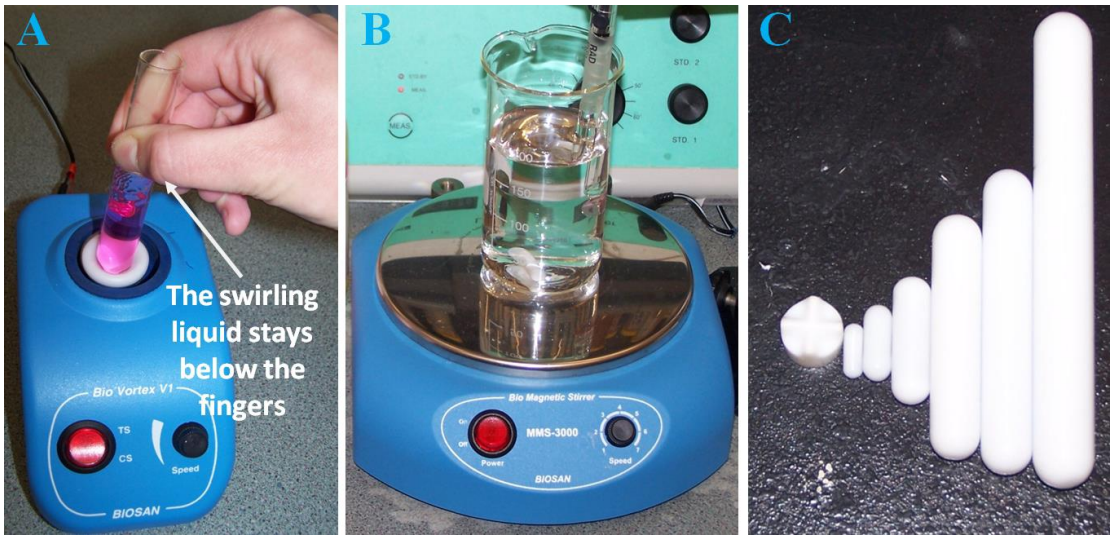


Figure 3.6. A, Mixing of a solution with a Vortex mixer. B, Mixing of a solution with a magnetic stirrer. C, Stirring bars of different size and shape.

3.5 Centrifuges

Cells can readily be harvested from liquid cultures by using different centrifuges Similarly, any suspension or floating colloidal particles (e.g. precipitated proteins) in a solution can be separated into fractions by spinning the sample in a centrifuge. The resulting fractions are referred to as "supernatant" (i.e. the solution) and "pellet" (i.e. the particles collected at the bottom of the centrifuge tube, pressed together into a compact mass). The rotation speed of centrifuges is often specified as the number of revolutions per minute (RPM).

However, as the force applied to the sample depends not only on the actual RPM value but also on the radius of the rotor, the relative centrifugal force (RCF) is more informative about a particular experiment. This defines acceleration according to the mass of particles floating in the sample. Therefore, RCF values are given as relative acceleration values (the centrifugal acceleration compared to *g*, ~9.8 m/s2, the acceleration due to

gravity on the surface of Earth). Thus, if the same sample is spun at equal RPM values in two centrifuges with different rotor geometries (different rotor radii), the results will be different. However, if equal RCF values are applied, sedimentation forces will be identical. Therefore, RPM values are only informative when specified together with the rotor type or radius.

Figure 3.7 different centrifuges

Preparation of Human Genomic DNA

4

LECTURE OUTLINE

INTRODUCTION

The goal of this experiment is to isolate and purify a large quantity of high molecular weight human DNA. The source of the DNA will be your cheek cells obtained from a saline mouthwash (a bloodless and non-invasive procedure). You will also learn how to determine DNA concentration and purity.

4.1 BACKGROUND

DNA constitutes a small percent of the cell material and is usually localized in a defined part of the cell.

- In procaryotic cells DNA is highly condensed and localized in a structure called the nucleoid, which is not separated from the rest of the cell sap by a membrane.
- In eucaryotic cells the bulk of DNA is localized in the nucleus, which is separated from the rest of the cell sap by a complicated membrane structure.
- In viruses and bacteriophages, DNA is encapsulated by the protein coat and constitutes between 30 and 50 percent of the total mass of the virus.

The goal of DNA purification is to separate DNA from all of the components of the cell. “major impurities” and should be removed are protein and RNA. There are several methods of purification of DNA that exploit differences in the physical properties between DNA and proteins.

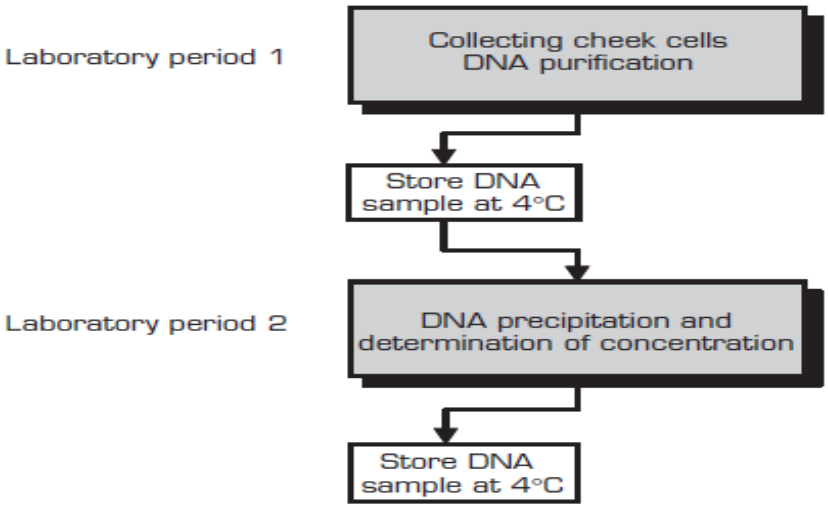


Figure 4.1 Schematic outline of the DNA isolation procedure.

All methods of purification involve five essential steps.

1. Cell breakage.
2. Removal of protein.
3. Removal of RNA.
4. Concentration of DNA.
5. Determination of the purity and quantity of DNA.

4.2 Cell Breakage

- Cell breakage is one of the most important steps in the purification of DNA.
- The usual means of cell opening, such as sonication, grinding, blending, or high pressure, cannot be used in DNA purification. These procedures apply strong forces to open cells that shear DNA into small fragments.
- The best procedure for opening cells and obtaining intact DNA is through application of chemical (detergents) and/or enzymatic procedures.

  Advantage of used Detergents

  1. It can solubilize lipids in cell membranes resulting in gentle cell lysis.
  2. In addition, detergents have an inhibitory effect on all cellular DNases and can denature proteins

  The lysis of animal cells is usually performed using anionic detergents such as SDS (sodium deodecyl sulfate) or Sarcosyl (sodium deodecyl sarcosinate).

4.3 Removal of Protein

The second step in purification involves removing a major contaminant, namely protein, from the cell lysate. This procedure is called deproteinization. Removal of proteins from the DNA solution depends on differences in the physical properties between nucleic acids and proteins.

4.3.1 Deproteinization using organic solvents

- The most frequently used methods for removing proteins explore the solubility differences between proteins and nucleic acids in organic solvents.
- Nucleic acids are predominantly hydrophilic molecules and are easily soluble in water. Proteins, on the other hand, contain many hydrophobic residues making them partially soluble in organic solvents.
- The organic solvents commonly used are phenol and/or chloroform containing 1 percent isoamyl alcohol.

Phenol-Chloroform Method

1. adding an equal volume of phenol-chloroform to an aqueous solution of lysed cells or homogenized tissue,
2. mixing the two phases, and allowing the phases to separate by centrifugation (Figure 4.2).
3. Centrifugation of the mixture yields two phases: the lower organic phase and the upper aqueous phase.
4. Nucleic acids are polar because of their negatively charged phosphate backbone, and therefore nucleic acids are soluble in the upper aqueous phase instead of the lower organic phase (water is more polar than phenol).
5. Conversely, proteins contain hydrophobic regions.
6. In the presence of phenol, the hydrophobic cores interact with phenol, causing the protein to swell and eventually to unfold or denature.
7. Chloroform denaturates proteins and lipids and makes DNA less soluble in the organic/phenolic phase
8. As a result, causing precipitation of proteins and polymers (including carbohydrates) to collect at the interface between the two phases.

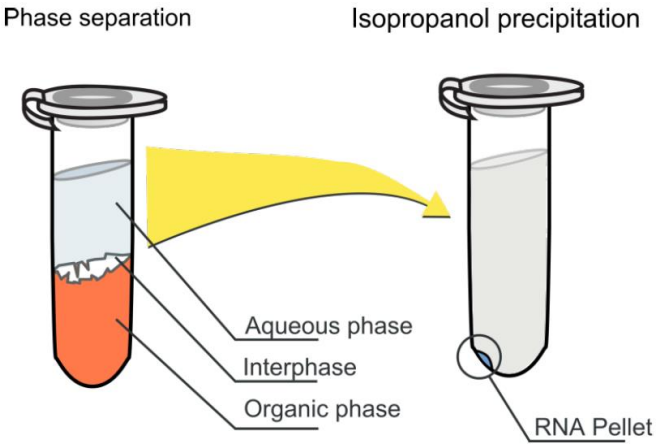


Figure 4.2 organic solvent deproteinization procedure.

Limitations using phenol-chloroform:

- it requires repeated time-consuming extractions when large amounts of protein are present.
- Phenol-chloroform extractions, require vigorous mixing that contributes to shearing of large DNA molecules.

4.3.2 Deproteinization using enzymes

A. Proteins can be removed from DNA preparations using a protease that can digest all proteins, i.e. a general-purpose protease.
B. Two such enzymes are in use, proteinase K and pronase.
C. These proteases are active in the presence of low concentrations of anionic detergent, high concentrations of salts, and EDTA and exhibit broad pH (6.0–10.0) and temperature (50–67°C) optima.
D. They can digest intact (globular) and denatured (polypeptide chain) proteins and do not require any co-factors for their activities.

4.4 Removal of RNA

The removal of RNA from DNA preparations is usually carried out using an enzymatic procedure.
Two ribonucleases that can be used, namely ribonuclease A and ribonuclease T1.

4.5 Concentrating the DNA

Precipitating with alcohol is usually performed for concentration of DNA from the aqueous phase

A. Two alcohols are used for DNA precipitation: ethanol and isopropanol.
B. Polar water molecules surround the DNA molecules in aqueous solutions. This interaction promotes the solubility of DNA in water.
C. Ethanol is completely miscible with water. Ethanol molecules cannot interact with the polar groups of nucleic acids as strongly as water, making ethanol a very poor solvent for nucleic acids.
D. Replacement of 95 percent of the water molecules in a DNA solution will cause the DNA to precipitate.
E. To precipitate DNA at a lower ethanol concentration, the activity of water molecules must be decreased. This can be accomplished by the addition of salts to DNA solutions.

DNA precipitation is customarily carried out with 70 percent ethanol (final concentration) in the presence of the appropriate concentration of sodium or ammonium salts.

Advantages and disadvantages using each of these salts

1. convenience and low cost,
2. The use of sodium chloride is recommended if a high concentration of SDS has been used for lysing the cells. SDS remains soluble in ethanol in the presence of 0.2 M NaCl.
3. The disadvantage of sodium chloride is its limited solubility in 70 percent ethanol making it difficult to completely remove from the DNA samples.

4.6 Determination of the Purity and Quantity of DNA

4.6.1 Quantity of DNA (concentration)

The most common technique to determine DNA yield and purity is measurement of absorbance.

1. Absorbance readings are performed at 260nm (A260) where DNA absorbs light most strongly, and the number generated allows one to estimate the concentration of the solution.
2. DNA concentration is estimated by measuring the absorbance at 260nm multiplying by the dilution factor, and using the relationship that an A260 of 1.0 = 50µg/ml pure dsDNA

Concentration (µg/ml) = (A260 reading – A320 reading) × dilution factor × 50µg/ml

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume.

DNA yield (µg) = DNA concentration × total sample volume (ml)

4.6.2 Purity of DNA

The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm (Proteins absorb maximally at 280 nm.) Good-quality DNA will have an A260/A280 ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present.

DNA purity (A_{260}/A_{280}) = (A_{260} reading – A_{320} reading) ÷ (A_{280} reading – A_{320} reading)

* 1 ng/µl =1 µg/ml

* For more accurate readings of the nucleic acid sample of interest, dilute the sample to give readings between 0.1 and 1.0.

* A260 = 1, give concentration of nucleic acids as fallowing (Table 4.1):

Table 4.1: Concentration per A260 Unit

Nucleic Acid	Concentration (µg/ml) per A260 Unit
ds DNA	50
ss DNA	33
ss RNA	40

Table 4.2: A260/A280 ratio for given molecules

Purity of	Target A_{260}/A_{280} Ratio
DNA	1.8
RNA	2.0
Protein	0.6

Example of Calculation 4.1

A sample of dsDNA was diluted 60X. The diluted sample gave a reading of 0.65 on a spectrophotometer at OD260. To determine the concentration of DNA in the original sample, perform the following calculation:

Concentration (µg/ml) = (A260 reading – A320 reading) × dilution factor × 50µg/ml

dsDNA concentration = OD260 × dilution factor × 50 µg/mL

dsDNA concentration = 0.65 × 60 × 50 µg/mL

dsDNA concentration = 1.95 mg/mL

5 Electrophoresis

Agarose gels, stained by Ethidium bromide visuallized by Transilluminator

5.1 INTRODUCTION

The movement of particles under spatially uniform electric field in a fluid is called **electrophoresis**. In 1807, Ferdinand Frederic Reuss observed clay particles dispersed in water to migrate on applying constant electric field for the first time. It is caused by a charged interface present between the particle surface and the surrounding fluid.

The rate of migration of particle depends on

1. the strength of the field.
2. on the net charge size and shape of the molecules.
3. the ionic strength
4. viscosity and temperature of medium in which the molecules are moving.

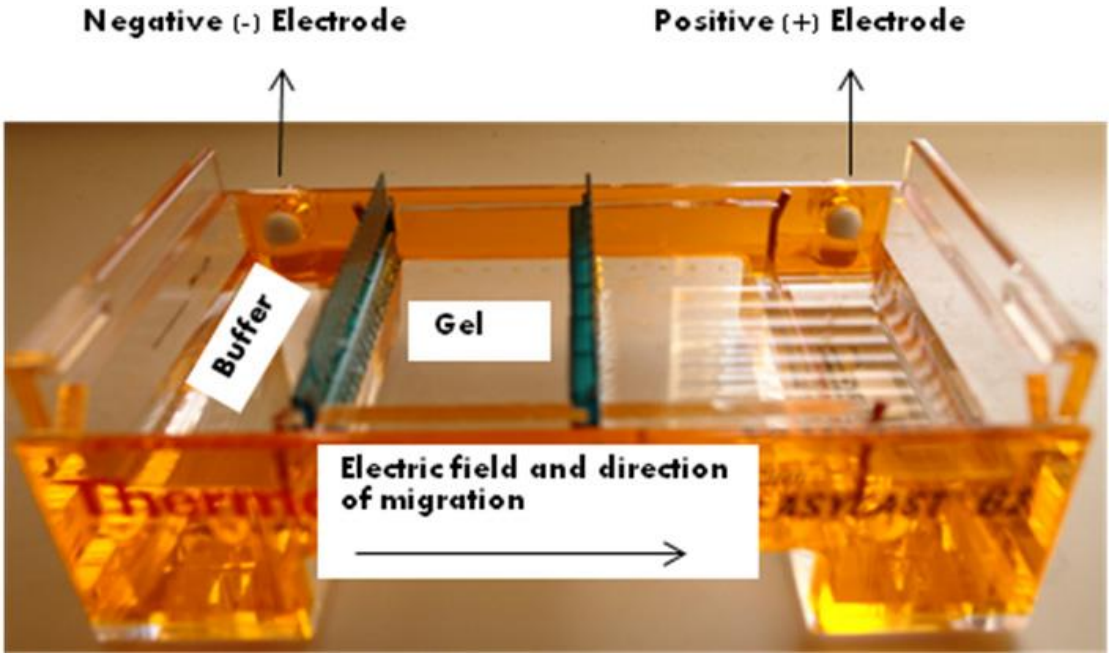


Fig. 5.1 Schematic illustration of a typical horizontal gel electrophoresis setup for the separation of nucleic acids.

5.2 ELECTROPHORESIS USES:

As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It is used analytically to

1. study the properties of a single charged species.
2. as a separation technique: separating molecules by size, charge, or binding affinity, (e. g. separation of DNA, RNA or protein) using an electric field applied to a gel matrix.

   * Gel matrix used mainly is polyacrylamide and agarose.

5.3 Principle and Theory of Electrophoresis

- Conducting medium (aqueous buffer/ electrolyte/run buffer)
- Applied Electric Field
- Positively charged species move to the cathode (-)
- Negatively charged species move to the anode (+)
- Large size molecules move slower than small one, So the distance of small size molecules that travel is greater

5.4 TYPES OF ELECTROPHORESIS

1. Affinity electrophoresis
2. Capillary electrophoresis
3. Pulsed field gel electrophoresis
4. SDS-PAGE
5. DNA Agarose Gels

5.5 General characteristics of matrices used in electrophoresis (agarose and polyacrylamide)

5.5.1 Agarose gel electrophoresis (AGE)

Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool. Therefore, agarose gels are simple and rapid to prepare. They are the most popular medium for the separation of moderate and large-sized nucleic acids.

Table 5.1 Advantages and disadvantages of Agarose gel

Advantages	Disadvantages
Nontoxic gel medium	High cost of agarose
Gels are quick and easy to cast	Fuzzy bands
Good for separating large DNA molecules	Poor separation of low molecular weight samples
Can recover samples by melting the gel, digesting with enzyme agarose	

Fig. 5.2 Polysaccharide gels (agarose) are formed by boiling followed by cooling.

5.5.2 Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N'-methylenebisacrylamide.

The reaction usually carried out with:

a) ammonium persulfate as the initiator
b) N,N,N',N'-tetramethylethylendiamine (TEMED) as the catalyst.

Although the gels are generally more difficult to prepare and handle, involving a longer time for preparation than agarose gels.

They have major advantages over agarose gels:

a) a greater resolving power
b) The DNA recovered from polyacrylamide gels is extremely pure.

Table 5.2 Advantages and disadvantages of polyacrylamide gel electrophoresis.

Advantages	Disadvantages
Stable chemically cross-linked gel	Toxic monomers (neurotoxin when unpolymerized).
Sharp bands	Gels are tedious to prepare and often leak
Good for separation of low molecular weight fragments	Need new gel for each experiment

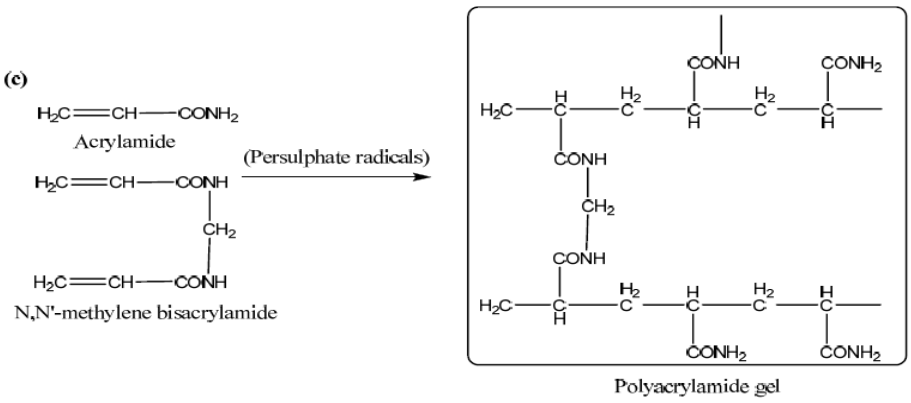


Fig. 5.3 Polymerization of acrylamide to form polyacrylamide gel.

5.6 Gel concentration

5.6.1 Agarose gel concentration

The percentage of agarose used depends on the size of fragments to be resolved. The concentration of agarose is referred to as a percentage of agarose to volume of buffer (w/v), and agarose gels are normally in the range of 0.2% to 3%. The lower the concentration of agarose, the faster the

DNA fragments migrate. In general, if the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended.

Table 5.3 Agarose gel concentration for resolving linear DNA molecules.

Concentration of agarose (%)	DNA size range (bp)
0.2	5000-40000
0.4	5000-30000
0.6	3000-10000
0.8	1000-7000
1	500-5000
1.5	300-3000
2	200-1500
3	100-1000

5.6.2 Polyacrylamide gel concentration

The choice of acrylamide concentration is critical for optimal separation of the molecules. Choosing an appropriate concentration of acrylamide and the cross-linking agent "methylenebisacrylamide" the pore sized in the gel can be controlled. With increasing the total percentage concentration (T) of monomer (acrylamide and cross-linker) in the gel, the pore size decreases in a nearly linear relationship.

Table 5.4 Polyacrylamide gel concentration for resolving DNA/RNA molecules.

Acrylamide/Bis Ratio	Gel %	Native DNA/RNA (bp)
19:1	4	100-1500
	6	60-600
	8	40-500
	10	30-300
	12	20-150
29:1	5	200-2000
	6	80-800
	8	60-400
	10	50-300
	12	40-200
	20	>40

5.7 Loading buffer

This is the buffer to be added to the DNA fragment that will be electrophoresed.

- This buffer contains glycerol or sucrose to increase the density of the DNA solutions.
- The gel loading buffer also contains dyes that facilitate observation of the sample during gel loading and electrophoresis, such as bromophenol blue.
- Because these molecule is small, they migrate quickly through the gel during electrophoresis, thus indicating the progress of electrophoresis.

5.8 Voltage/current applied

The higher the voltage/current, the faster the DNA migrates. If the voltage is too high, band streaking, especially for DNA≥12-15kb, may result. Moreover, high voltage causes a tremendously increase in buffer temperature and current in very short time. The high amount of the heat and current built up in the process leads to the melting of the gel, Therefore, it is highly recommended not exceed 5-8 V/cm and 75 mA for standard size gels or 100 mA for minigels. On the other side, when the voltage is too low, the mobility of small (≤1kb) DNA is reduced and band broadening will occur due to dispersion and diffusion.

5.9 Visualizing the DNA

After the electrophoresis has been completed there are different methods that may be used to make the separated DNA species in the gel visible to the human eye.

5.9.1 Ethidium bromide staining (EBS)

The localization of DNA within the agarose gel can be determined directly by staining with low concentrations of intercalating fluorescent ethidium bromide dye under ultraviolet light. It is important to note that ethidium bromide is a potent mutagen and moderately toxic after an acute exposure. Therefore, it is highly recommended to handle it with considerable caution.

5.9.1 Silver staining (SS)

Silver staining is a highly sensitive method for the visualization of nucleic acid and protein bands after electrophoretic separation on polyacrylamide gels. Nucleic acids and proteins bind silver ions, which can be reduced to insoluble silver metal granules. Sufficient silver deposition is visible as a dark brown band on the gel.

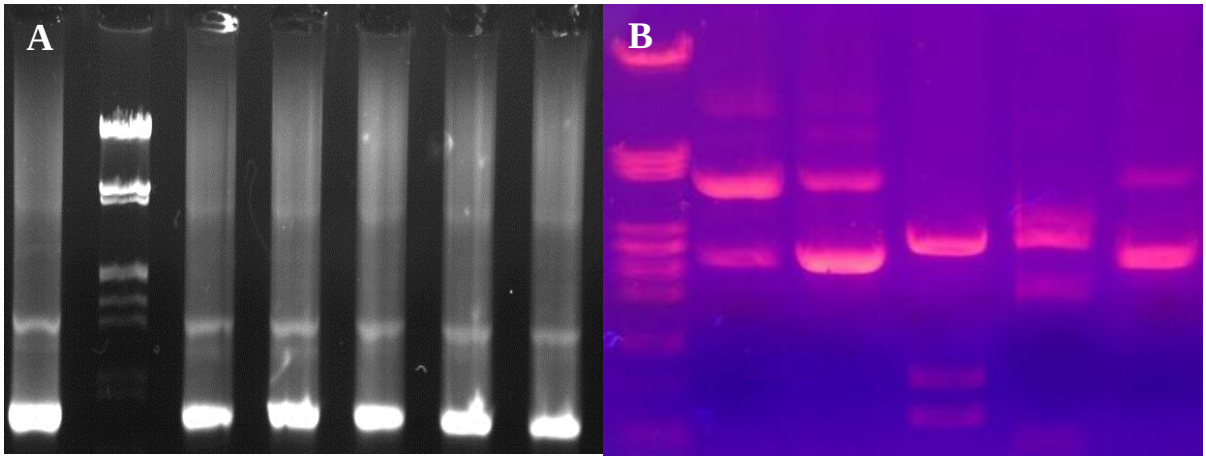


Fig. 5. Gel electrophoresis based image analysis. Agarose gels, stained by Ethidium bromide (A) and UV light (B).

Enzymes for cutting DNA: Restriction endonucleases

6

LECTURE OUTLINE

6.1 INTRODUCTION

Many experiments in molecular biology and genetic engineering requires that DNA molecules be cut in a very precise and reproducible fashion. Purified restriction endonucleases allow the molecular biologist to cut DNA molecules in the precise, reproducible manner required for gene cloning. The discovery of these enzymes, which led to Nobel Prizes for W. Arber, H. Smith, and D. Nathans in 1978, was one of the key breakthroughs in the development of genetic engineering.

6.2 THE DISCOVERY AND FUNCTION OF RESTRICTION ENDONUCLEASES

The initial observation that led to the eventual discovery of restriction endonucleases was made during the early 1950s, when some strains of bacteria were shown to be immune to bacteriophage infection, a phenomenon referred to as **host-controlled restriction**.

Restriction occurs because the bacterium produces an enzyme that degrades the phage DNA before it has time to replicate and direct the synthesis of new phage particles (Figure 6.1a). The bacterium's own DNA – the destruction

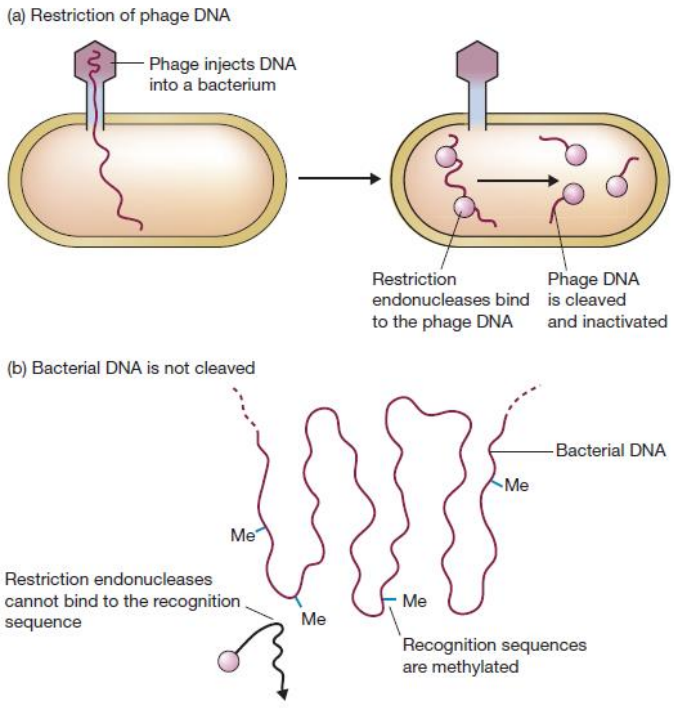


Fig. 6.1 The function of a restriction endonuclease in a bacterial cell. (a) Phage DNA is cleaved, but (b) bacterial DNA is not.

6.3 Nomenclature

The guidelines for naming restriction enzymes are based on the original suggestion by Smith and Nathans.

1. The enzyme names begin with an italicized three-letter acronym;
2. the first letter of the acronym is the first letter of the genus of bacteria from which the enzyme was isolated,
3. the next two letters are the two letters of the species.
4. These are followed by extra letters or numbers to indicate the serotype or strain
5. a space, then a Roman numeral to indicate the chronology of identification.
   For example,
   A. the first endonuclease isolated from *Escherichia coli*, strain RY13 is named as *Eco*R I.
   B. *Hin*d III is the third endonuclease of four isolated from *Haemophilus influenza*, serotype d.

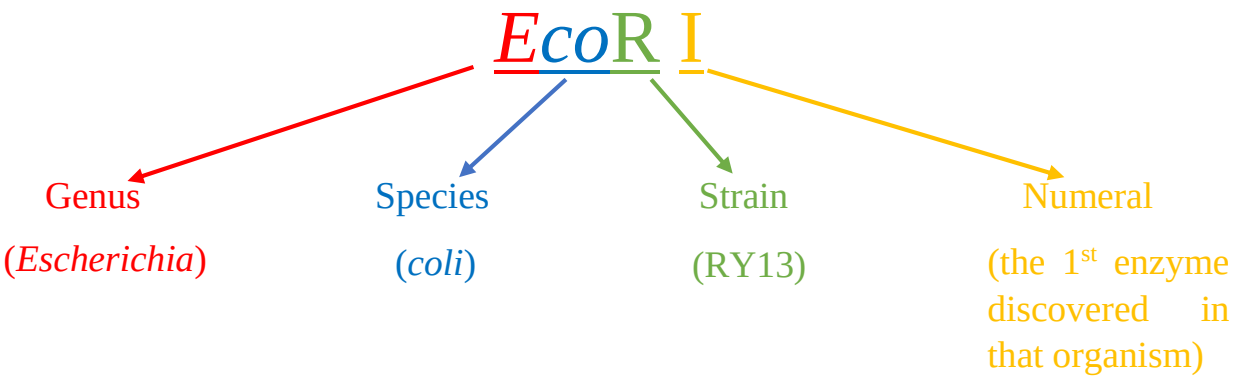


Fig. 6.2 scheme describe restriction endonucleases Nomenclature

6.4 Types of restriction endonucleases

Three different classes of restriction endonuclease are recognized

- Types I and III are rather complex and have only a limited role in genetic engineering
- type II restriction endonucleases are the cutting enzymes that are so important in gene cloning.

6.5 Type II restriction endonucleases cut DNA at specific nucleotide sequences

The central feature of type II restriction endonucleases is that each enzyme has a specific recognition sequence at which it cuts a DNA molecule. A particular enzyme cleaves DNA at the recognition sequence and nowhere else. For example, the restriction endonuclease called *Pvu*I (isolated from *Proteus vulgaris*) cuts DNA only at the hexanucleotide CGATCG; in contrast, a second enzyme from the same bacterium, called *Pvu*II, cuts at a different hexanucleotide, in this case CAGCTG.

The recognition sequences for some of the most frequently used restriction endonucleases are listed in Table 6.1

Table 6.1 The recognition sequences for some of the most frequently used restriction endonucleases.

Enzyme	Organisms	Recognition sequence	Blunt or sticky end
***Eco*R I**	*Escherichia coli*	GAATTC	Sticky
***Bam*H I**	*Bacillus amyloliquefaciens*	GGATCC	Sticky
***Bgl*II**	*Bacillus globigii*	AGATCT	Sticky
***Pvu*II**	*Proteus vulgaris*	CAGCTG	Blunt
***Hin*d III**	*Haemophilus influenzae* R_d	AAGCTT	Sticky
***Hae*III**	*Haemophilus aegyptius*	GGCC	Blunt

6.6 Blunt ends and sticky ends

Many restriction endonucleases make a simple double-stranded cut in the middle of the recognition sequence (Figure 6.3a), which results in a **blunt end** or a **flush end**. *Pvu*II and *Alu*I are examples of blunt end cutters. Other restriction endonucleases the cleavage is staggered, so that the resulting DNA fragments have short single-stranded overhangs at each end (Figure 6.3b). These are called **sticky ends** or **cohesive ends**.

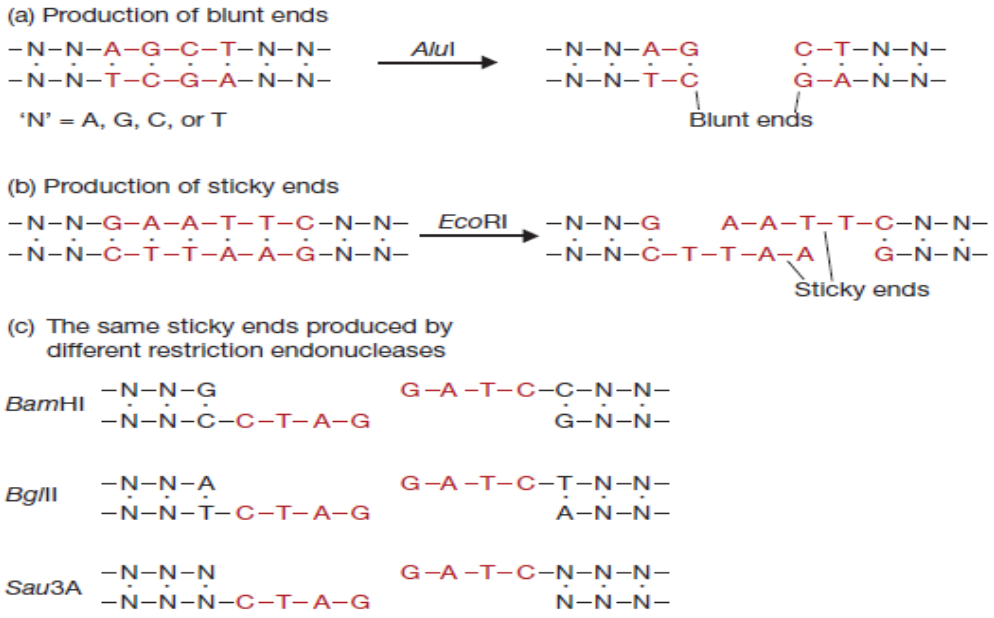


Fig. 6.3 The ends produced by cleavage of DNA with different restriction endonucleases. (a) A blunt end produced by *Alu* I. (b) A sticky end produced by *Eco*R I. (c) The same sticky ends produced by *Bam*HI, *Bgl*I and *Sau*3A

6.7 Setting up a Restriction Enzyme Digestion

An analytical-scale restriction enzyme digestion is usually performed in a volume of 20µl with 0.2–1.5µg of substrate DNA and a two- to tenfold excess of enzyme. If an unusually large volume of DNA or enzyme is used, aberrant results may occur. The following protocol is an example of a typical RE digestion.

1. In a sterile tube, assemble the following components in the order listed below.

Component	Volume
Sterile, deionized water	16.3µl
Restriction Enzyme 10X Buffer	2µl
Acetylated BSA, 10µg/µl	0.2µl
DNA, 1µg/µl	1.0µl
Mix by pipetting, then add:	
Restriction Enzyme, 10u/µl	0.5µl
Final volume	20µl

2. Mix gently by pipetting, close the tube and centrifuge for a few seconds in a microcentrifuge. Incubate at the enzyme's optimum temperature for 1–4 hours.
3. Add loading buffer to a 1X final concentration and proceed to gel analysis.

Note: Overnight digestions are usually unnecessary and may result in DNA degradation.

Nucleic Acids Amplification: The Polymerase Chain Reaction

7

LECTURE OUTLINE

7.1 Introduction: The Polymerase Chain Reaction Is a Powerful Technique for Copying DNA

In 1986, a technique, called the **polymerase chain reaction (PCR)**, was developed. This advance revolutionized recombinant DNA methodology and further accelerated the pace of biological research. The significance of this method was underscored by the awarding of the 1993 Nobel Prize in Chemistry to Kary Mullis, who developed the technique.

PCR is a rapid method of DNA amplification and cloning, in which DNA polymerase copies a target DNA sequence from a template molecule in vitro. The amplification products of each cycle provide new templates for the next round of amplification. Thus, the concentration of the target DNA sequence increases exponentially over the course of PCR.

7.2 PCR COMPONENTS

The typical PCR mixture contains

1. DNA polymerase (*Taq* polymerase)
2. Primers
3. each of the four deoxynucleotide triphosphates (dNTPs)
4. reaction buffer
5. DNA template (genomic DNA, cDNA, or a cell lysates).

7.2.1 a thermostable DNA polymerase (Taq polymerase).

DNA polymerase enzyme is required for DNA synthesis during the primer extension step of PCR. *Taq* DNA polymerase (isolated from *T. aquaticus*).

Taq polymerase properties:

- 5′→3′ polymerase activity
- thermostability,
- optimum performance at 70–80°C.
- Temperature, pH, and ion concentrations (Mg2+) can influence the activity of *Taq* polymerase.

7.2.2 forward and reverse oligodeoxynucleotide primers.

Some parameters need to be considered when designing a set of oligonucleotide primers:

- **Primer Length**: the optimal length of PCR primers is 18-22 bp.
- **Primer Melting Temperature:** Primers with melting temperatures in the range of 52-58 ^{o}C generally produce the best results. ***Tm* = 2(A+T) +4(G+C),**
- **Primer Annealing Temperature:** Too high T_a will produce insufficient primer-template hybridization resulting in low PCR product yield. Too low T_a may possibly lead to non-specific products caused by a high number of base pair mismatches ***Ta* = *Tm*** -5
- **GC Content:** The GC content (the number of G's and C's in the primer as a percentage of the total bases) of primer should be 40-60%.
- **Primer secondary structure:** (i. Hairpins ii. Self Dimer iii. Cross Dimer)
- **Repeats** For example: ATATATAT.

7.2.3 Deoxynucleotide triphosphates:

each of the four deoxynucleotide triphosphates (dNTPs): A, T, G, and C (dATP, dTTP, dGTP, dCTP) provide the energy for polymerization and the building blocks for DNA synthesis.

7.2.4 reaction buffer:

The PCR buffer consists of 50 m*M* KCl, 1.5 m*M* MgCl2, 10–50 m*M*Tris-HCl (pH 8.3), and 50–200 µ*M* dNTPs.

7.2.5 DNA template:

(genomic DNA, cDNA, or a cell lysates).

7.3 Procedure of PCR (steps of PCR)

All the PCR components are mixed together and are taken through series of 3 major cyclic reactions conducted in an automated, self-contained thermocycler machine.

1. **Denaturation** :
   This step involves heating the reaction mixture to 94°C for 15-30 seconds. During this, the double stranded DNA is denatured to single strands due to breakage in weak hydrogen bonds.
2. **Annealing** :
   The reaction temperature is rapidly lowered to 54-60°C for 20-40 seconds. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.
3. **Elongation** :
   Also known at extension, this step usually occurs at 72-80°C (most commonly 72°C). In this step, the polymerase enzyme sequentially

adds bases to the 3′ each primer, extending the DNA sequence in the 5′ to 3′ direction. Under optimal condi+tions, DNA polymerase will add about 1,000 bp/minute.

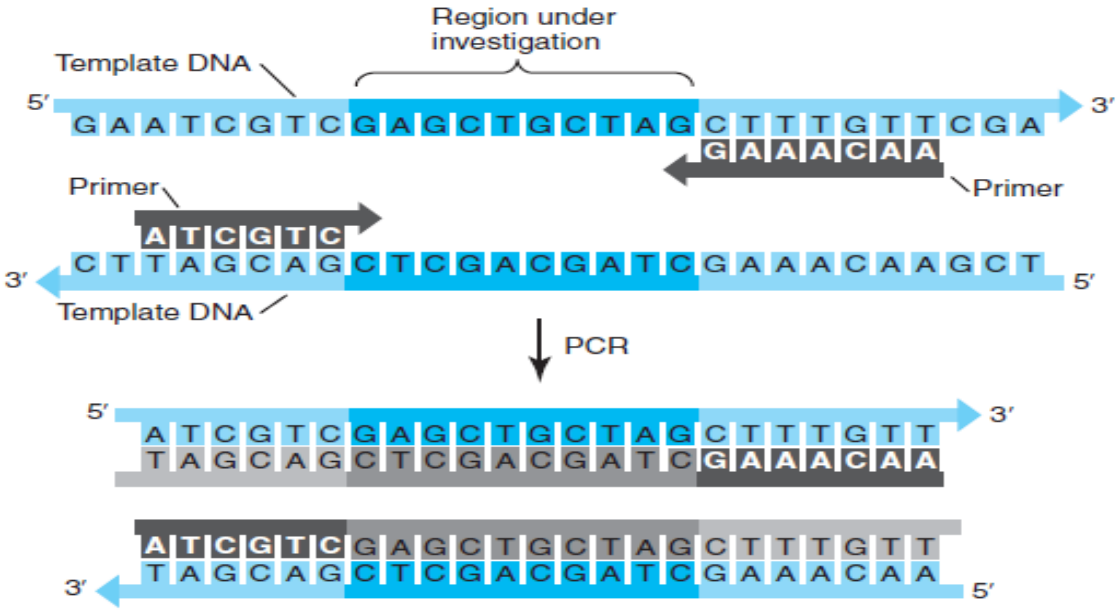


Fig. 7.1 The components and result of a PCR.

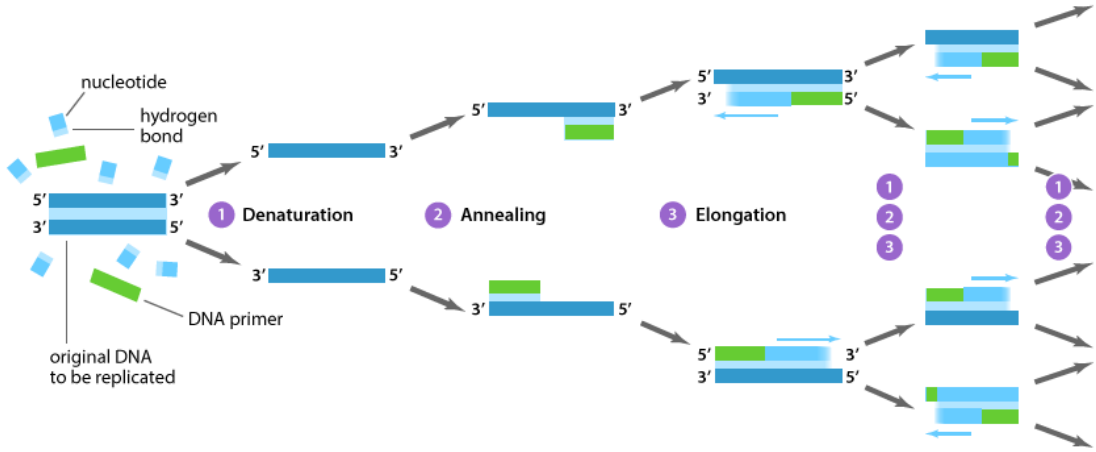


Fig. 7.2 The PCR Steps

Table 7.1 Components of a Typical PCR Reaction

Component	Purpose
0.25 mM each primer (oligodeoxynucleotides)	Directs DNA synthesis to the desired region
0.2 mM each dATP, dCTP, dGTP, dTTP	Building blocks that extend the primers
50 mM KCl	Monovalent cation (salt), for optimal hybridization of primers to template
10 mM Tris, pH 8.4	Buffer to maintain optimal pH for the enzyme reaction
1.5 mM $MgCl_2$	Divalent cation, required by the enzyme
2.5 units polymerase	The polymerase enzyme that extends the primers (adds dNTPs)
10^2–10^5 copies of template	Sample DNA that is being tested.

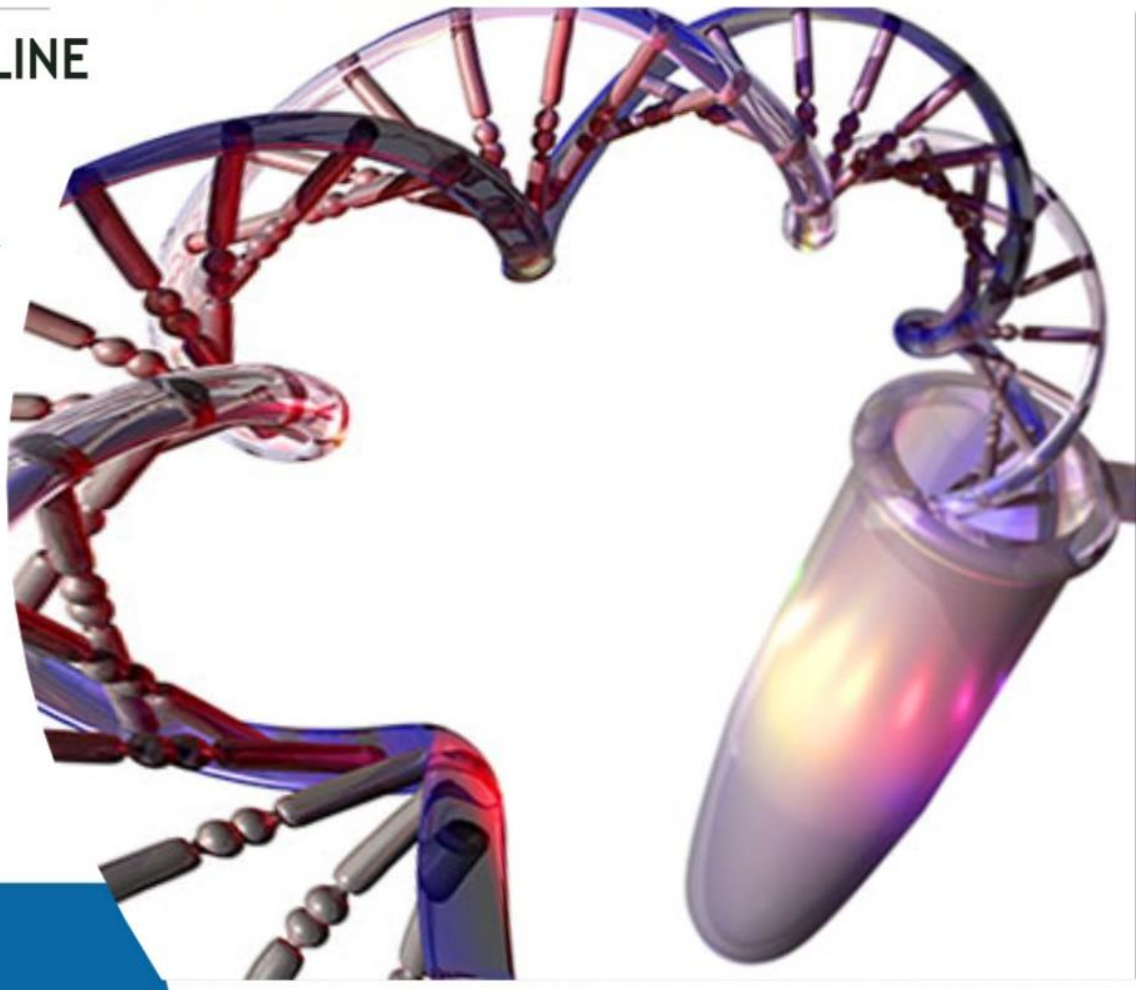
8 Types of PCR and PCR Amplications

LECTURE OUTLINE

8.1 Introduction

8.2 Types of PCR

8.3 Applications of PCR



8.1 Introduction

PCR today has been adapted for various applications. Several modifications are used in the clinical laboratory. Of the large (and increasing numbers) of PCR modifications, following is a description of those in standard use in the clinical molecular laboratory. These methods are capable of detecting multiple targets in a single run (multiplex PCR), using RNA templates (reverse transcriptase PCR), or such amplified products as templates (nested PCR) and quantitating starting template (quantitative PCR, or real-time PCR).

8.2 Types of PCR

8.2.1 Multiplex PCR

Multiplex PCR is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture.

Uses:

1. Pathogen Identification
2. SNP Genotyping
3. Mutation Analysis
4. Gene Deletion Analysis
5. Forensic Studies

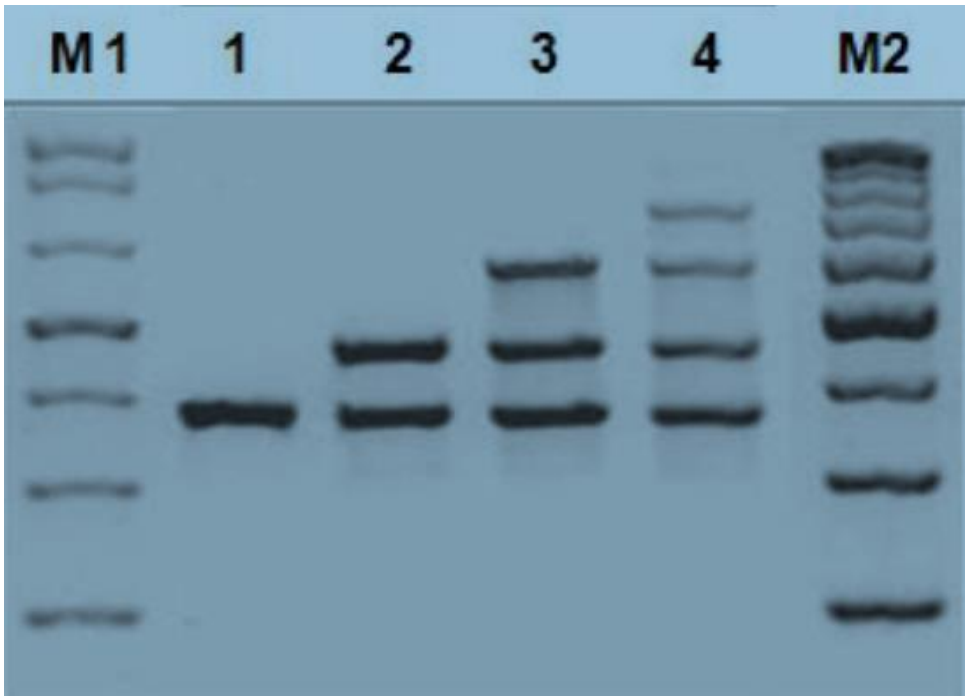


Fig. 8.1 Multiplex PCR in Agarose Gel

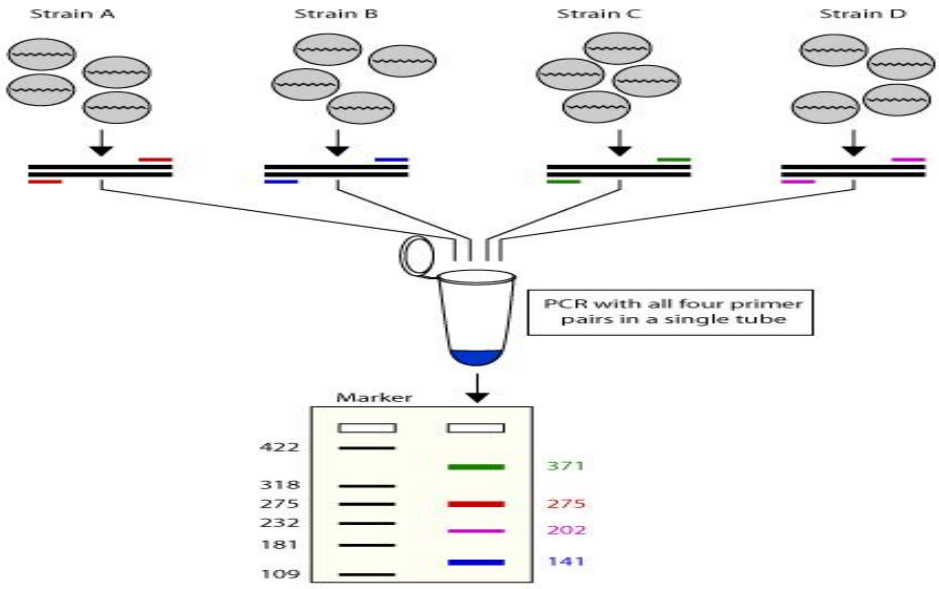


Fig. 8.2 Primer Design Parameters for Multiplex PCR

8.2.2 NESTED PCR

This PCR increases the ***specificity*** of DNA amplification, in which Two sets (instead of one pair) of primers are used in two successive PCRs.

- In the first reaction, on pair of primers "outer pair" is used to generate DNA products
- The product(s) are then used in a second PCR after the reaction is diluted with a set of second set "nested or internal" primers, and produce a second PCR product that will be shorter than the first one

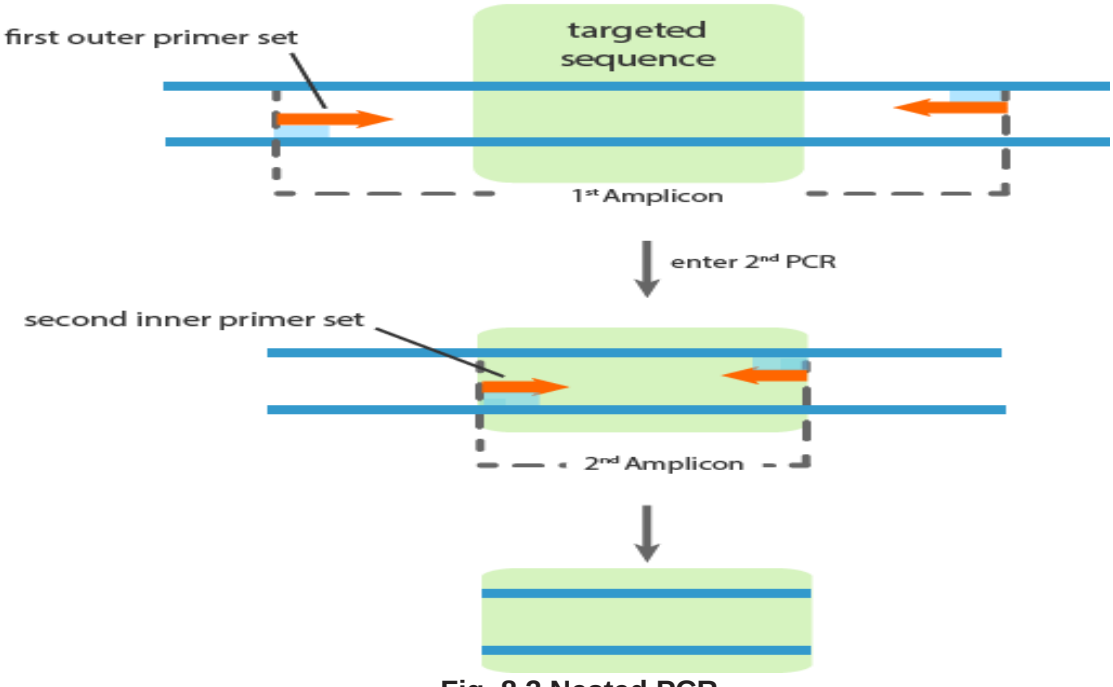


Fig. 8.3 Nested PCR

8.2.3 HOT-START PCR

A technique that reduces non-specific amplification during the initial set up stages of the PCR.

- It may be performed manually by heating the reaction components to the melting temperature (e.g., 95°C) before adding the polymerase.
- Specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature, either by the binding of an antibody or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step.

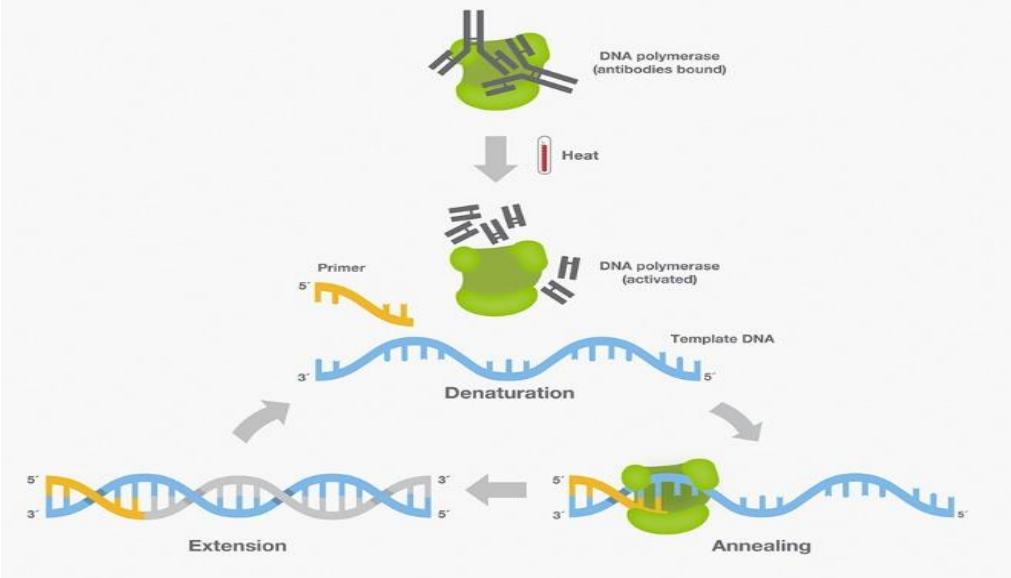


Fig. 8.4 Hot-start PCR

8.2.4 REVERSE TRANSCRIPTION PCR (RT-PCR)

A PCR designed for amplifying DNA from RNA. Reverse transcriptase reverse transcribes RNA into cDNA, which is then amplified by PCR.

Uses

1. to determine the expression of a gene
2. to identify the sequence of an RNA transcript, including transcription start and termination sites.

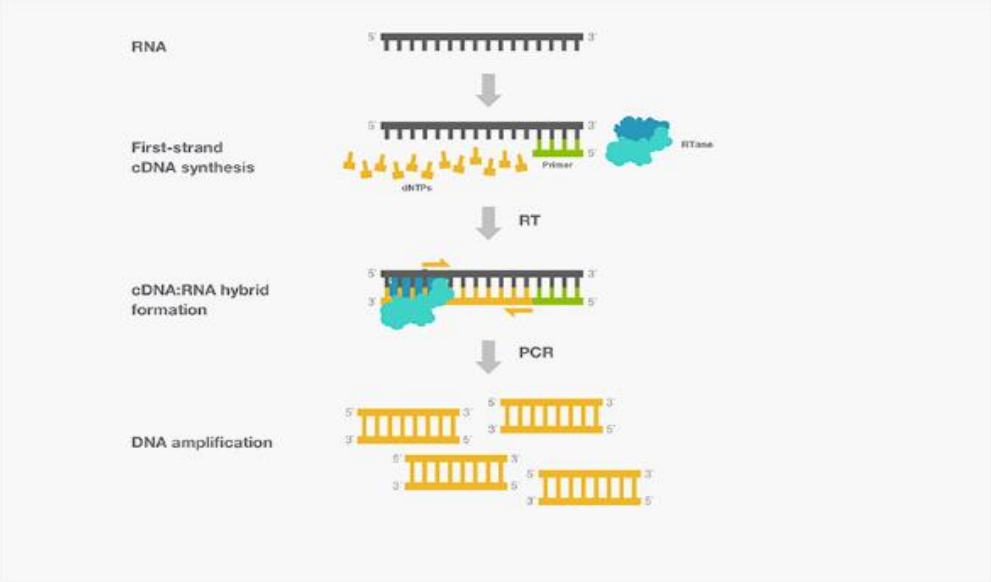


Fig. 8.5 Reverse Transcription PCR (RT-PCR)

8.2.5 QUANTITATIVE PCR (Q-PCR)

Used to measure the quantity of a PCR product (commonly in real-time). It quantitatively measures starting amounts of DNA, cDNA or RNA. Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample. Quantitative real-time PCR has a very high degree of precision. q*RT*-PCR methods use fluorescent dyes, such as Sybr Green, EvaGreen or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

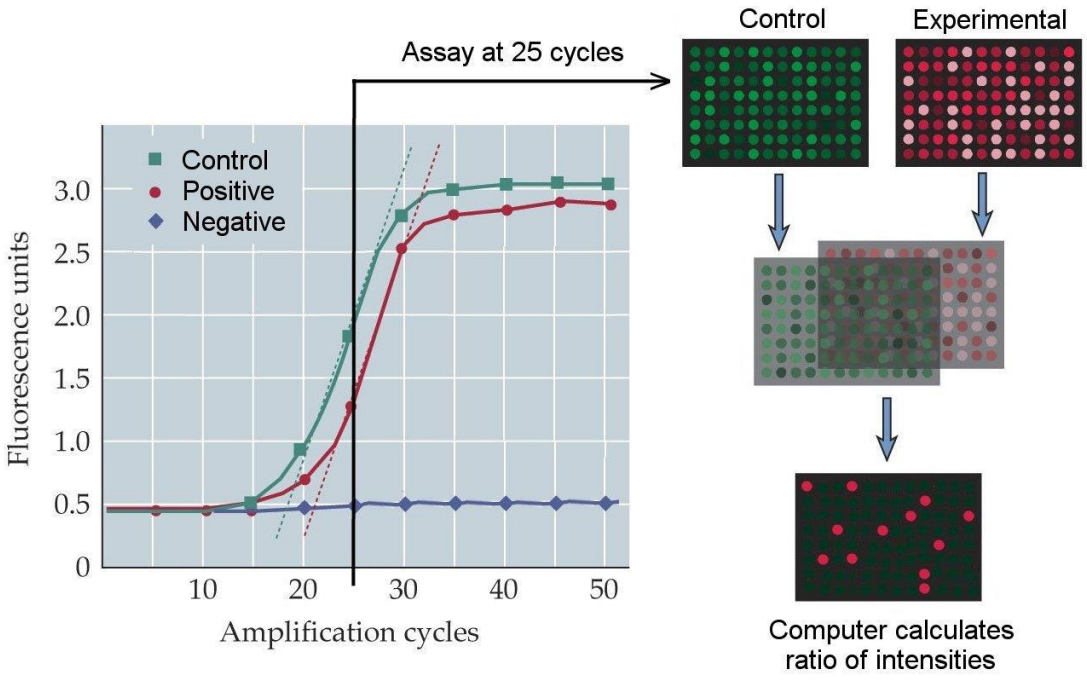


Fig. 8.6 quantitative Real time PCR (qRT-PCR)

8.3 APPLICATIONS OF PCR

PCR can be applied in many fields

1. In diagnosis therapy
2. In medical field
3. In agricultural sciences
4. In mycology-parasitology
5. In virological diagnostics
6. In molecular systematic evolution
7. In cancer therapy
8. In forensic medicine
9. In PCR-fingerprinting
10. In the detection of microbiological gene.

9.1 Introduction

A mutation can be defined as an alteration in DNA sequence. Any base-pair change in any part of a DNA molecule can be considered a mutation. A mutation may comprise a single base-pair substitution, a deletion or insertion of one or more base pairs, or a major alteration in the structure of a chromosome.

9.2 Types of mutation

9.2.1 **Point Mutations** is a change in a single DNA base.

A. It is a **transition** if a purine replaces a purine (A to G or G to A) or a pyrimidine replaces a pyrimidine (C to T or T to C).
B. It is a **transversion** if a purine replaces a pyrimidine or vice versa (A or G to T or C).

other terms used to describe the point mutation

C. **missense mutation:** Is a point mutation that changes a codon that normally specifies a particular amino acid into one that codes for a different amino acid.
D. **nonsense mutation.** Is a point mutation that changes a codon specifying an amino acid into a "stop" codon—UAA, UAG, or UGA in mRNA. A premature stop codon shortens the protein product, which can greatly influence the phenotype.
E. **silent mutation**: is a point mutation alters a codon but does not result in a change in the amino acid at that position in the protein (due to degeneracy of the genetic code).

9.2.2 **Frameshift mutations:** is **insertion or deletion** of one or more nucleotides at any point within the gene. the loss or addition of a single nucleotide causes all of the subsequent three-letter codons to be changed.

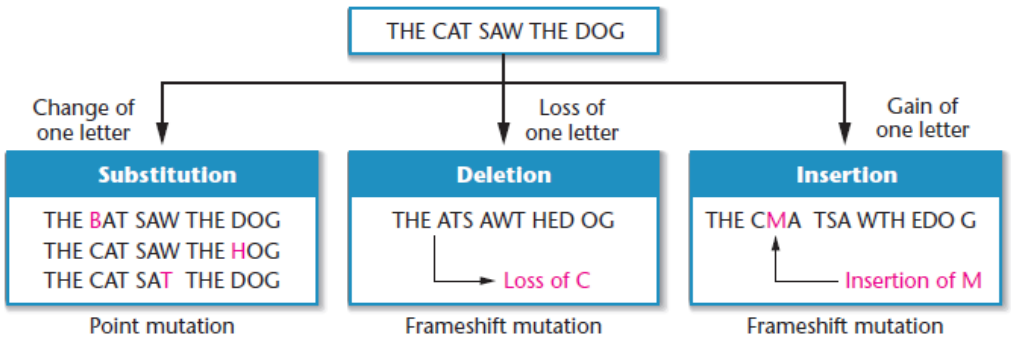


Fig. 9. 1 | Analogy showing the effects of substitution, deletion, and insertion of one letter in a sentence composed of three-letter words to demonstrate point and frameshift mutations.

These are called **frameshift mutations** because the frame of triplet reading during translation is altered.

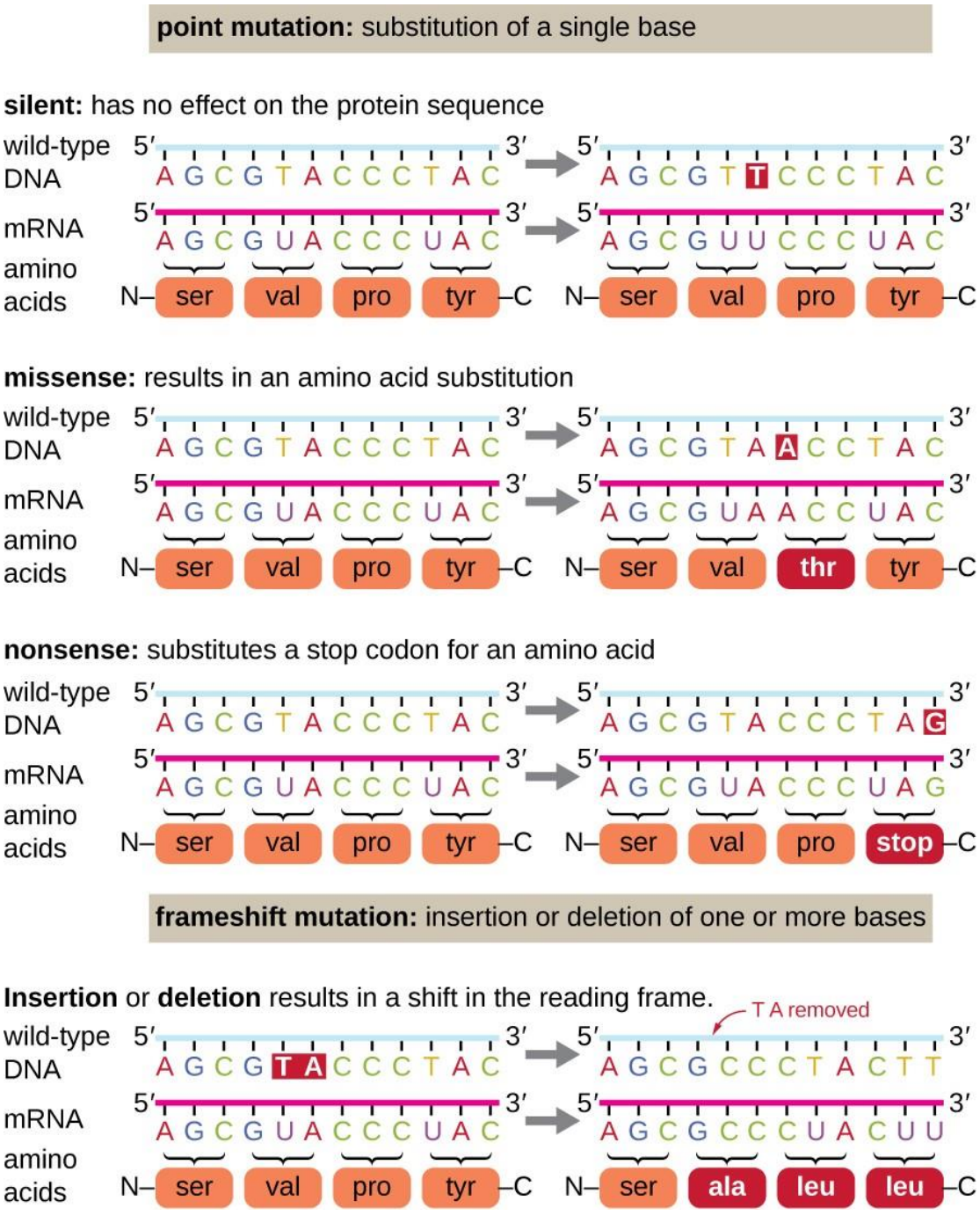


Fig. 9.2: type of mutations

9.3 Detection of Gene Mutations

Sequence detection methods can be generally classified according to three broad approaches: **hybridization-based** methods, **sequence (polymerization)-based** methods, and **enzymatic** or **chemical cleavage** methods.

9.3.1 Hybridization-Based Methods

Single-Strand Conformation Polymorphism

Single-strand conformational polymorphism (SSCP) analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation. Altered conformation due to a single base change in the sequence can cause single-stranded DNA to migrate differently under non-denaturing electrophoresis conditions. Therefore, wild-type and mutant DNA samples display different band patterns.

SSCP analysis involves the following four steps:

1. polymerase chain reaction (PCR) amplification of DNA sequence of interest.
2. denaturation of double-stranded PCR products.
3. cooling of the denatured DNA (single-stranded) to maximize self-annealing.
4. detection of mobility difference of the single-stranded DNAs by electrophoresis under non-denaturing conditions.

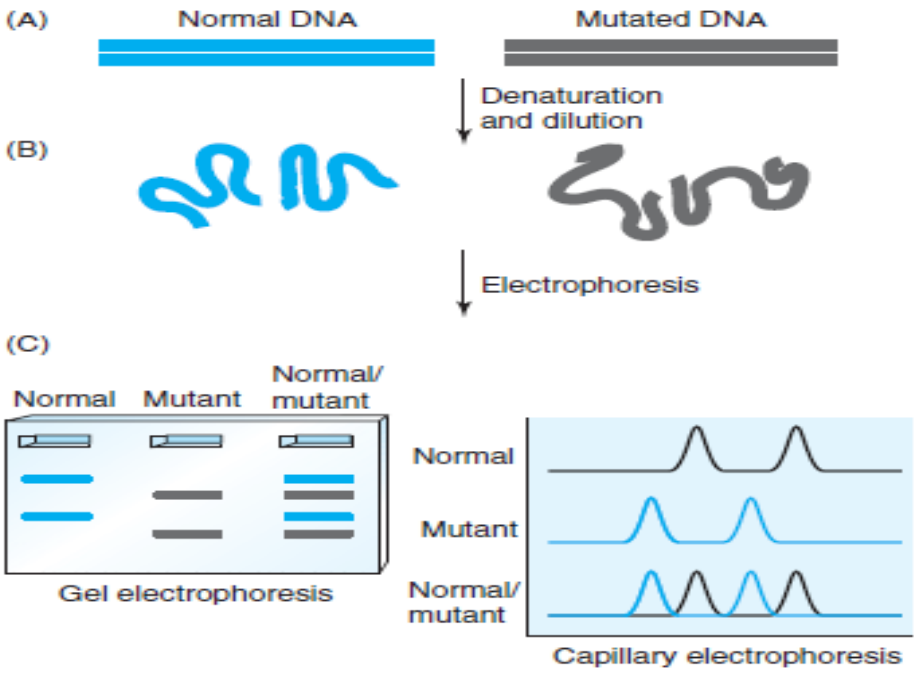


Fig. 9.3: Single-strand conformation polymorphism analysis. Double-stranded PCR products (A) of normal or mutant sequences are denatured and form conformers (B) through intrastrand hydrogen bonding. These conformers can be resolved (C) by gel (left) or capillary (right) electrophoresis.

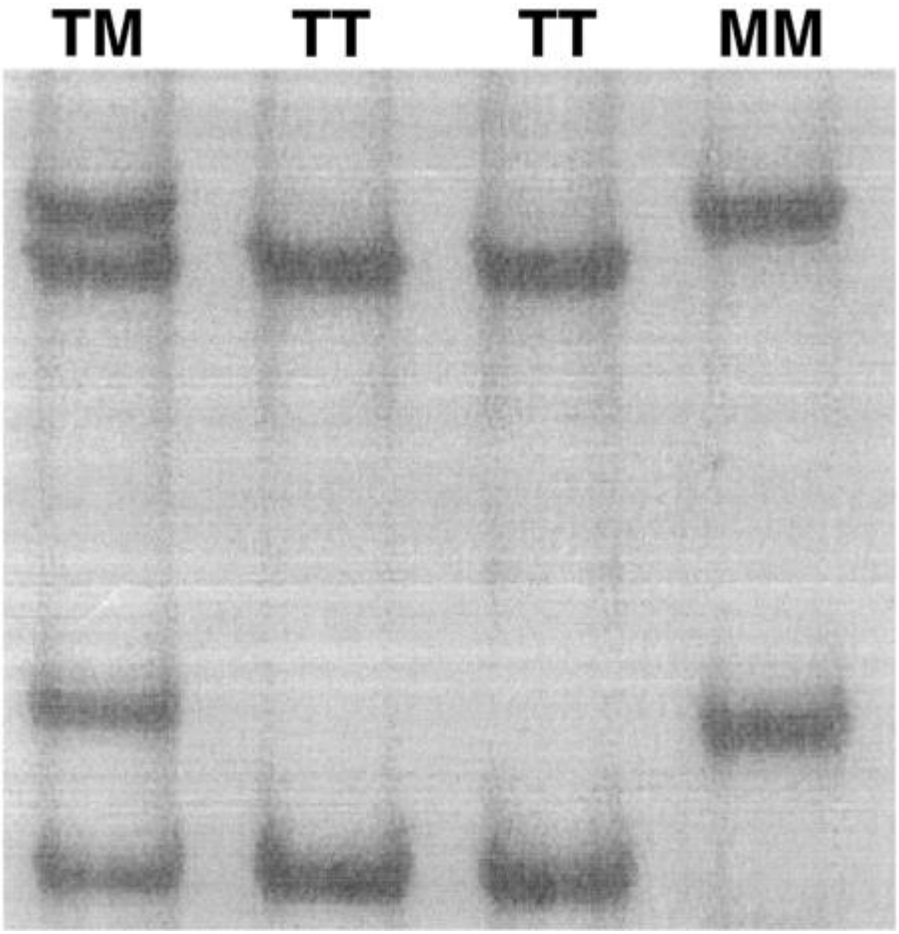


Fig. 9.4. Silver staining SSCP analysis for detection of the T594M mutation in the epithelial sodium channel -subunit. The PCR fragment is 245 bp. TM, heterozygous mutant; TT, wild type; MM, homozygous mutant.

9.3.2 Sequencing (Polymerization)- Based Methods

Sequence-Specific PCR

Sequence-specific PCR (**SSP-PCR**) is commonly used to detect point mutations and other single nucleotide polymorphisms.

SSP-PCR involves careful design of primers such that the primer 3′ end falls on the nucleotide to be analyzed. The 3′ end of a primer must match the template perfectly to be extended by *Taq* polymerase (Fig. 9-5).

The result: the presence or absence of product can be interpreted as the presence or absence of the mutation.

Note: Normal and mutant sequences can be analyzed simultaneously by making one primer longer than the other, resulting in differently sized products (Fig. 9-6).

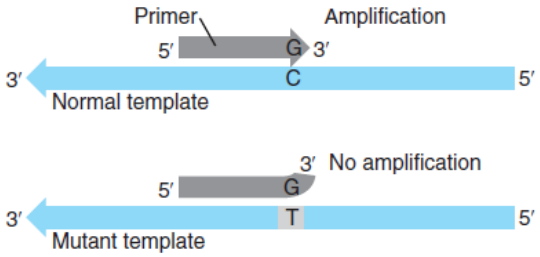


Fig. 9. 5 Sequence-specific primer amplification. Successful amplification will occur only if the 3′ end of the primer matches the template.

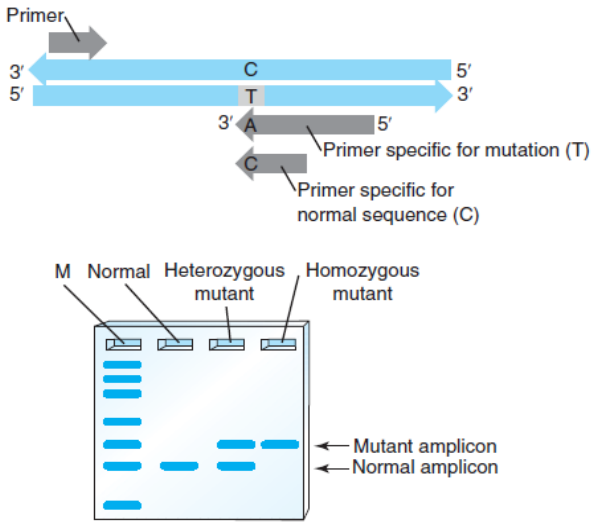


Fig. 9.6 Allele-specific primer amplification of a C→T mutation. A longer primer is designed with the mutated nucleotide (A) at the 3′end. This primer is longer and gives a larger amplicon than the primer binding to the normal sequence (top). The resulting products can be distinguished by their size on an agarose gel (bottom). First lane: molecular weight marker; second lane: a normal sample; third lane: a heterozygous mutant sample; fourth lane: a homozygous mutant.

9.3.3 Cleavage Methods

Restriction Fragment Length Polymorphisms

If a mutation changes the structure of a restriction enzyme target site or changes the size of a fragment generated by a restriction enzyme, restriction fragment length polymorphism (RFLP) analysis can be used to detect the sequence alteration.

To perform **PCR-RFLP**:

1) The gene fragment concerned (the region surrounding the mutation) is amplified using PCR.
2) The PCR product is digested using the appropriate restriction enzyme.
3) Analyzed using gel electrophoresis.

The results Mutations can inactivate a naturally occurring restriction site or generate a new restriction site so that digestion of the PCR product

results in cutting of the mutant amplicon but not the normal control amplicon or vice versa.

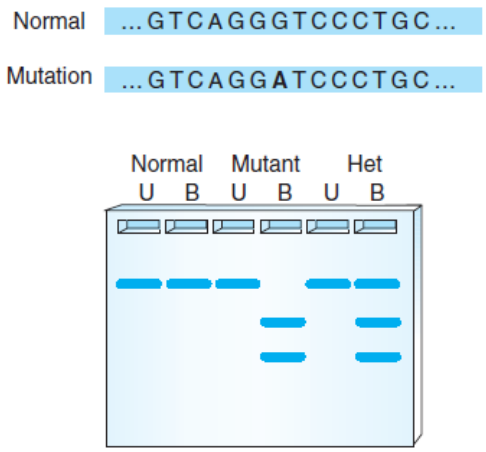


Fig. 9.7 PCR-RFLP. The normal sequence (top line) is converted to a *Bam*H1 restriction site (GGATCC) by a G_A mutation. The presence of the mutation is detected by testing the PCR product with *Bam*H1. The bottom panel shows the predicted gel patterns for the homozygous normal, homozygous mutant, and heterozygous samples uncut (U) or cut with *Bam*H1 (B).

اسم المحاضرة: **DNA Sequencing**

DNA Sequencing

10

The British biochemist Fred Sanger

LECTURE OUTLINE

5

10.1 Introduction

In the clinical laboratory, DNA sequence information (the order of nucleotides in the DNA molecule) is used routinely for a variety of purposes, including detecting mutations, typing microorganisms, identifying human haplotypes, and designating polymorphisms.

10.2 What is DNA Sequencing?

DNA sequencing is the process of determining the sequence of nucleotide bases (As, Ts, Cs, and Gs) in a piece of DNA.

10.3 Why we need to DNA Sequencing?

Direct determination of the order, or sequence, of nucleotides in a DNA polymer is the most specific and direct method for identifying genetic lesions (mutations) or polymorphisms.

10.4 Types of " DNA sequencing " methods

1. The Sanger method
2. Maxam-Gilbert method

10.5 The chain termination (Sanger method) method:

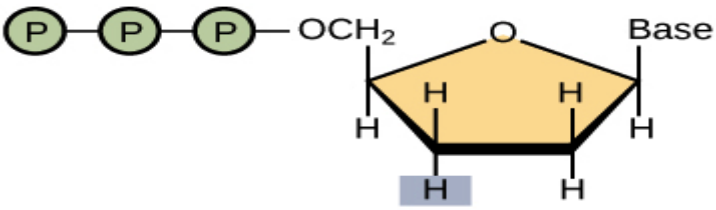
Regions of DNA up to about 900 base pairs in length are routinely sequenced using a method called Sanger sequencing or the chain termination method. Sanger sequencing was developed by the British biochemist Fred Sanger and his colleagues in 1977.

10.5.1 Ingredients for Sanger sequencing

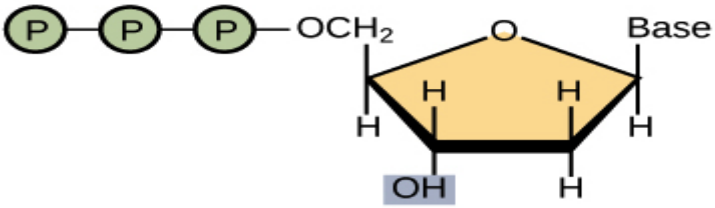
Sanger sequencing involves making many copies of a target DNA region. Its ingredients are similar to those needed for DNA replication in an organism, or for polymerase chain reaction (PCR), which copies DNA in vitro. They include:

- A DNA polymerase enzyme
- A primer, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase
- The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)
- The template DNA to be sequenced

However, a Sanger sequencing reaction also contains a unique ingredient:



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

- Dideoxy, or chain-terminating, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye

Fig. 10.1 **Dideoxy nucleotides(ddNTP)and deoxynucleotides(dNTP)**

Note: Dideoxy nucleotides are similar to regular, or deoxy, nucleotides, but with one key difference: they lack a hydroxyl group on the 3’ carbon of the sugar ring.

10.5.2 Procedure of Sanger Method

The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labeled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.

DNA polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.

The result: After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called **capillary gel electrophoresis**