MICROBIAL GENETICS AND MOLECULAR BIOLOGY (MCB 302)

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NUCLEIC ACID STRUCTURE

- The nucleic acids, DNA and RNA, are polymers of nucleotides linked together by phosphodiester bond.
- There are two classes of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
- However, DNA and RNA differ in terms of the nitrogenous bases they contain, the sugar component of their nucleotides, and whether they are double or single stranded.
- Deoxyribonucleic acid (DNA) contains the bases adenine, guanine, cytosine, and thymine. The sugar found in the nucleotides is deoxyribose, and DNA molecules usually are double stranded.
- Ribonucleic acid (RNA), on the other hand, contains the bases adenine, guanine, cytosine, and uracil (instead of thymine. Its sugar is ribose, and most RNA molecules are single stranded.
- DNA is the hereditary molecule in all cellular life-forms, as well as in many viruses
- DNA serves as the storage molecule for the genetic instructions that enable organisms to carry out metabolism and reproduction.
- RNA functions in the expression of genetic information so that enzymes and other proteins can be made.
- These proteins are used to build cellular structures and to do other cellular work. The pathway from DNA to RNA and RNA to protein is conserved in all cellular forms of life and is often called the central dogma.

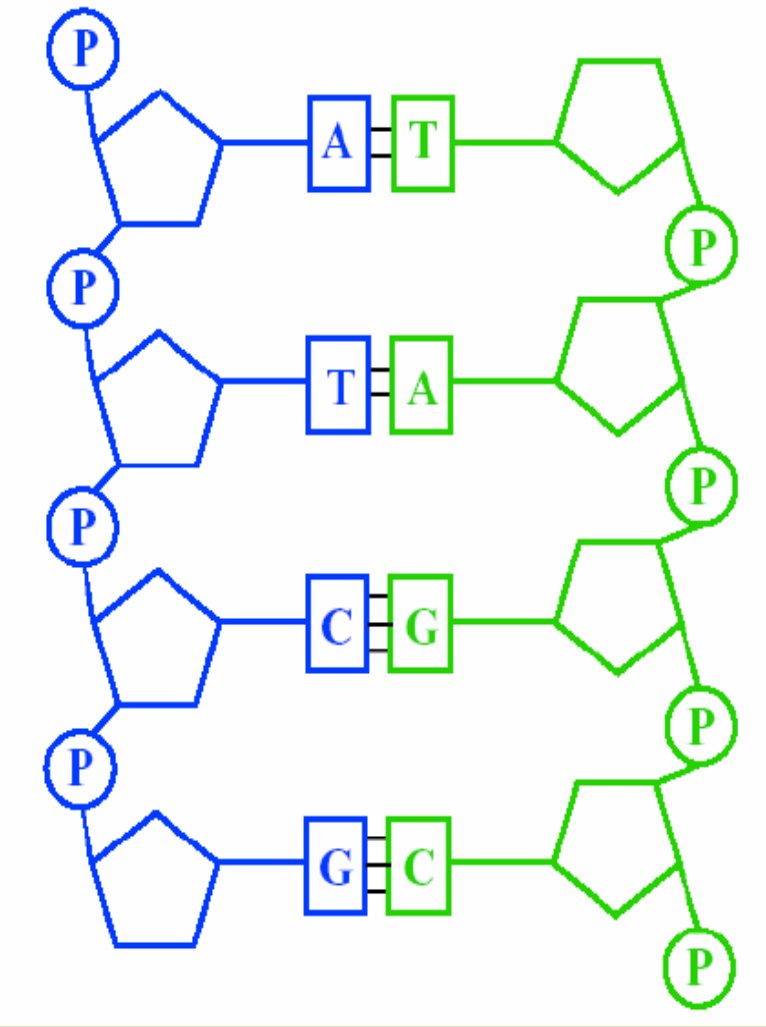

Fig. 1: DNA structure showing the sugar phosphate backbone

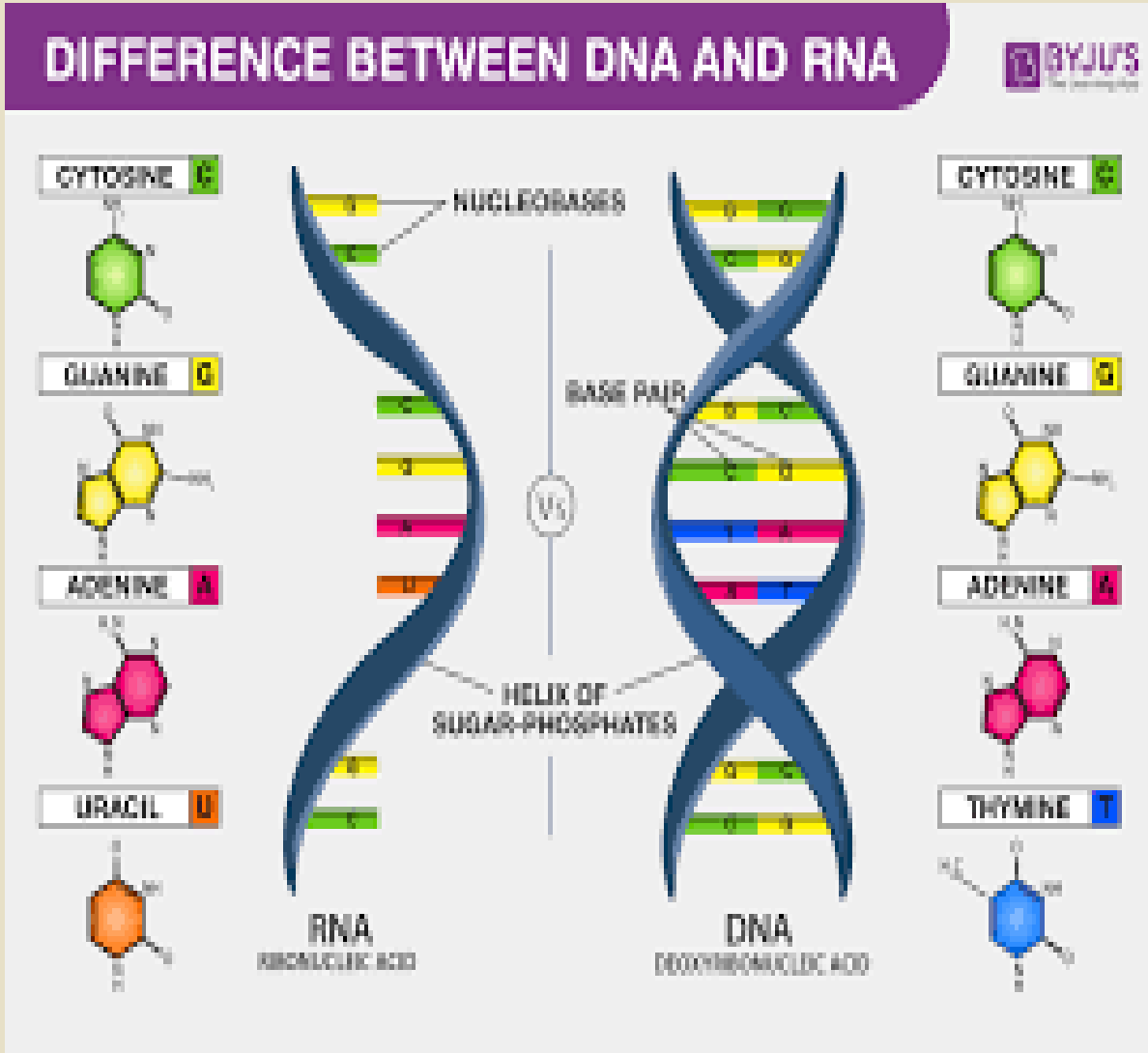

Fig. 2: Illustrating differences between DNA and RNA

NUCLEIC ACID STRUCTURE CONT'D

- The sugar and phosphate groups of DNA form what is often called a backbone to support the bases, which jut out from the chain.
- This structure allows the bases from one single strand of DNA to form hydrogen bonds with another strand of DNA, thereby holding together two separate nucleotide chains.
- The concentration of guanine (G) always equals the concentration of cytosine (C) and the concentration of adenine (A) always equals the concentration of thymine (T).
- Each A-and-T pair and each G-and-C pair in DNA is called a complementary base pair.

DNA replication

- https://youtu.be/3jslVQDGkLU?si=vs52ouOwu5YEc5uZ
- The link above explains DNA replication in details.
- DNA replication takes place in three sequential steps: Initiation, Elongation, and Termination.

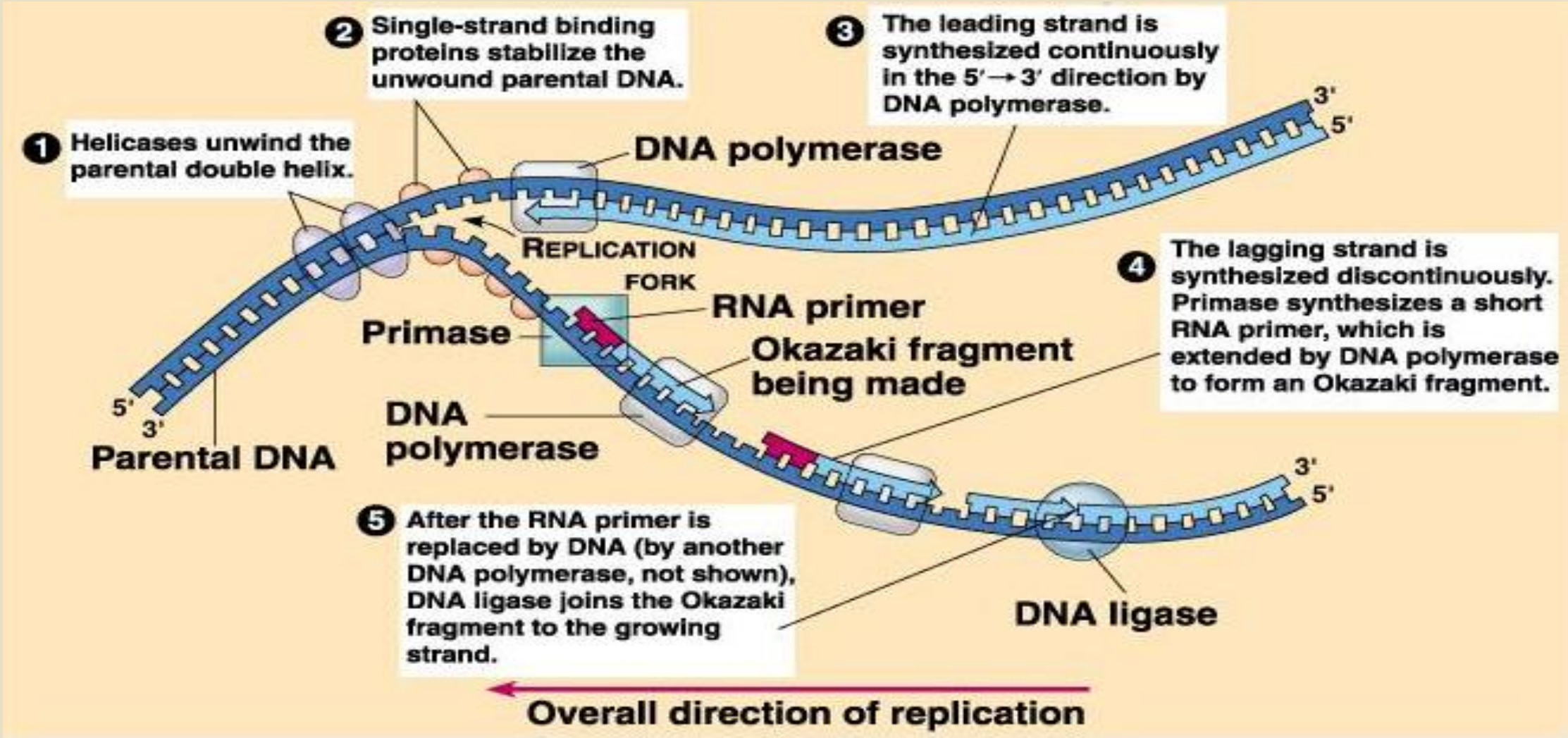

Fig. 3. DNA replication

PLASMIDS

- Plasmids are small, double-stranded DNA molecules that can exist independently of the chromosome.
- Plasmids are not essential for normal bacterial growth and bacteria may lose or gain them without harm.
- They provide an advantage under certain environmental conditions e.g antibiotics resistance
- Some yeasts and fungi also contain plasmids.
- Both circular and linear plasmids have been documented, but most known plasmids are circular.
- Plasmids are able to replicate autonomously but they rely on host enzymes to catalyze their replication.
- Actually, plasmids are quite common in nature and can be found in bacteria, archaea, fungi, and even some plants and animals.

Types of plasmids

- **Conjugative plasmids**: They have genes for the construction of pili and can transfer copies of themselves to other bacteria during conjugation. Perhaps the best-studied conjugative plasmid is the F factor of *E. coli*.
- **Some conjugative plasmids are also R plasmids** (resistance factors, R factors). R plasmids confer antibiotic resistance to the cells that contain them.
- **Col plasmids** contain genes for the synthesis of bacteriocins known as colicins, which are produced by and directed against strains of *E. coli*.
- **Virulence plasmids** encode factors that make their hosts more pathogenic. For example, enterotoxigenic strains of E. coli cause traveler's diarrhea because they contain a plasmid that codes for an enterotoxin.
- **Metabolic plasmids** carry genes for enzymes that degrade substances such as aromatic compounds (toluene), pesticides (2,4-dichlorophenoxyacetic acid), and sugars (lactose).

TRANSPOSABLE ELEMENTS

- DNA segments that carry the genes required for transposition are transposable elements or transposons, sometimes called "jumping genes."
- The simplest transposable elements are insertion sequences (IS). An IS element is a short sequence of DNA. It contains only the gene for the enzyme transposase, and it is bounded at both ends by inverted repeats—identical or very similar sequences of nucleotides in reversed orientation.
- Inverted repeats are usually about 15 to 25 base pairs long and vary among IS elements so that each type of IS has its own characteristic inverted repeats.
- A transposon contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called transpoase.
- Transpoase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition.

TRANSPOSABLE ELEMENTS CONT'D

- **Composite transposons** contain genes in addition to those required for transposition (e.g., antibiotic resistance or toxin genes).
- The process of transposition in procaryotes occurs by two basic mechanisms.
- **A. Simple transposition, also called cut-and-paste transposition**, involves transposase-catalyzed excision of the transposon, followed by cleavage of a new target site and ligation of the transposon into this site. Target sites are specific sequences about five to nine base pairs long.
- **B**. **Replicative transposition:** In this mechanism, the original transposon remains at the parental site on the chromosome and a replicate is inserted at the target DNA site.

MUTAGENESIS

- A mutation is a permanent alteration in the DNA sequence of a gene or chromosome.
- Mutations can occur in both somatic cells (non-reproductive cells) and germ cells (reproductive cells)
- Mutations occur in one of two ways: Spntaneous and Induced mutation.
- **Spontaneous mutations** arise occasionally in all cells and in the absence of any added agent.
- This can result from errors in DNA replication or from the action of mobile genetic elements such as transposons.
- **Induced Mutations arise from the action of** any agent that damages DNA, alters its chemistry, or in some way interferes with its functioning will induce mutations.
- Three common types of chemical mutagens are **Base analogs (5-bromouracil, an analog of thymine), DNA-modifying agents**(methyl-nitrosoguanidine)**, Intercalating agents**(acridines) such as proflavin and acridine orange.

Point mutations: mutation in protein coding genes

- Point mutations in protein-coding genes can affect protein structure in a variety of ways.
- The most common types of point mutations are **silent mutations, missense mutations, nonsense mutations, and frameshift mutations.**
- **Silent mutations** change the nucleotide sequence of a codon but do not change the amino acid encoded by that codon.
- **Missense mutations** involve a single base substitution that changes a codon for one amino acid into a codon for another.
- **Nonsense mutations** convert a sense codon (i.e., one that codes for an amino acid) to a nonsense codon (i.e., a stop codon: one that does not code for an amino acid).
- **Frameshift mutations** arise from the insertion or deletion of one or two base pairs within the coding region of the gene. Since the code consists of a precise sequence of triplet codons, the addition or deletion of fewer than three base pairs cause the reading frame to be shifted for all codons downstream.

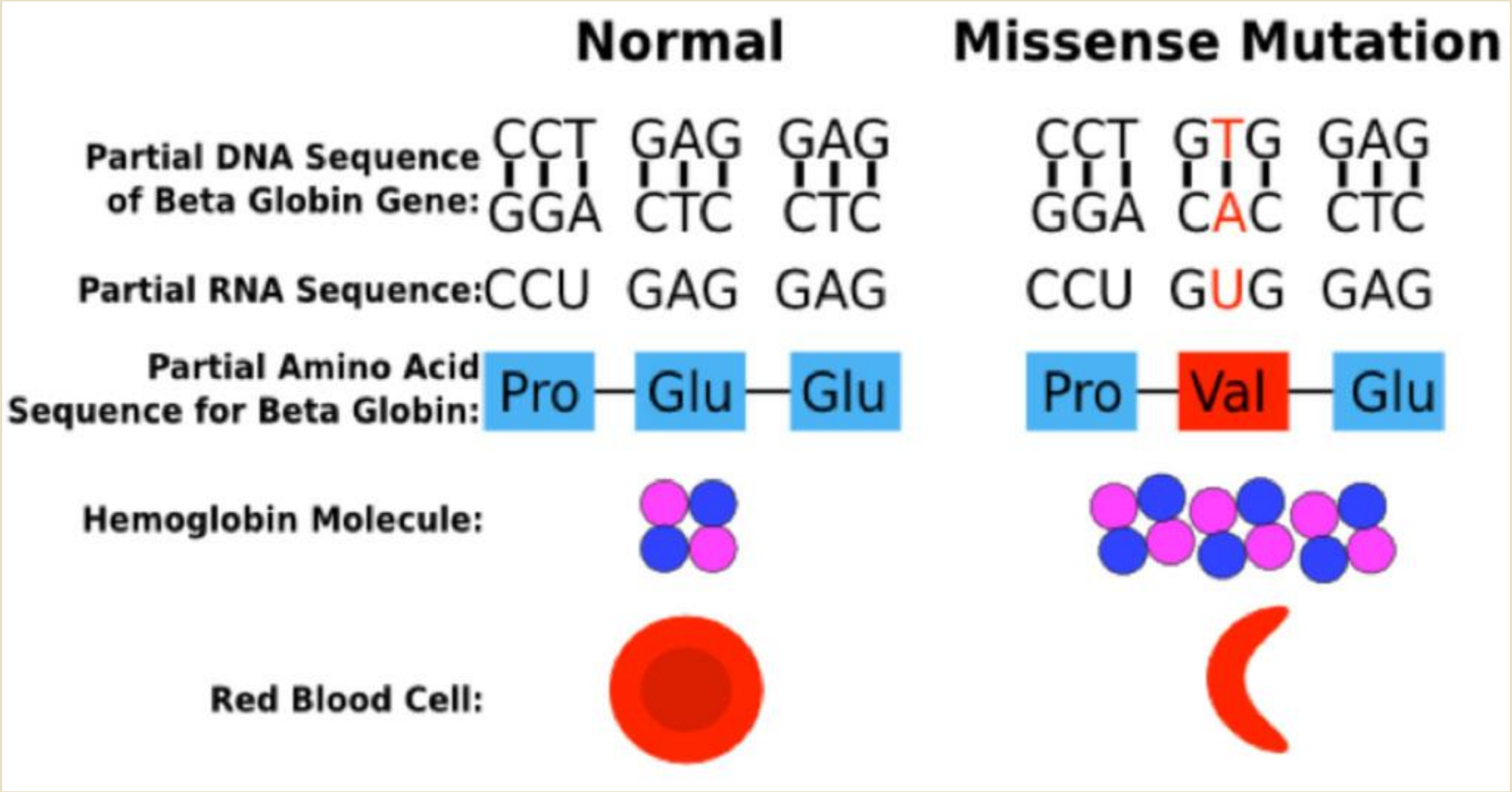

Fig. 4: Missense mutation that leads to sickle cell anaemia

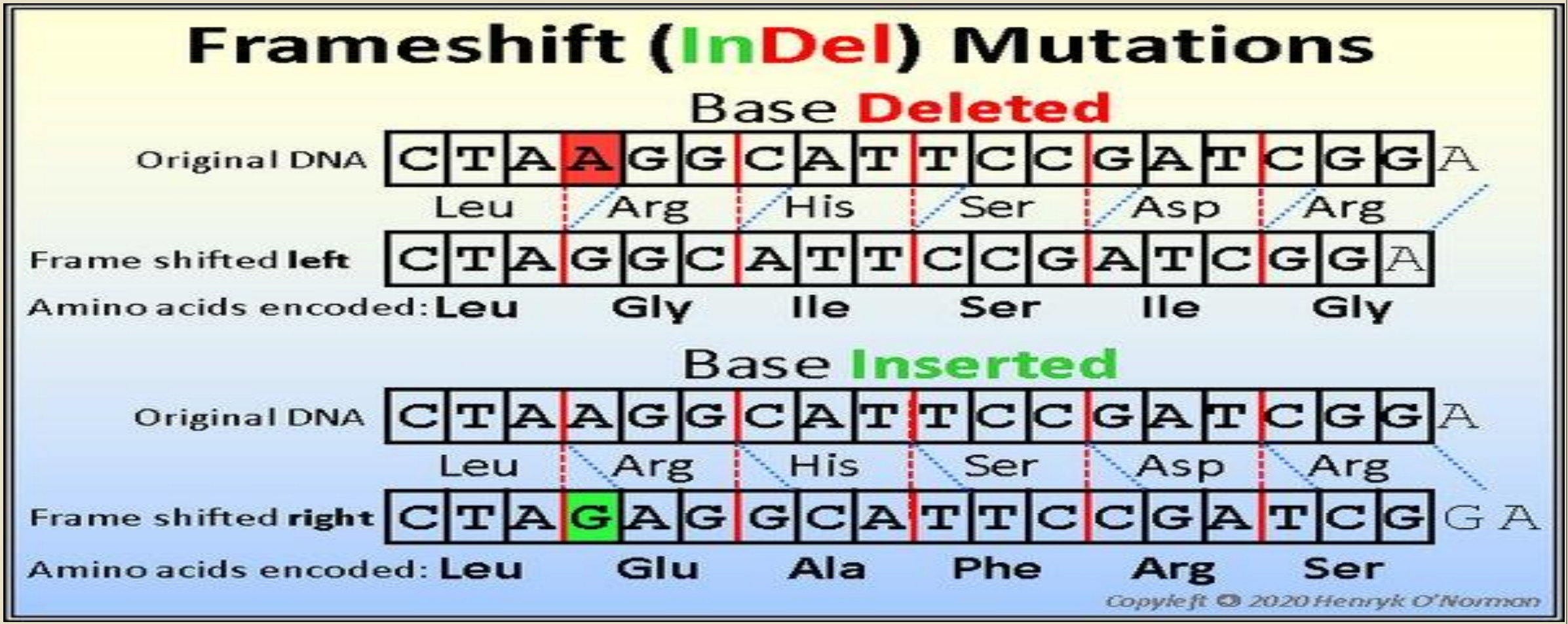

Fig 5: Frameshift mutation

Detection of mutants

- **The replica plating technique** is used to screen for auxotrophic mutants.
- It distinguishes between mutants and the wild-type strain based on their ability to grow in the absence of a particular biosynthetic end product.
- A lysine auxotroph, for instance, grows on lysine-supplemented media but not on a medium lacking an adequate supply of lysine because it cannot synthesize this amino acid.
- **Mutant Selection**
- An effective selection technique **uses incubation conditions under which the mutant grows**, because of properties conferred by the mutation, whereas the wild type does not.
- Selection methods often involve **reversion mutations** or the **development of resistance to an environmental stress**. For example, if the intent is to isolate revertants from a lysine auxotroph the approach is quite easy.
- **Substrate utilization mutations** also are employed in bacterial selection. Many bacteria use only a few primary carbon sources e. g. Glucose. With such bacteria, it is possible to select mutants by plating a culture on medium containing an alternate carbon source. Any colonies that appear can use the substrate and are probably mutants.

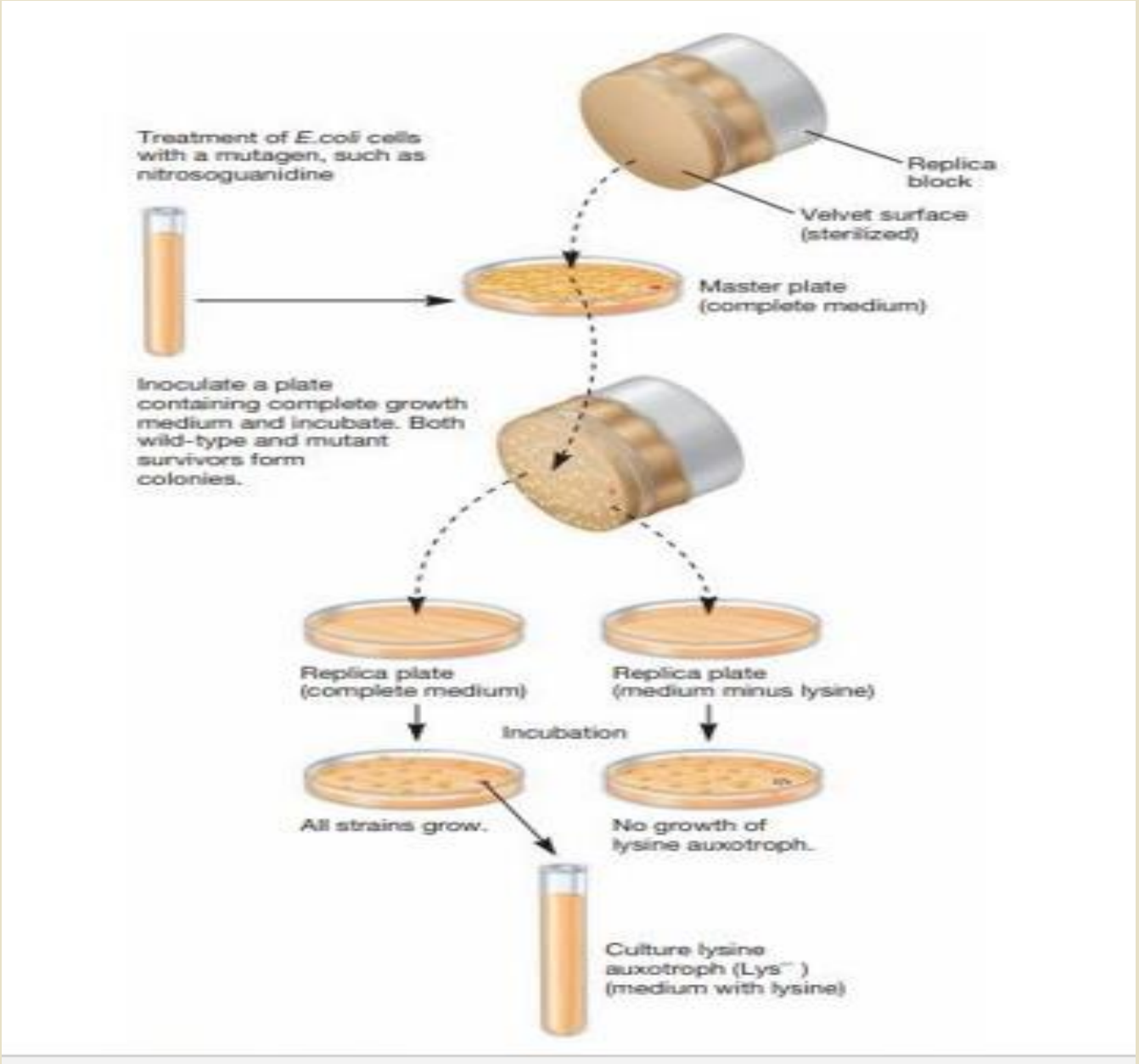

Fig 6: Replica plating technique

DNA REPAIR

- Because replication errors and mutagens can alter nucleotide sequences, a microorganism must be able to repair any changes that might be lethal.
- **Mechanisms of DNA repair:**
- **A. Excision Repair (nucleotide excision repair and base excision repair)**
- **b. Direct Repair**
- **C. Mismatch Repair**
- **D. Recombinational Repair**

GENETIC EXCHANGE IN PROKARYOTES

- Genetic exchange in prokaryotes, such as bacteria, is a process that allows these organisms to share and recombine genetic material, which can lead to increased genetic diversity and the acquisition of new traits.
- Procaryotes have evolved three different mechanisms for creating recombinants. These mechanisms are referred to collectively as **horizontal (lateral) gene transfer (HGT)**.
- In HGT, genes from one independent, mature organism are transferred to another, often creating a stable recombinant having characteristics of both the donor and the recipient.
- During HGT, a piece of donor DNA, the exogenote, enters a recipient cell.
- The transfer can occur in three ways: direct transfer between two cells temporarily in physical contact (conjugation), transfer of a naked DNA fragment (transformation), and transport of DNA by viruses (transduction).

GENE EXPRESSION IN PROKARYOTES

- Gene expression is the process by which the information encoded in a gene is used to direct the synthesis of a functional gene product, typically a protein. This process involves multiple steps, each regulated to ensure that genes are expressed at the right time, place, and levels within an organism.
- **Key Stages of Gene Expression**
- **Transcription**: The synthesis of RNA from a DNA template.
- **1. Initiation**

Promoter Recognition: RNA polymerase binds to the promoter region of the DNA. Prokaryotic promoters typically contain conserved sequences, such as the -10 (TATAAT) and -35 (TTGACA) boxes, which are recognized by the sigma factor of RNA polymerase.

Open Complex Formation: The DNA strands are unwound to form an open complex, allowing RNA polymerase to access the template strand.

- **2. Elongation**

RNA Synthesis: RNA polymerase synthesizes the RNA strand in the 5' to 3' direction, complementary to the DNA template strand. The RNA sequence is the same as the coding (non-template) strand of DNA, except that uracil (U) replaces thymine (T).

- **3. Termination**

Rho-Dependent Termination: The rho protein binds to the RNA and moves along it until it reaches the RNA polymerase, causing the release of the RNA transcript.

Rho-Independent Termination: A GC-rich region followed by a series of uracils forms a hairpin loop in the RNA transcript, causing RNA polymerase to dissociate from the DNA.

GENE EXPRESSION IN PROKARYOTES

- **Translation in Prokaryotes**
- **1. Initiation**

Ribosome Binding: The small ribosomal subunit binds to the Shine-Dalgarno sequence on the mRNA, a purine-rich sequence located upstream of the start codon (AUG).

Initiator tRNA Binding: The initiator tRNA carrying formylmethionine (fMet) binds to the start codon.

Assembly of Ribosomal Subunits: The large ribosomal subunit joins the complex, forming the complete ribosome ready for elongation.

- **2. Elongation**

Codon Recognition: The ribosome moves along the mRNA, and each codon is recognized by a complementary tRNA carrying the appropriate amino acid.

Peptide Bond Formation: The ribosome's peptidyl transferase activity forms peptide bonds between adjacent amino acids.

Translocation: The ribosome moves one codon down the mRNA, shifting the tRNA from the A site to the P site and from the P site to the E site, where it is then released.

- **3. Termination**

Stop Codon Recognition: When the ribosome encounters a stop codon (UAA, UAG, UGA), release factors bind to the ribosome, causing the release of the newly synthesized polypeptide.

Disassembly: The ribosomal subunits dissociate, and the mRNA is released.

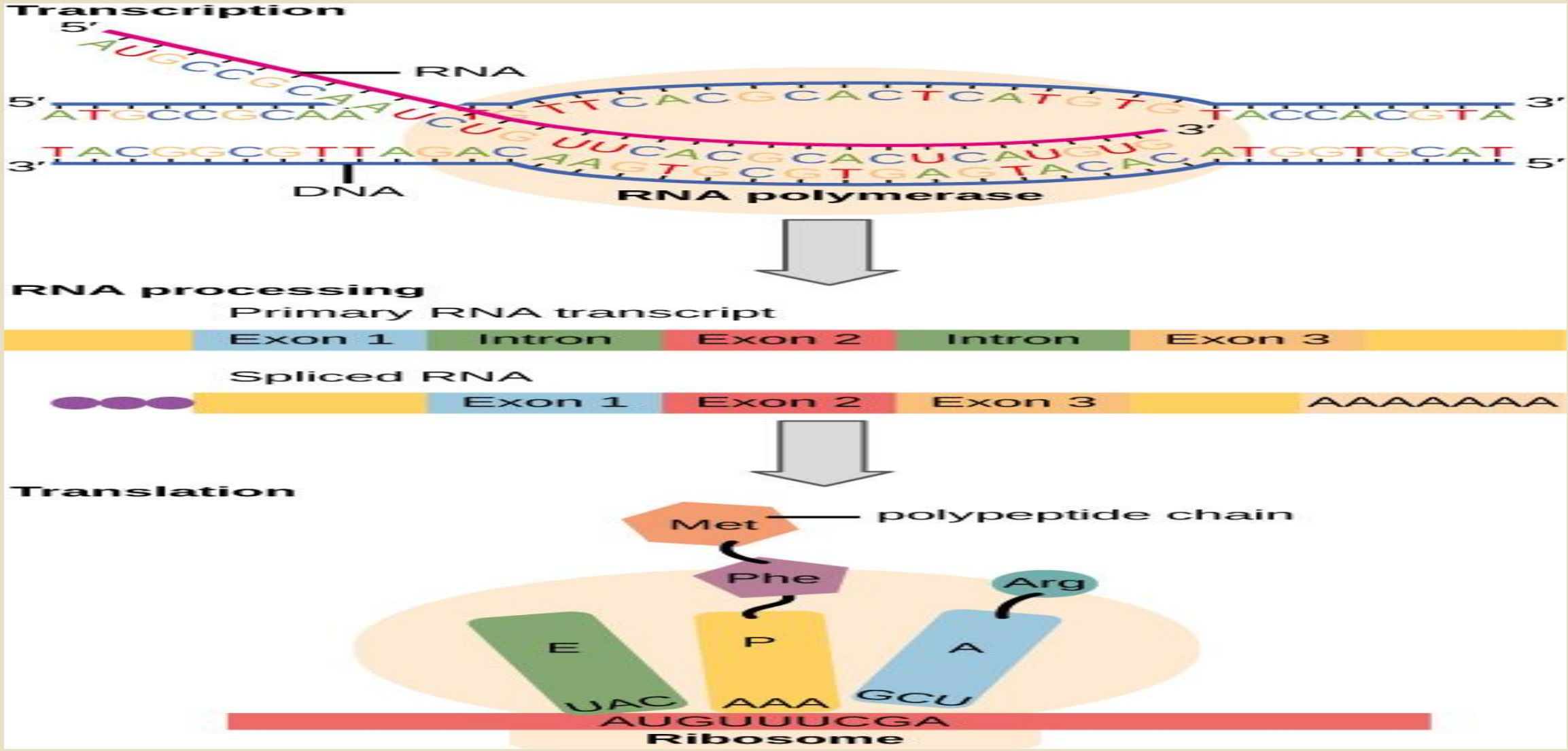

Fig 7: Gene expression in prokaryotes

Regulation of Gene Expression in Prokaryotes

- **1. Operons**
- **Operon Structure**: An operon is a cluster of genes under the control of a single promoter, transcribed as a single mRNA. It includes structural genes, a promoter, and an operator.
- **Lac Operon**: An example is the lac operon in E. coli, which is regulated by the presence or absence of lactose and glucose. It includes genes involved in lactose metabolism, regulated by the repressor protein and catabolite activator protein (CAP).
- **2. Transcriptional Regulation**
- **Repressors and Activators**: Proteins that bind to DNA sequences near the promoter to inhibit or enhance transcription. Repressors block RNA polymerase binding, while activators facilitate it.
- **Sigma Factors**: Different sigma factors recognize different promoter sequences, allowing for the regulation of groups of genes in response to environmental changes.

GENETIC CODE

- The genetic code is the set of rules by which information encoded within genetic material (DNA or RNA sequences) is translated into proteins by living cells. This code defines how sequences of nucleotide triplets, called codons, specify which amino acid will be added next during protein synthesis. Here's a detailed explanation of the genetic code and its features:
- **Key Features of the Genetic Code**
- **Codons**: The genetic code is based on triplets of nucleotides, known as codons. Each codon specifies a single amino acid or a stop signal for translation.
  - **Start Codon**: The codon AUG serves as the start signal for translation and also codes for the amino acid methionine.
  - **Stop Codons**: Three codons (UAA, UAG, UGA) signal the end of translation and do not code for any amino acids.
- **Redundancy**: The genetic code is redundant, meaning that most amino acids are encoded by more than one codon. For example, leucine is encoded by six different codons (UUA, UUG, CUU, CUC, CUA, CUG).
- **Universality**: The genetic code is nearly universal, meaning that almost all organisms use the same code. There are some exceptions, such as in mitochondrial genomes and some protozoans, where slight variations in the code occur.
- **Non-overlapping**: The genetic code is read in a non-overlapping manner, with each nucleotide being part of only one codon.
- **Continuous**: The code is read continuously, without gaps or interruptions, from a fixed starting point on the mRNA.

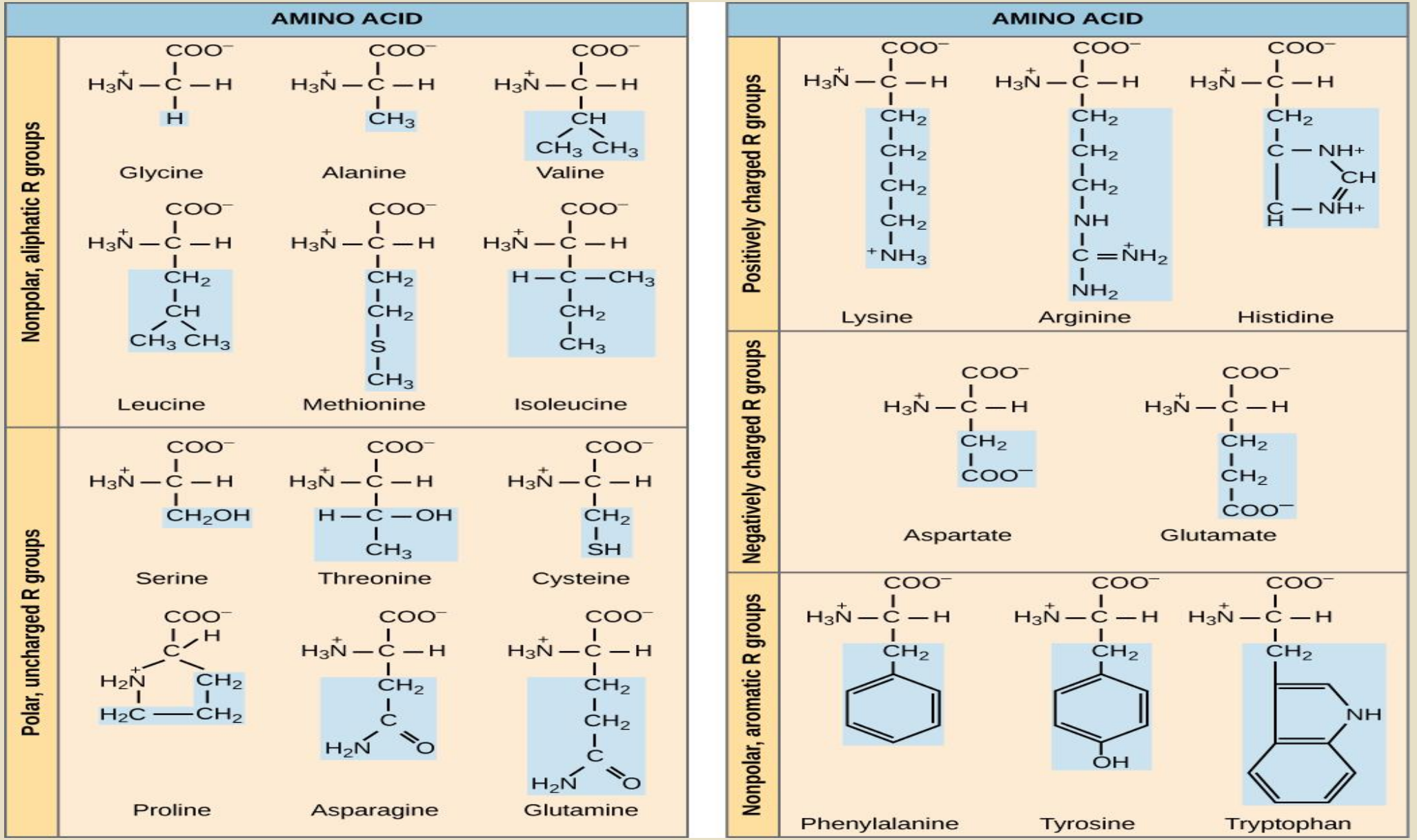

Fig 8: Codon table

Recent techniques in microbial genetics

- **DNA Extraction:**
- Although the order of nitrogen bases is what determines DNA's instructions, or genetic code, so the DNA sequences contain the information (gene or genes) required for building organism.
- During molecular techniques, the extraction of DNA is the first step to preformed cloning, DNA must be isolated from the organism and purified in order to prevent interaction with other molecules such as RNA, proteins, polysaccharide. DNA can extracted by different methods.
- **MOLECULAR CLONING**
- Molecular cloning refers to the isolation of DNA sequence from any species (often a gene), and its insertion into a vector for propagation, without alteration of the original DNA sequence. It is also a laboratory technique used to create copies of a specific DNA sequence or gene of interest.
- General steps involved in molecular cloning: Isolate DNA, Vector preparation, DNA digestion, Ligation, Transformation, Selection and screening, Amplification and expression.
- **DNA sequencing**
- The process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery.

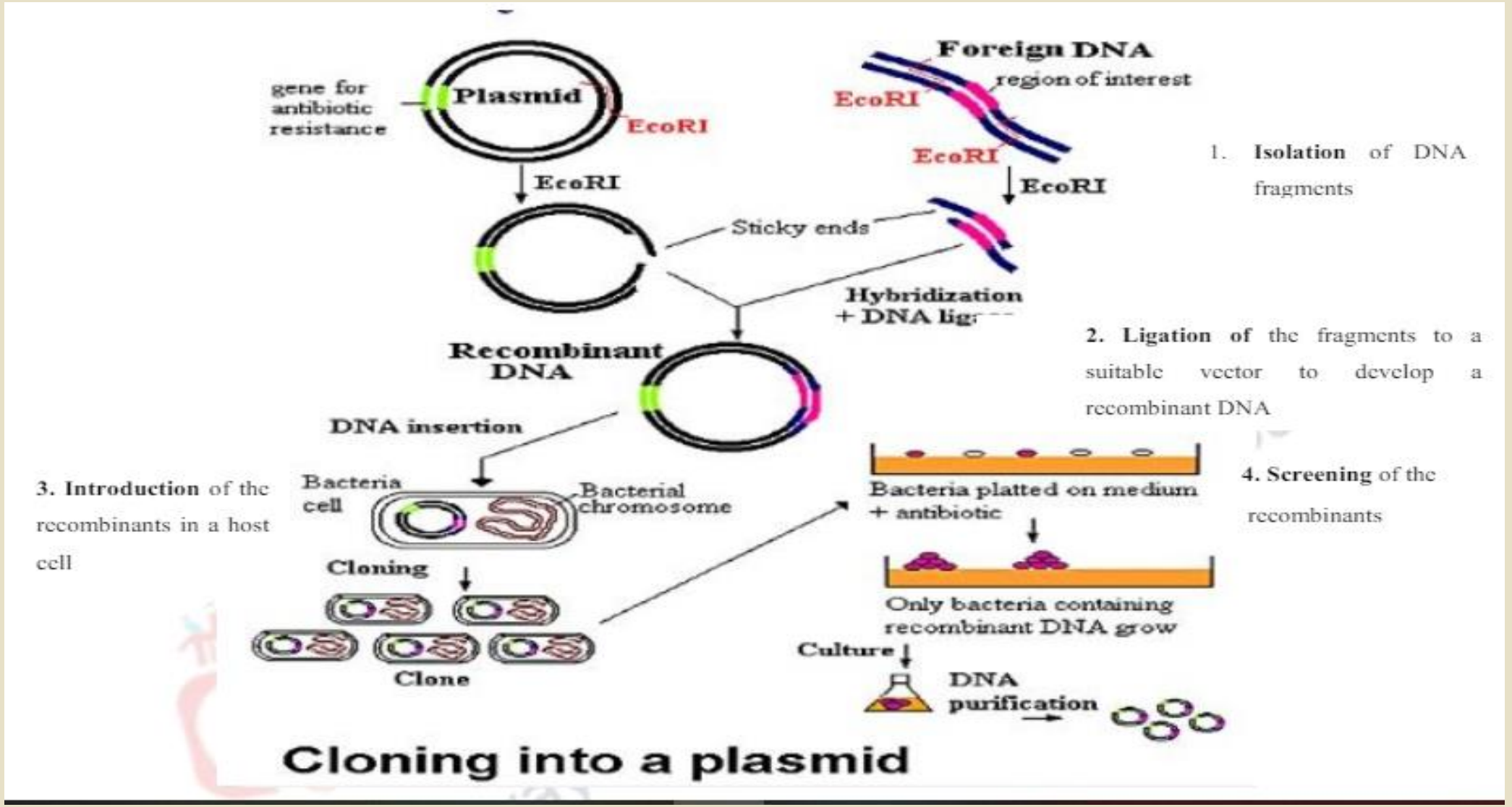

Fig 9: Cloning into a plasmid

Recent techniques in microbial genetics

- **The polymerase chain reaction (PCR)** :-
- is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- **PCR principles and procedure**:-
- PCR technique is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size.
- **PCR steps:-**
- **Denaturation step**: This step is the first regular cycling event and consists of heating causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
- **Annealing step:** The reaction temperature is lowered for allowing annealing of the primers to the single-stranded DNA template.
- **Extension/elongation step**: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary (opposite) to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction.

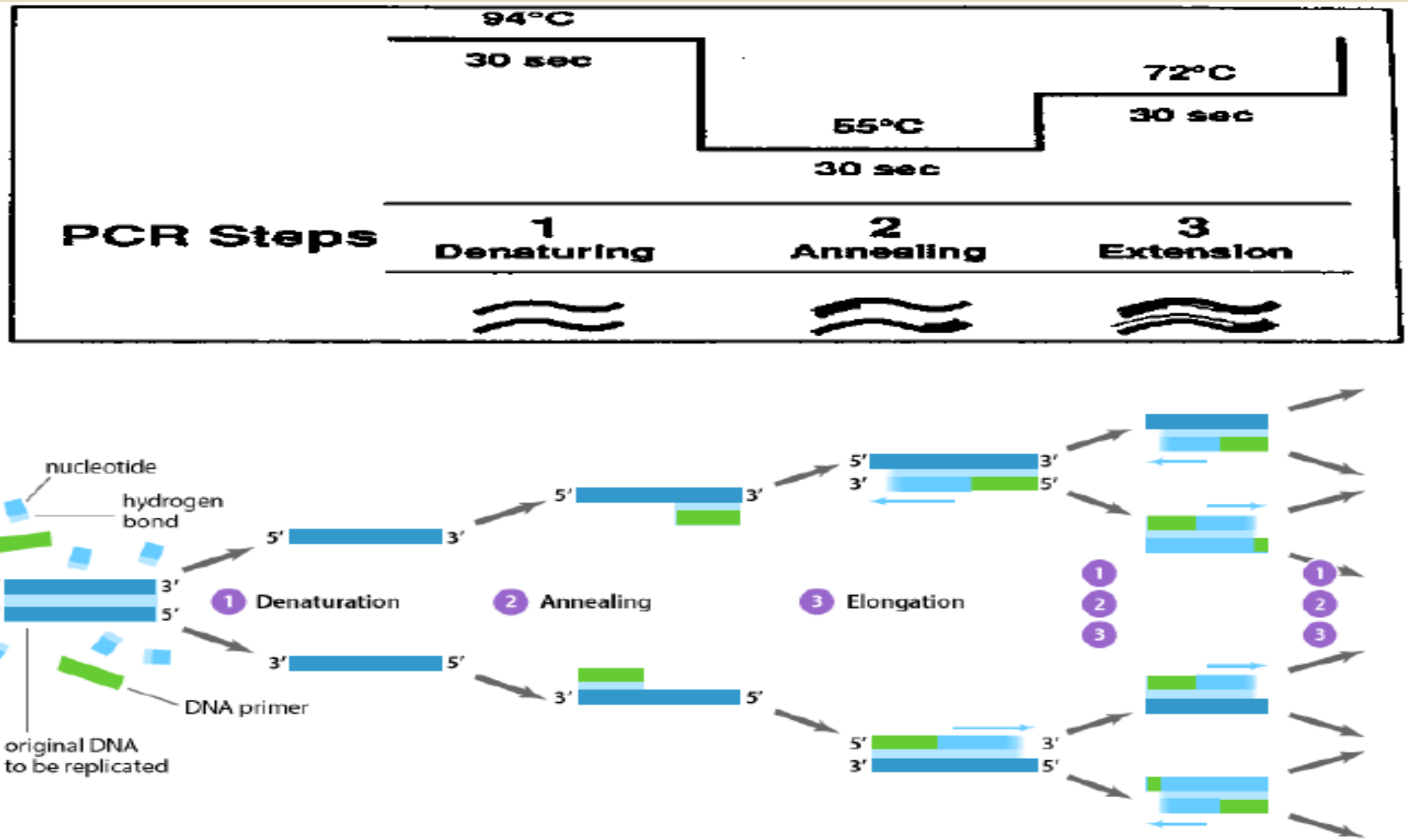

Fig. 10: illustration of Polymerase Chain reaction

Recent techniques in microbial genetics

- **Agarose Gel Electrophoresis**
- Agarose Gel Electrophoresis is a laboratory technique used to separate DNA fragments or RNA fragments based on their size. The DNA or RNA samples are loaded into wells in a gel made of agarose and an electrical current is applied. The fragments move through the gel and are separated based on their size, with smaller fragments moving further down the gel.
- **Southern Blotting Technique**
- Southern Blotting is a laboratory technique used to detect a specific DNA sequence in a sample. DNA samples are cut into fragments using restriction enzymes and separated using agarose gel electrophoresis.
- **Northern Blotting Technique**
- Northern Blotting is a similar technique to Southern Blotting, but is used to detect specific RNA sequences instead of DNA sequences. RNA samples are separated using agarose gel electrophoresis and transferred to a membrane.
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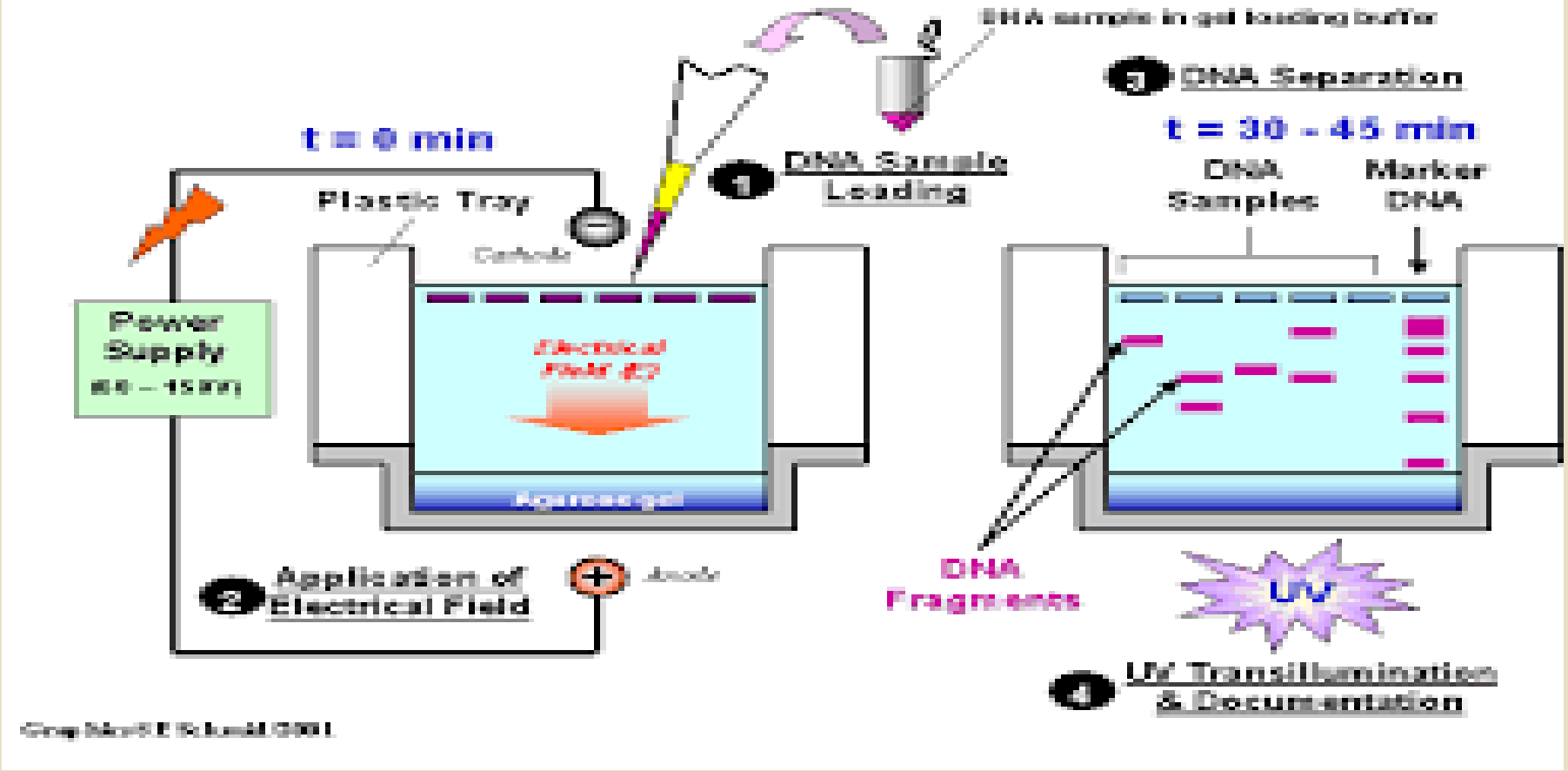

Fig 11: Agarose gel electrophoresis

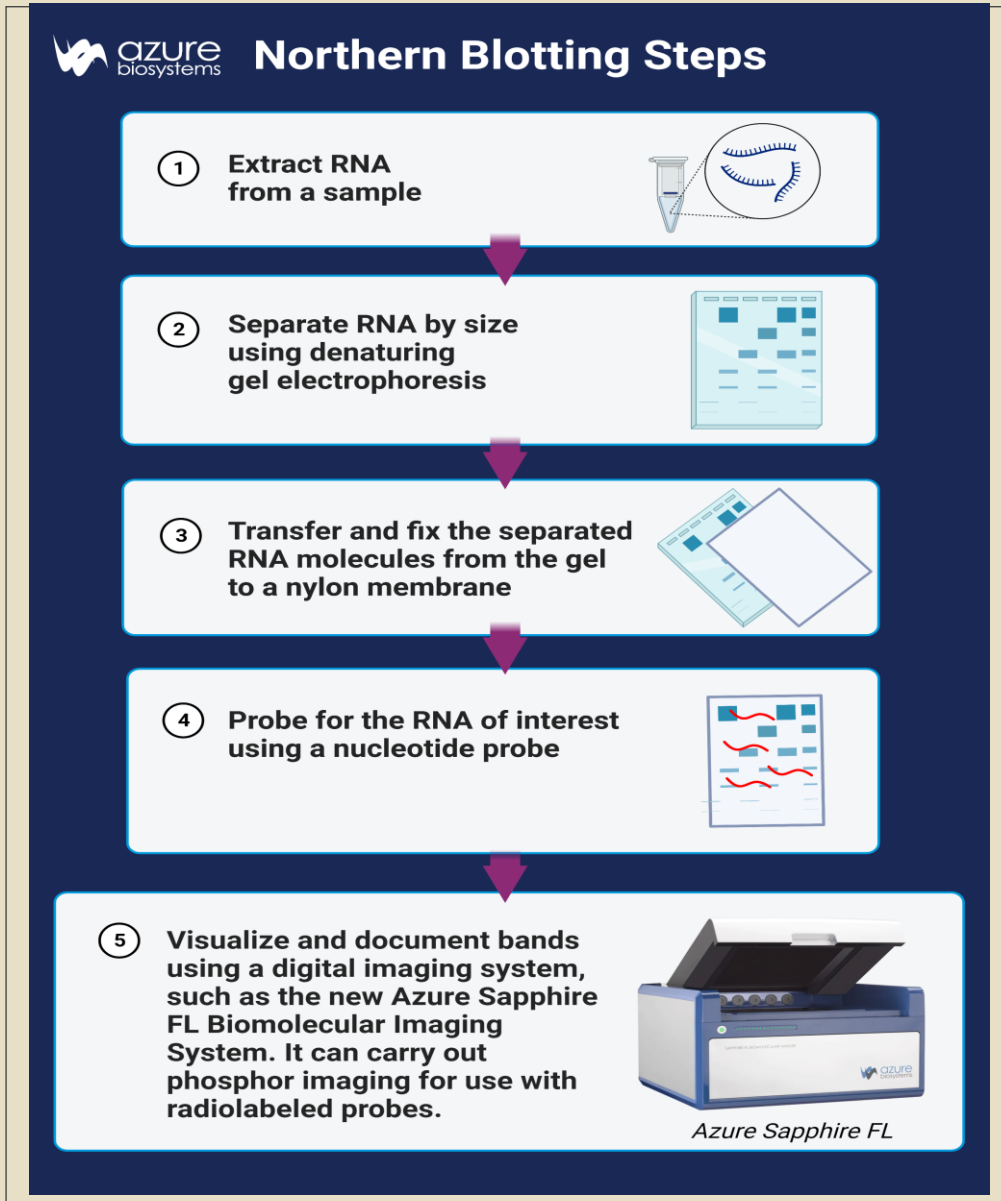

Fig 12: Northern blotting

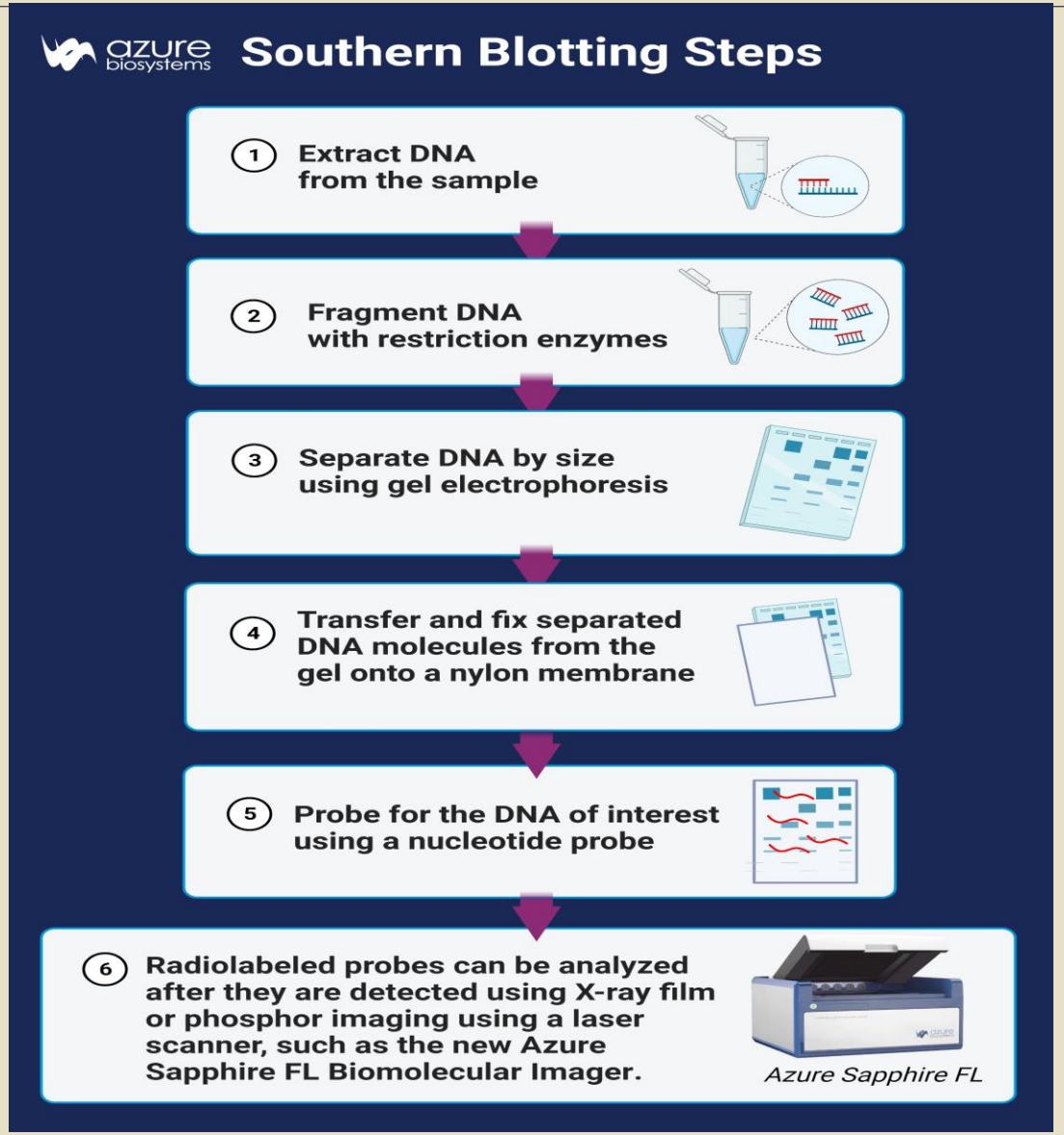

Fig 13: Southern blotting

Recent techniques in microbial genetics

- **Dot Blot**
- Dot Blot is a simplified version of Southern and Northern blotting techniques. It involves the direct transfer of the DNA or RNA sample onto a membrane, without prior separation on a gel.
- **DNA Micro-array Analysis**
- DNA Micro-array Analysis is a laboratory technique used to analyze gene expression. It involves the use of a microchip containing thousands of DNA probes that can detect specific gene sequences.
- **SDS-PAGE**
- SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) is a laboratory technique used to separate proteins based on their size. The protein samples are treated with a detergent called SDS, which denatures the proteins and gives them a uniform negative charge.
- **Western Blotting**
- Western Blotting is a laboratory technique used to detect a specific protein in a sample. Proteins are separated using SDS-PAGE and transferred to a membrane. The membrane is then treated with a primary antibody that binds specifically to the target protein.

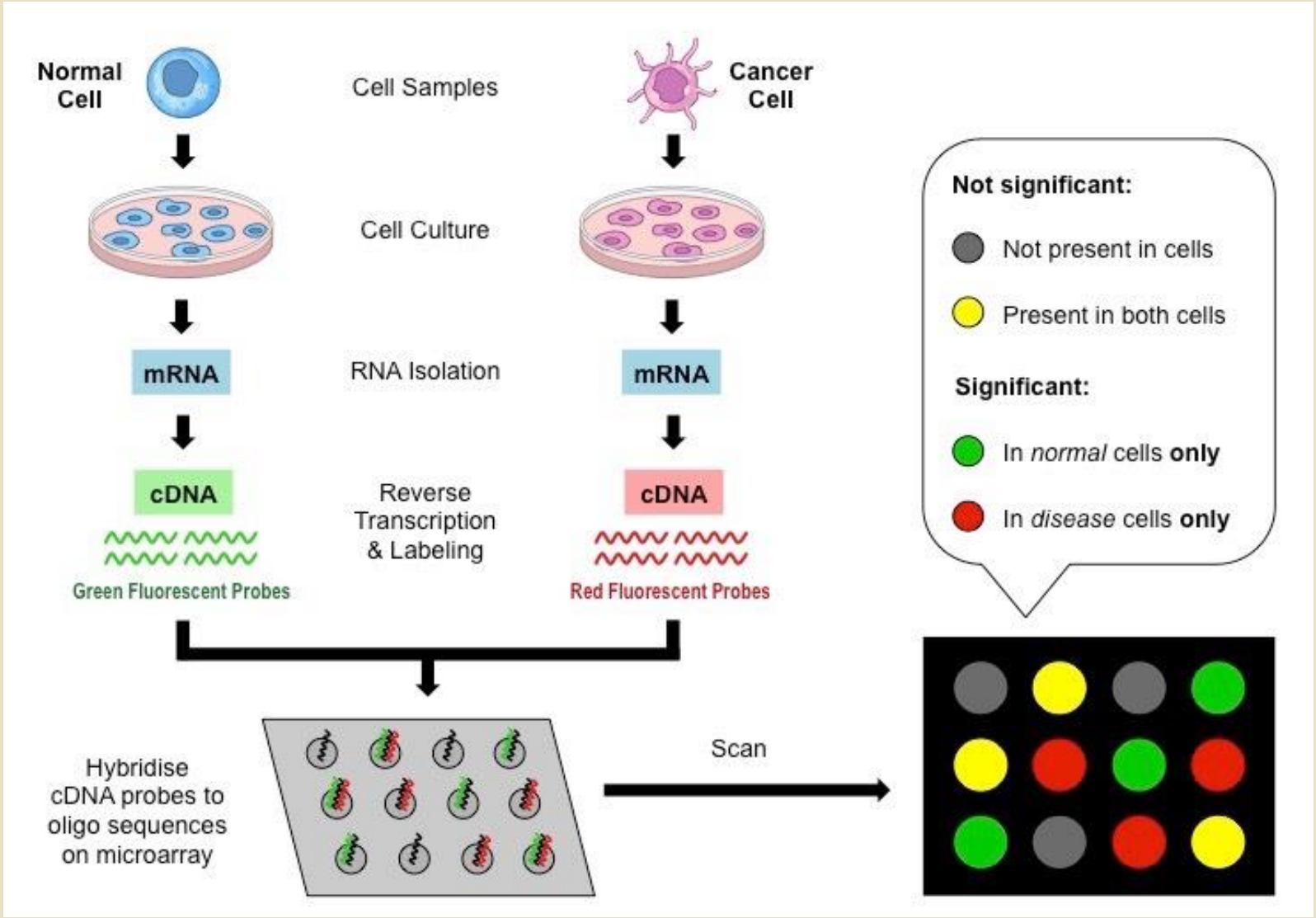

Fig 14: Hybridization technique

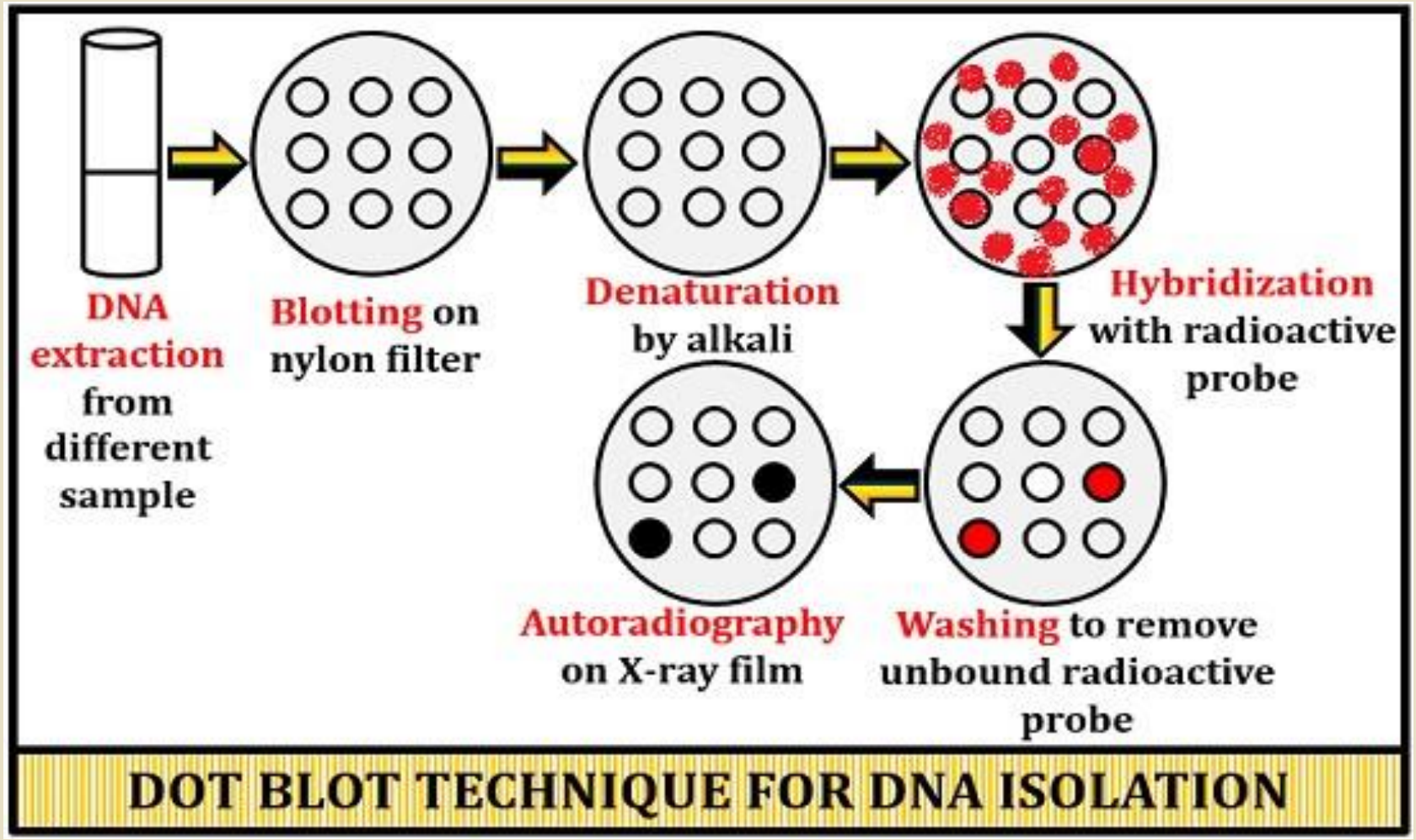

Fig. 15: DOT Blot technique

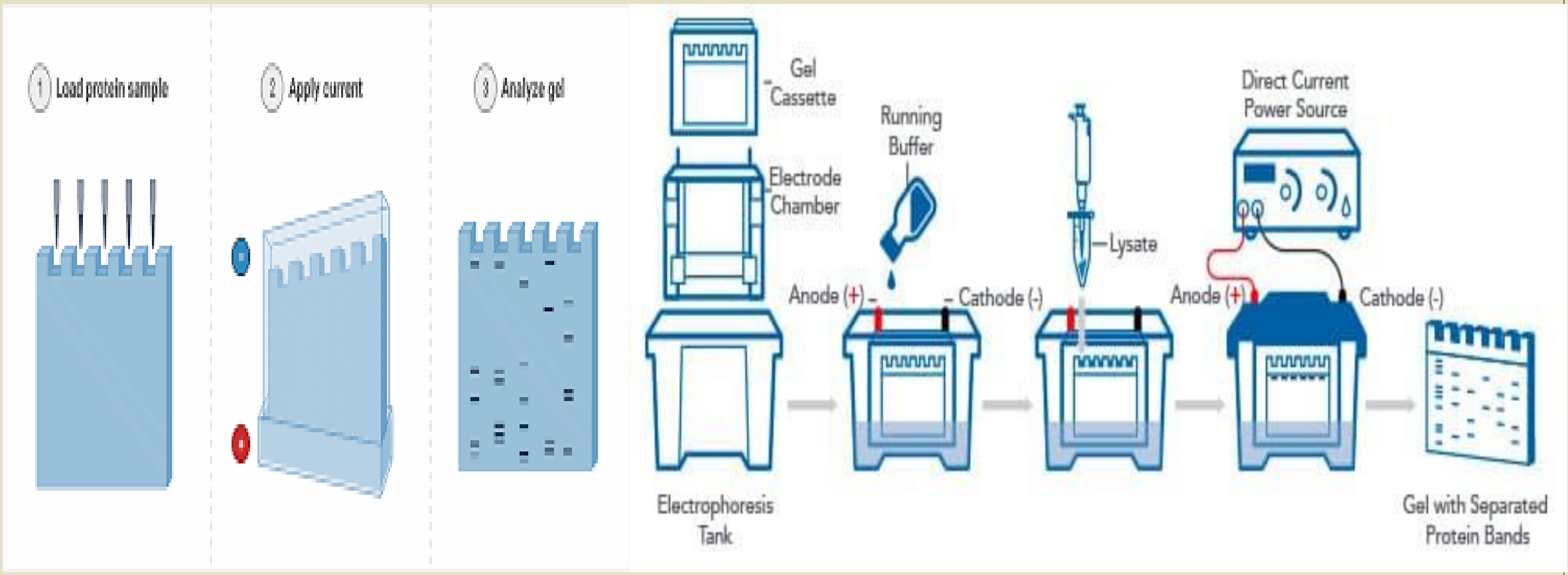

Fig. 16: SDS Page workflow

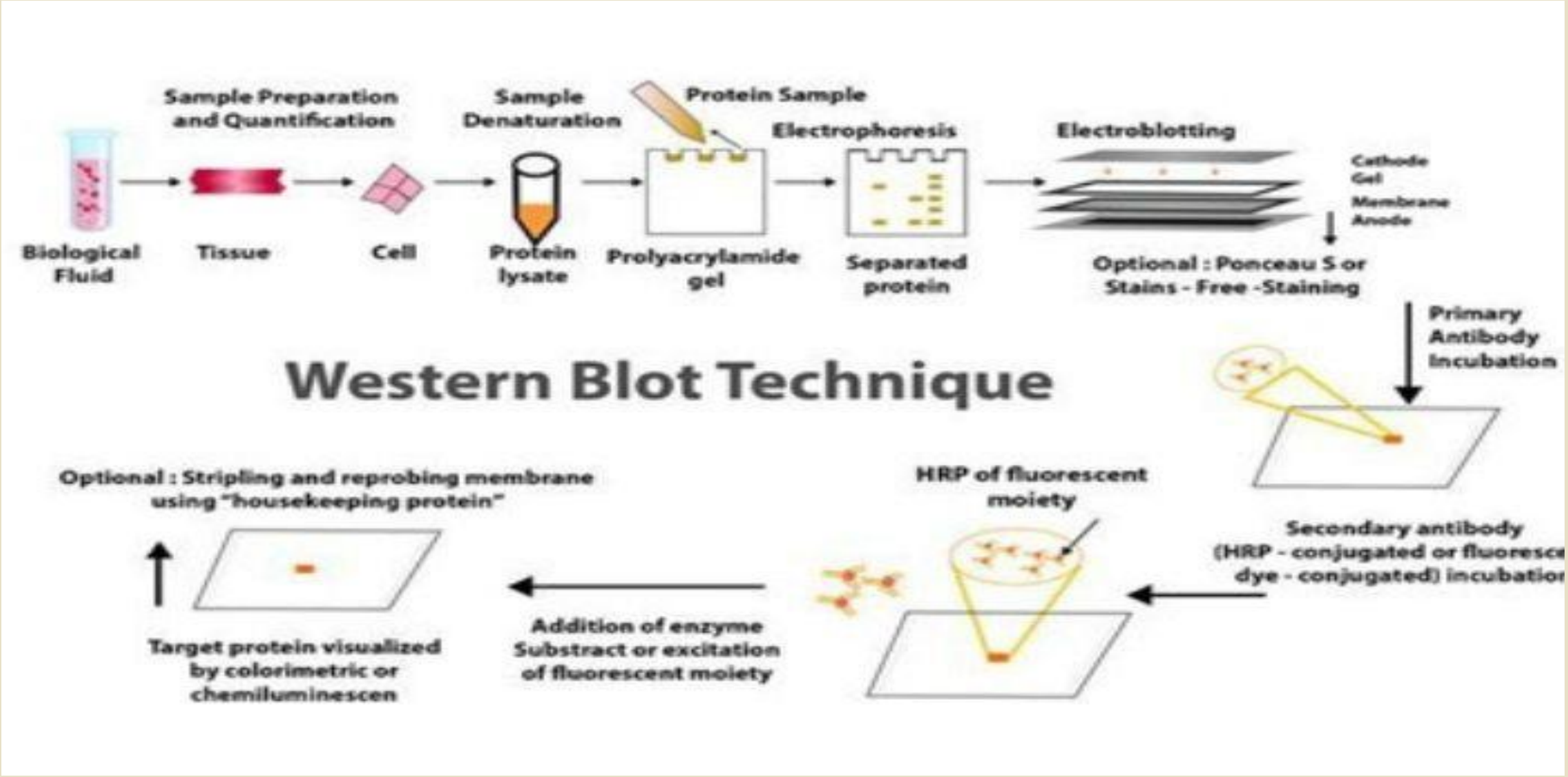

Fig. 17: Western Blot Technique

Recent techniques in microbial genetics

- **Hybridization** is the process by which a single-stranded nucleic acid molecule (DNA or RNA) forms a double-stranded molecule by pairing with a complementary strand.
- This technique is fundamental in various molecular biology methods, including Southern blotting, Northern blotting, and in situ hybridization.
- hybridization of biomolecules for applications such as identifying species' relatedness, discovering evolutionary relationships among the organisms, detection and/or location of specific nucleotide sequences, detection of infectious agents in several diagnostic assays.
- **Restriction enzymes**:- One of the important tools of hybridization technique is restriction or called **restriction endonuclease** can be used for cleaving DNA molecules double strand at specific recognize nucleotide sequences that **know restriction sites** .
- In order to be able to sequence DNA , it is first necessary to cut the strand into smaller fragments, this produces a heterogeneous collection of fragments of varying size.
- For example, the bacterium *Haemophilus aegypticus* produce enzyme was named Hae III That cuts DNA wherever it encounters sequence

Recent techniques in microbial genetics

- **Genetic Engineering:** Genetic engineering, also called recombinant DNA technology, involves the group of techniques used to cut up and join together genetic material, especially DNA from different biological species, and to introduce the resulting hybrid DNA into an organism in order to form new combinations of heritable genetic material.
- Example of genetically engineering (transgenic) organisms including:
- 1- One example of a transgenic microorganism is the bacterial strain that produces human insulin.
- 2- Plants with resistance to some insects, plants that can tolerate herbicides, and corps with modified oil content.

Applications of Genetic engineering

- **1. Application in Agriculture:** alter the genotype of crop plants to make them more productive, nutritious, rich in proteins, disease resistant, and less fertilizer consuming. Recombinant DNA technology and tissue culture techniques can produce high yielding cereals, pulses and vegetable crops. E.g. Golden rice, Bt. cotton, Bt. Corn.
- **2. Application to Medicine:** Production of antibiotics, vaccines, enzymes and proteins, Digestive enzymes, Single Cell Protein (SCP), Gene therapy.
- **3. Energy Production:** It is now possible to bioengineer energy crops or biofuels that grow rapidly to yield huge biomass that used as fuel or can be processed into oils, alcohols, diesel, or other energy products.
- The waste from these can be converted into methane. Genetic engineers are trying to transfer gene for cellulase to proper organisms which can be used to convert wastes like sawdust and cornstalks first to sugar and then to alcohol.
- **4. Application in Industries:** Genetically modified plants supply different resources like starch, fuel, pharmaceuticals in huge quantity • Proteases are used in detergents • Biofertilizers production