STRUCTURE AND DIAGNOSTIC APPLICATIONS OF DNA

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Overview of Deoxyribonucleic Acid (DNA) structure

What are the Nitrogenous bases in DNA?

- Four Nitrogenous bases in DNA:
- Two Purines and Two Pyrimidines;
  - **Purine bases** are:
    - **Adenine (A)**
    - **Guanine (G)**;
  - **Pyrimidine bases** are:
    - **Thymine (T)**
    - **Cytosine (C);**

What are the Nucleosides in DNA?

- **Nucleoside**: Pyrimidine or Purine base covalently bonded to a sugar;
  - **Nucleoside** = Nitrogenous base + Sugar;
- In **DNA**, sugar is **Deoxyribose**,
- DNA contains **Deoxy-Nucleosides**:
  - **Nitrogenous base + Deoxy-ribose**;
- **Four** types of Deoxy-Nucleosides in DNA:
  - **Deoxy-Adenosine,**
  - **Deoxy-Guanosine,**
  - **Deoxy-Thymidine**
  - **Deoxy-Cytidine**

What are the Nucleotides in DNA?

- **Nucleotide**: Nucleoside bonded to Phosphate group
  - **Nucleotide** = **Nucleoside + Phosphate**
- In **DNA**, Nucleotides are **Deoxy-Nucleotides**, because the sugar is **Deoxyribose**;
- **Deoxy-Nucleotide = Deoxy-Nucleoside + Phosphate**
- **Four** types of **Deoxy-Nucleotides in DNA**:
  - **Deoxy-Adenosine Monophosphate (dAMP),**
  - **Deoxy-Guanosine Monophosphate (dGMP),**
  - **Deoxy-Thymidine Monophosphate (dTMP),**
  - **Deoxy-Cytidine Monophosphate (dCMP);**

What type of bonds link the Deoxy-Nucleotides in DNA?

- DNA contains deoxy-nucleotides covalently linked by **3’5’- Phosphodiester Bonds**;
- The bonds form the repetitive **Sugar-Phosphate Chain** that is the **Back-bone** to which Nitrogenous bases are attached;

What base pairs are formed between complementary strands in DNA?

- **Purine** base pairs with specific **Pyrimidine**,
- **Adenine** pairs with **Thymine**
  - **A = T** {Two hydrogen bonds}
- **Guanine** pairs with **Cytosine**
  - **G ≡ C** {Three hydrogen bonds}

Brief describe Watson and Crick mode of DNA structure

- In 1953 Watson and Crick proposed the Three-dimensional structure of DNA,
- DNA is made up of Two Strands wound round each other to form a Double Helix,
- DNA strands are in an **anti-parallel** arrangement, because the strands run in opposite directions,
    - One strand is oriented 5’ ⟶ 3’ direction
    - The other is oriented 3’ ⟶ 5’ direction

- Nitrogenous bases are on the inside and the Sugar-Phosphate backbone on the outside of the double helix,
- Complementary base pairings are formed between Nitrogenous bases on one strand and corresponding bases on the other strand;
  - **A:T base pair**
  - **G:C base pair**
- On each strand the Helical structure is repeated after every 10 residues,
- After every 10 residues the Helix turns 360 degrees;

What do you understand by the term DNA sequence?

- **DNA sequence**: Arrangement of bases **A, C, G, T** along DNA strands;
  - Precise sequence of bases carries the Genetic Information,
- In DNA structure each deoxy-nucleotide unit represents a Single Letter in an Alphabet that has only Four Letters: A, G, C, T
  - Different Genes have different sequential arrangements of these Four Letters,
  - Therefore each gene codes for different biological message;

- Deoxynucleotides in DNA differ only in the sequence of bases they carry, thus they are recorded simply according to their base sequences;
- For example:
  - ACTTCAGACC is part of the base sequence of one gene that codes for a particular protein;
  - TGGAACCGTCA is part of the base sequence of a different gene that code for a different protein;
- By tradition base sequence is written in the order from the 5’ end of the DNA strand to the 3’ end (i.e., 5’ $\longrightarrow$ 3’ direction);

DNA REPLICATION

What are the two possible ways of Replication of DNA?

- **DNA REPLICATION : Formation of Complementary DNA Strands,**
  - DNA Replication occurs when Double-helical DNA strands separate and act as Templates for formation of New Complementary DNA Stands;
- Two possible ways of Replication of DNA are:
  - **Conservative Replication,**
  - **Semi-Conservative Replication,**

- **Conservative Replication**:
  - After replication:
    - Parental DNA strands stay together, and
    - Newly synthesized DNA strands stay together;
- **Semi-Conservative Replication**:
  - During replication the parental DNA strands are separated and each acts as template to its Newly synthesized Complementary strand;
  - Each new DNA contains one original strand and one newly synthesized DNA strand;

What are the basic components required for DNA Replication?

- **Enzyme**: DNA–dependent DNA polymerases (require a DNA template);
  - Catalyze DNA synthesis during replication;
  - Copy DNA Template by catalyzing addition of Deoxy-nucleotide units to DNA chain,
- **Substrates**: Four Deoxy-Nucleosides Triphosphates (dNTPs);
  - d ATP, d GTP, d CTP, d TTP;
  - Cleavage of 2 high-energy phosphate bonds provides energy for forming the Phosphodiester bond;

- **Template**: DNA replication requires a Template;
  - Template directs addition of complementary Deoxy-nucleotide to the newly synthesized DNA strand;
  - Semi-conservative replication, each strand of parent DNA serves as template,
- **Primer**: DNA replication requires a Primer,
  - Primer prepares the template strand for addition of Deoxy-nucleotides;
- Primer is a short piece of RNA with a free 3’-OH group;

What is the role of Primase in DNA replication?

- **Primase:** RNA Polymerase that synthesizes the Primer for DNA replication;
  - Primase synthesizes RNA directly on the single-stranded DNA template because it does not require a Primer to begin synthesis;
- New Deoxy-nucleotides are added to 3’ end Primer,
- DNA Replication occur in 5’ to 3’ direction;

Briefly state how DNA replication occurs in Eukaryotes

- DNA Replication is semi-conservative,
  - It occurs bi-directionally from many origins;
  - Use of multiple origins ensure that Chromosomal DNA is replicated within the necessary time period,
- At each origin, replication bubble forms consisting of two replication forks moving in opposite direction,

- DNA replication under control of a single origin is called a **Replicon**,
  - **Replicons are basic units of replication,**
- DNA replication proceeds until replication bubbles merge together;
- **DNA replication takes place in 5’ to 3’ direction**;
  - DNA Polymerase reads the parental DNA stand in the 3’ to 5’ direction;

What are the functions of DNA Polymerases in Eukaryotic cells?

- Eukaryotic cells have 5 different DNA Polymerases;
- **DNA polymerase α**:

  Synthesizes RNA Primer,

  - Involves in replication of lagging strand in chromosomal DNA,
- **DNA polymerase δ**:
  - Involve in replication of leading strand in chromosomal DNA;
  - Contains proofreading activity (3’ to 5’ Exonuclease)

- **DNA polymerase β**: Involve in DNA repair;
- **DNA polymerase ε**: Involve in DNA repair;
- **DNA polymerase γ**: Involve in replication of mitochondrial DNA;

What is the leading strand in DNA replication?

- Leading Strand in DNA:
  - Complementary DNA strand that is copied in the direction of the advancing replication fork,
- Leading strand is synthesized continuously by DNA polymerase δ;

What is the lagging strand in DNA replication?

- Lagging Strand:
  - Complementary DNA strand that is copied in the opposite direction of advancing replication fork,
  - Synthesis proceeds discontinuously, by DNA polymerase α, creating small fragments of DNA called **Okazaki fragments**;
- RNA primers for lagging strand are made by DNA polymerase α which carries a Primase activity;
- DNA Ligase links Okazaki fragments together to form continuous DNA strand;

TRANSCRIPTION

What is Transcription?

- **Transcription:**
  - Formation of Messenger RNA (m-RNA) from DNA directed by DNA Template,

DNA (Gene) ======➔ m-RNA

- Transcription occurs in three phases:
  - Initiation,
  - Elongation,
  - Termination;

What are the major types of RNA?

- **Three major types of RNA**:
- **Messenger RNA (m-RNA)**:
  - Transcribed from DNA in Nucleus of eukaryotes
  - Carries information from Genes (DNA) to Ribosomes for translation into proteins,
  - Contain **Codons,**

- **Ribosomal RNA (r-RNA):**
  - Part of the structure of Ribosomes;

- **Transfer RNA (t-RNA)**:
  - Specific for each Amino Acid (AA),
  - Transfer Amino Acids to Ribosomes for protein synthesis,
  - Facilitate addition of Amino Acids into newly synthesizing proteins;
- Sequences (**Anti-codons**) that pair with the appropriate **Codons** in the Ribosome are unique for each t-RNA;

What are the types of RNA Polymerases in eukaryotes?

- **RNA Polymerase I:**
  - Synthesizes Ribosomal RNA (r-RNA);
- **RNA Polymerase II:**
  - Synthesizes Messenger RNA (m-RNA);
- **RNA Polymerase III:**
  - Synthesizes Transfer RNA (t-RNA);

Briefly describe the Initiation phase in Transcription

- **Initiation**:
  - RNA polymerase II binds to specific site (**Promoter site**) on DNA;
  - **Promoter region** is located close to the Gene to be Transcribed;
  - Promoter region is known as **TATA box** [**TATAAT**];
  - **RNA polymerase II unwinds the DNA locally to expose a single-stranded DNA template that it can copy;**

Briefly describe the Elongation phase in Transcription

- After Initiation, the Promoter site identifies the correct DNA strand to be copied by RNA polymerase;
- RNA polymerase moves along the gene, synthesizing a complementary RNA copy of the DNA template;
- DNA strand copied is called **Antisense (-) strand;**
- **RNA produced has the same sequence as the non-template DNA strand, called Sense (+) strand**
  - (**m-RNA contains U instead of T**);

Briefly describe Termination phase of Transcription

- RNA polymerase encounters a termination signal and stop the Transcription,
- Transcribed RNA (m-RNA) is released, and it dissociates from the DNA template;

What are the basic requirements for Transcription?

- **Template:**
  - One of the DNA strands acts as template to direct formation of complementary RNA,
- **Substrates:**
  - Ribonucleoside-Triphosphates: **ATP, GTP, CTP, UTP;**
  - Two high-energy bonds provide the energy needed for addition of nucleotides to growing RNA chain;
- **Direction of Transcription:**
  - Transcription proceed in the **5' to 3' direction**

- **Enzymes: RNA polymerases**:
- **Prokaryotes**:
  - Single RNA polymerase synthesizes all cellular RNA,
- **Eukaryotes: Four RNA polymerases:**
  - One Mitochondrial RNA polymerase,
  - Three Nuclear RNA polymerases,
  - No proofreading activity in the RNA polymerases;

- **Promoter sequences:**
  - Initiation of Transcription does not require a Primer,
  - Promoter sequences direct RNA polymerase to initiate transcription at a particular point,
- **Initiation factors:**
  - Needed to initiate transcription,
  - Prokaryotes use a single factor called sigma;
  - Eukaryotes multiple factors are needed,
- **Post-transcriptional RNA processing:**
  - After transcription of a gene, post-translational modifications or processing events may be needed before the transcript is functional,

GENETIC INFORMATION

What is the "Gene"?

- Gene is the segment of a DNA (Chromosome) that contains information of how to synthesize a Polypeptide chain (Protein),
- Genes are located in:
  - DNA (Chromosomes) of Eukaryotes,
  - RNA of Prokaryotes,

What are Exons and Introns?

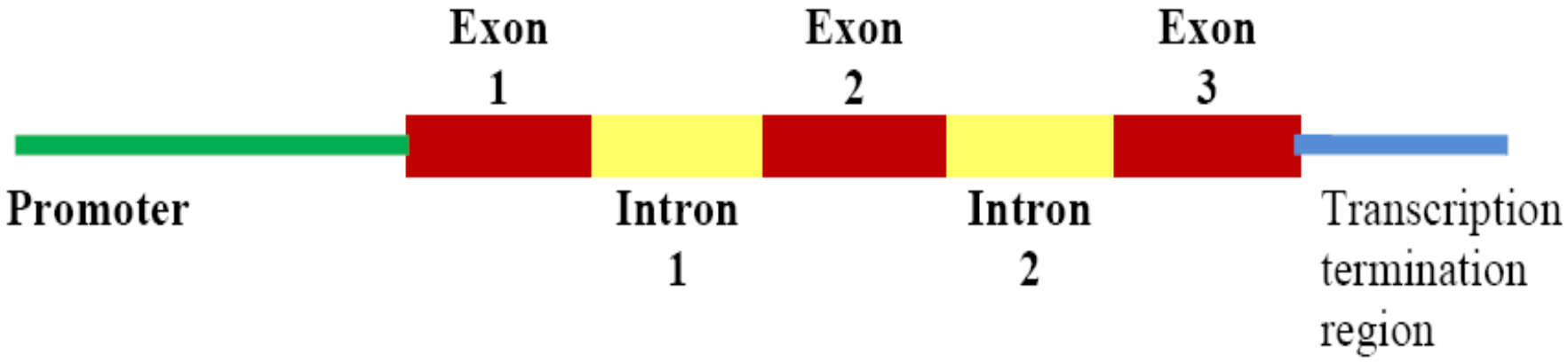
- **Exons are the sequences in DNA that codes for proteins;**
  - Exons are spliced together to form m-RNA,

- **Introns are the intervening sequences in DNA that do not code for protein**;
  - Introns are non-coding regions in the DNA,
  - Introns are not present in m-RNA,

Briefly describe the basic structure of a protein-coding gene in eukaryotes **(see Fig. 1)**

- Most protein-coding genes in eukaryotes are discontinuous,
- Exons are the coding sections of the gene,
- Introns are the non-coding sections of the gene,
- Coding sections (Exons) are interrupted by non-coding sections (Introns),
- Number of Introns in protein-coding genes varies,

Fig 1: Schematic diagram of the basic structure of protein coding gene;



What is the Genetic Information?

- **Genetic Information is the DNA sequences that specifies the correct Amino Acids for synthesis of a specific protein;**
- Genetic Information is encoded as the sequence of Nucleotide Bases on one Strand of DNA,
- Genetic Code is the sequence of Nucleotide bases in DNA,
- Function of a protein is determined by its amino acid sequence,
  - thus the DNA sequence (Genetic Information) must be exact;

Where is the genetic information located in the cell?

- Genetic Information is located in the genetic material: DNA of eukaryotes; RNA of Prokaryotes,
- DNA of eukaryotes is located in the Nucleus;
- In Eukaryotes:
  - Genetic information is Transcribed on Messenger RNA (m-RNA) in the nucleus,
  - m-RNA is then transferred to the Cytosol;
  - **m-RNA carries the genetic information from the nucleus to the cytosol for synthesis of protein**;

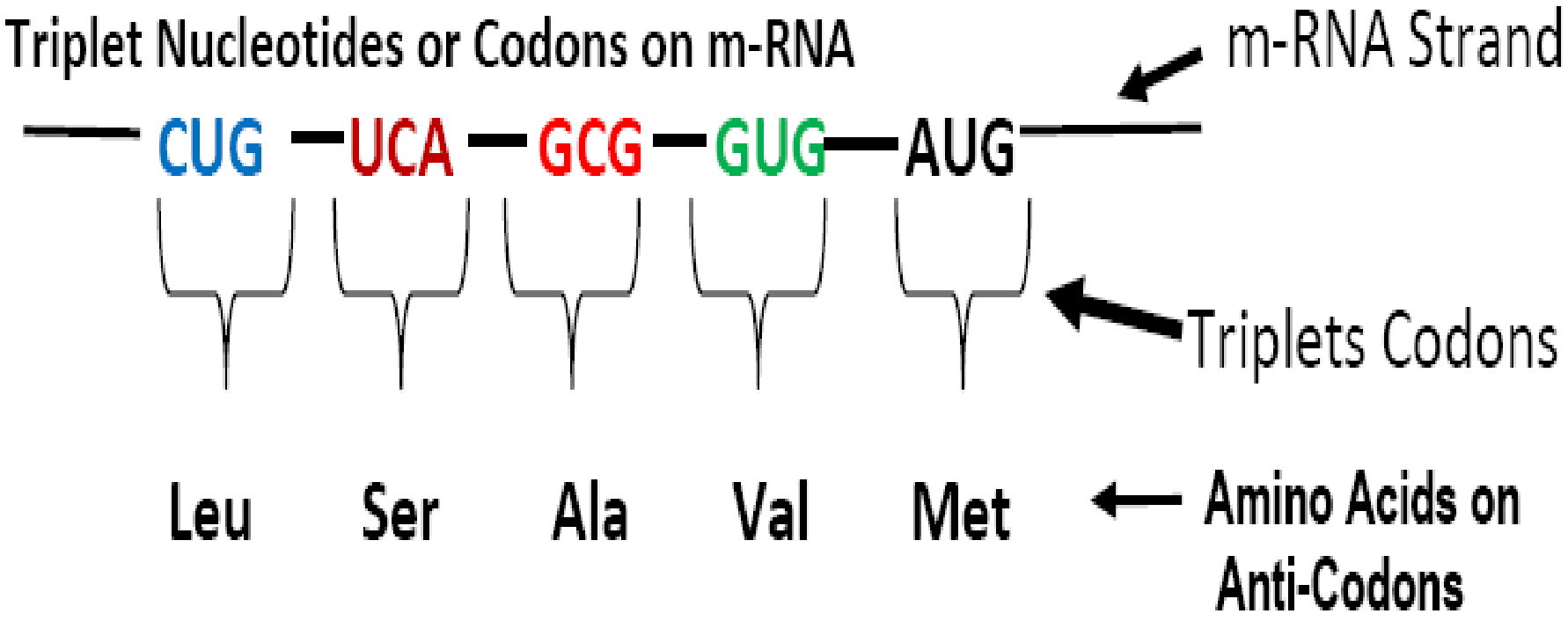
What is the genetic code?

- **Genetic Code represents the Genetic Information;**
- Genetic Code is the relationship between the Nucleotide sequence in m-RNA and Amino Acid sequence in Protein (Polypeptide);
- **Genetic Code is the set of rules that specify how the Nucleotide sequences of m-RNA is translated into the Amino Acid sequence of a polypeptide;**
- Genetic Code is made up of "**Triplet Nucleotides**" called **Codons**,
- **There are 64-Triplet Nucleotides (Codons) in the Genetic Code;**

What is a Codon?

- Nucleotide sequence in m-RNA is in groups of "**Three Nucleotides**" called **Codons**;
- Codon is the Three-Nucleotide bases (Triplet) set in a particular order that correspond to an Amino Acid;
  - Some Amino Acids have more that one Codon,
  - **Synonyms**: Codons that specify the same Amino Acid;
- Genetic Code contains **64-Codons**:
  - 61-Codons specify Amino Acids;
  - 3-Codons are Stop-Codons that terminates Translation
- **See Example in diagram below**

Example: **Genetic Code**: Genetic Information on m-RNA is sequence of Nucleotide bases transcribed from DNA (NOTE: m-RNA contains U instead of T)



- **IMPORTANT TO NOTE**:
- Codons in m-RNA are the Complement of the DAN base Triplets;
- **Transfer RNA (t-RNA)** contains **Anti-Codons** which are the **Complement of Codons in m-RNA**;
- **Anti-Codons in t-RNA are the same as DNA base triplets**;

TRANSLATION

What is Translation?

- **Translation:** conversion of information in **m-RNA** to formation of Polypeptide on Ribosome;
  - Conversion of Genetic Code in m-RNA into Protein;

Messenger RNA ======➔ Polypeptide

- Translation occurs on m-RNA attached to Ribosome in the Cytoplasm;
  - It involves attaching activated t-RNA containing Anti-Codons to appropriate Codons on m-RNA;

What are the three basic stages in Translation?

- **Initiation**:
  - m-RNA-Ribosome complex is formed,
  - First Codon (**AUG**) on m-RNA binds to Anti-codon on Initiator t-RNA;
- **Elongation**:
  - Anti-codon on t-RNA carrying amino acid binds to Codon on m-RNA on the Ribosome,
  - Process leads to sequential addition of amino acids to the C-terminal of the growing polypeptide chain;

- **Termination**:
  - Stop codon (UAA, UAG, UGA) on m-RNA signals termination of Transcription;
- Polypeptide formed is released from Ribosome

What is poly-cistronic m-RNA and Mono-cistronic m-RNA?

- **Poly-cistronic m-RNA:**
  - A single m-RNA that contains many coding regions with their corresponding initiation sites;
  - Poly-cistronic m-RNA are found in Prokaryotes;

- **Mono-cistronic m-RNA:**
  - A single m-RNA that codes for only one specific polypeptide,
  - **Mono-cistronic m-RNA are found in Eukaryotes**;

TOOLS FOR MOLECULAR BIOLOGY

What is the definition for Recombinant DNA (r-DNA)?

- There are several ways of defining Recombinant DNA (**r-DNA**);
  - **Recombinant DNA**: a DNA that contains parts originating from two or more sources;
  - **Recombinant DNA**: an artificially created DNA;
    - DNA from two or more sources that have been combined into a single recombinant molecule;

- Recombinant DNA is DNA intentionally made from different living sources;
- Guidelines from National Institute of Health (NIH) in USA states that:
  - Recombinant DNA (r-DNA) are molecules constructed outside the living cells by joining natural or synthetic DNA segments to DNA molecules that can Replicate in a living cell, or molecules that result from their replication;

What do you understand by Recombinant DNA Technology?

- Recombinant DNA technology refers to techniques used to manipulate, move, recombine, and propagate DNA molecules;
- **Recombinant DNA technology**:
  - Procedures by which DNA from different species can be Isolated, Cut and Spliced together;
  - New "Recombinant DNA“ formed are then multiplied in quantity in populations of rapidly dividing cells (e.g., in bacteria, or in yeast);
- Two important enzymes needed for this process are: **Restriction Enzymes** and **DNA Ligase**;

What are Restriction Enzymes (RE)?

- Restriction enzymes (**Restriction Endonucleases**) act like scissors, that can cut DNA at particular sites (**Recognition Sites**) in the DNA molecule;
- Recognition sites are **Palindromic** in nature; that is
  - Nucleotide sequence of each DNA strand is the same when each is read in 5’ to 3’ direction;
- **Restriction enzymes can cut the Phosphodiester bonds on each DNA strand in three different ways**;
- Several Restriction Enzymes have been isolated from bacteria; they are named according to the bacterial species from which they were isolated;

What are the three ways that DNA can be cleaved by Restriction Endonucleases?

- DNA stands can be cleaved in 3 different ways:
- When cleavage of DNA strands **is not along the axis of symmetry Two types** of Sticky ends are formed:
  - **"Cohesive or Sticky ends"**;
    - A staggered cut to leave a **5'-end** that overhangs the end of the double-stranded DNA (**Fig. 2**),
    - A staggered cut that leaves a **3'-end** that overhangs the end of the double-stranded DNA (**Fig. 3**),
- When cleavage of DNA strands is along the axis of symmetry then "**Blunt Ends**" are formed (**Fig. 4**);

Fig. 2: A staggered cut to leave a 5'-end that overhangs the end of the double-stranded DNA;

Bam H1	"Sticky Ends" 5' Overhang

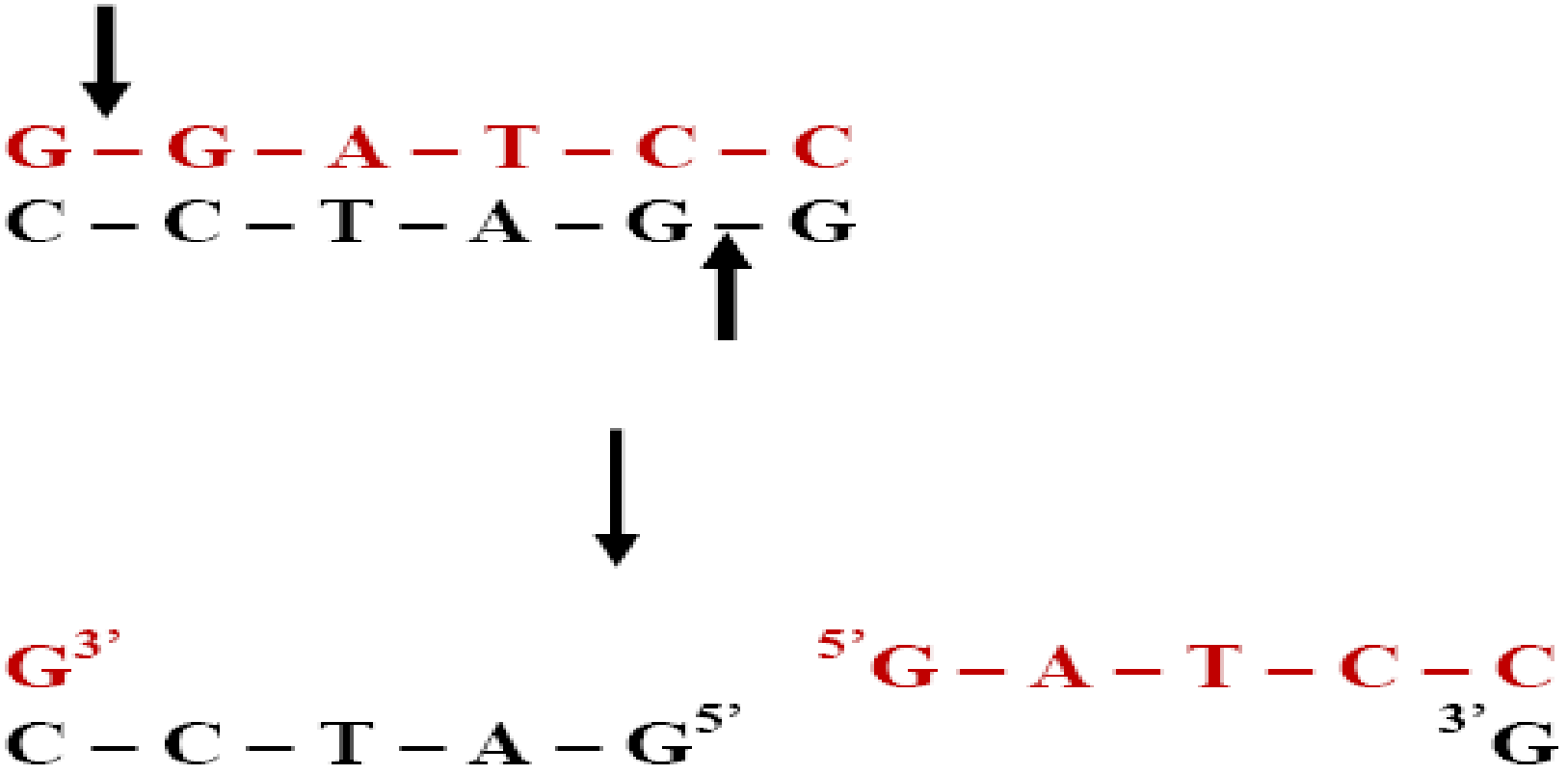


Fig. 3: A staggered cut to leave a 3'-end that overhangs the end of the double-stranded DNA

Kpn1	Sticky Ends 3'Overhang

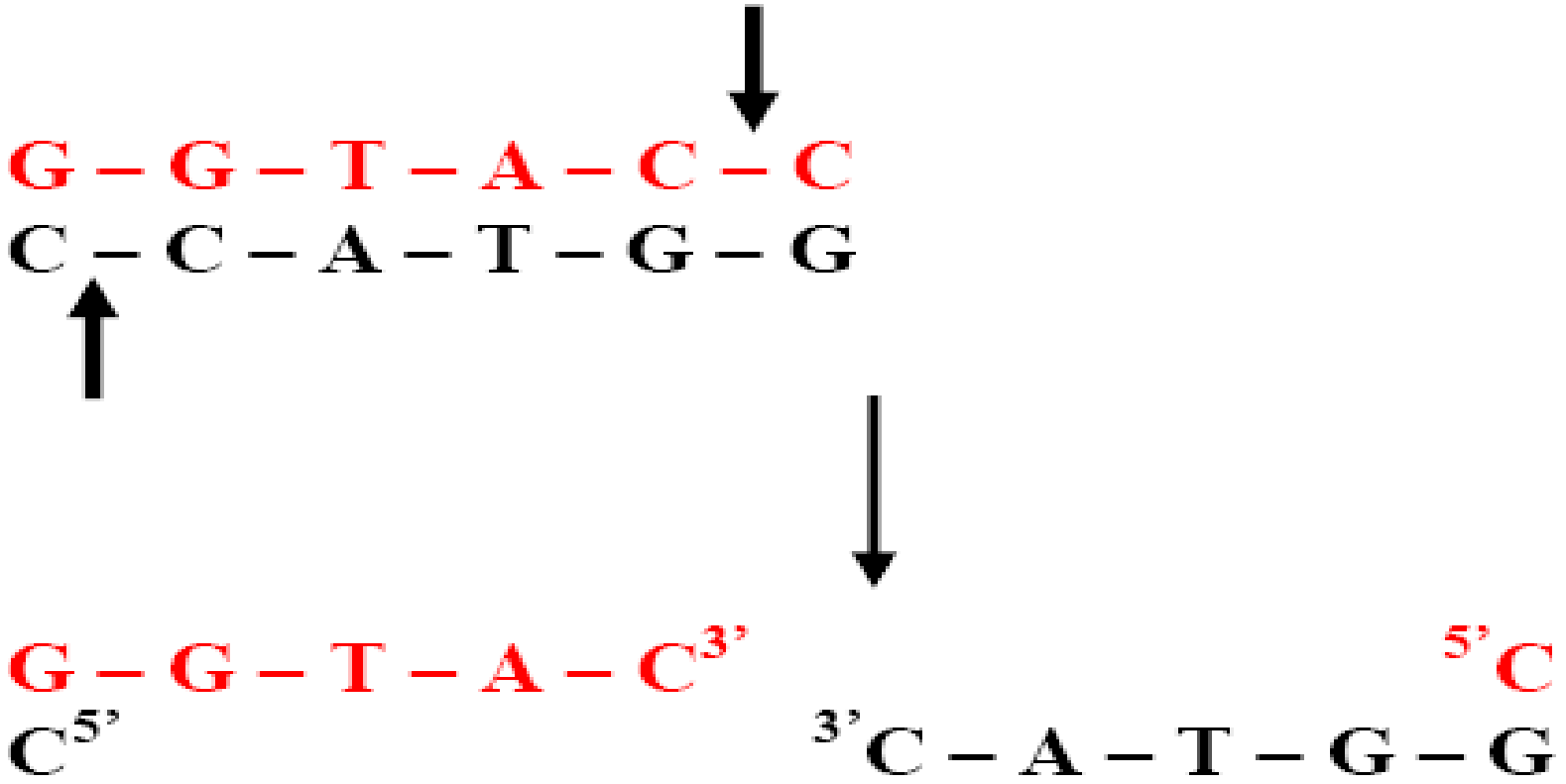
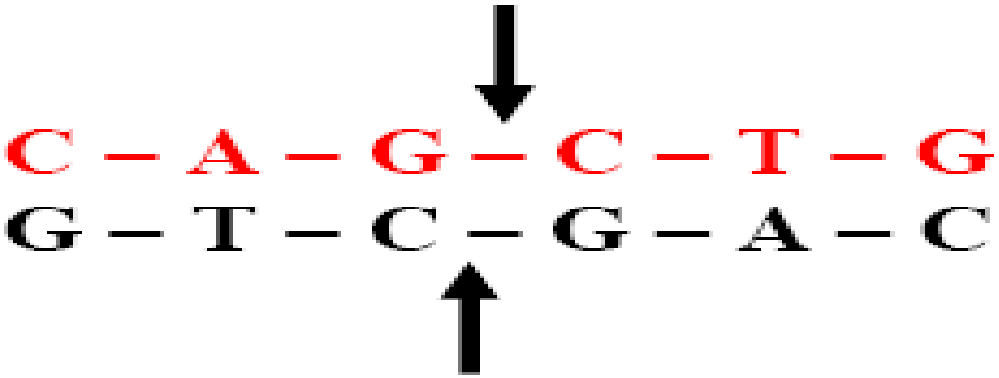
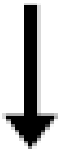
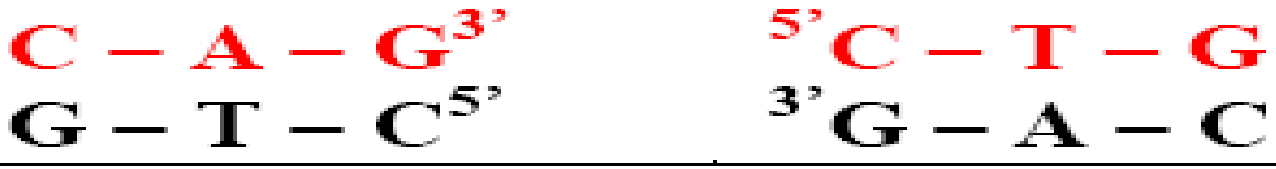


Fig. 4: DNA strands cleaved along the axis of symmetry to produce “Blunt Ends”

Pvu II	Blunt End





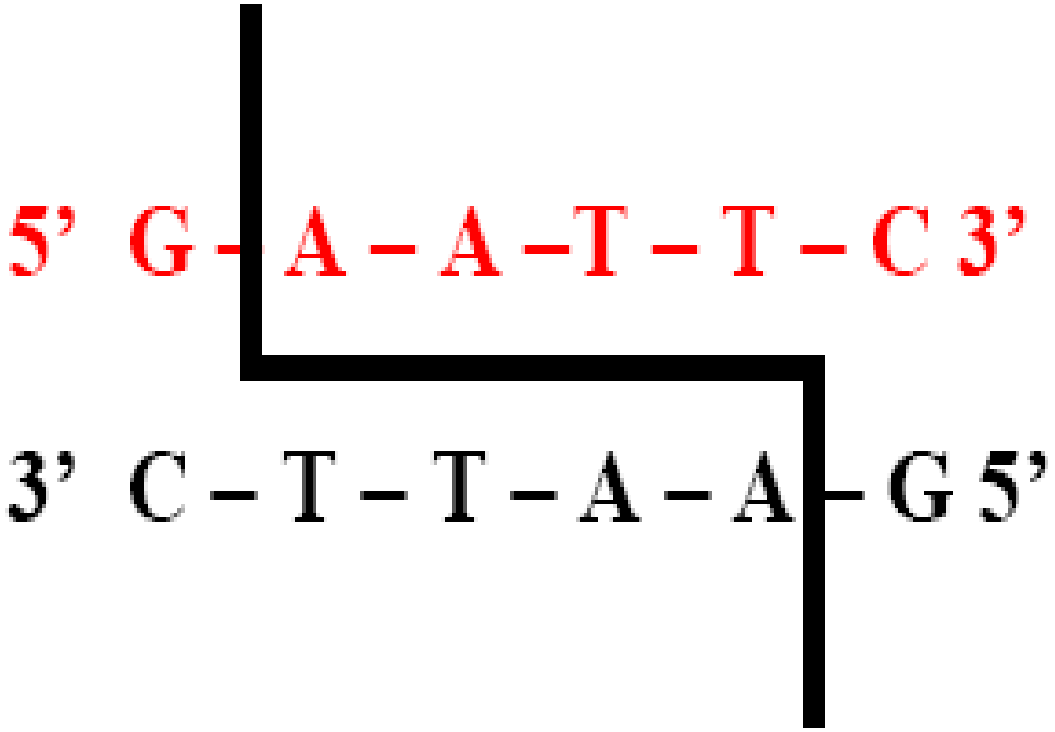


Outline the modes of actions of Restriction Endonucleases (Restriction enzymes)?

- Restriction Endonucleases (RE) act on specific Nucleotide Sequences (Recognition Sequences) in double stranded DNA molecules;
- Recognition sites are **Palindromic** in nature, that is, the Nucleotide sequence of each DNA strand is the same when each is read in a 5’ to 3’ direction;
- Restriction Endonucleases cut both DNA strands at the specific locations;

- Example:
- Restriction Endonuclease “Eco-RI” cuts DNA strands between Nucleotides with Guanine (G) and Adenine (A) bases, but only when they occur in the sequence:
  - **GAATTC** (5’⟶3’) on one strand
  - **CTTAAG** (3’⟶5’) on Complementary strand;
- Resulting in the formation of “**Sticky Ends**” (**Fig. 5**)

Fig. 5: Sticky ends showing Palindrome produced by Eco -R1 Restriction Endonuclease



What is the mode of action of DNA Ligase?

- **DNA Ligase** is the Glue that joins the ends of the DNA parts together;
- DNA Ligase forms the Phosphodiester bond that join the ends in the new DNA strand,
- if both DNA strands have been cut with the same Restriction Endonuclease, the ends will match up because they are sticky ends;

Why are DNA Polymerases considered as tools in Recombinant DNA methodology?

- Recombinant DNA technology uses DNA Polymerases with different activities for various functions;
- **DNA-dependent DNA Polymerases** are used:
  - To make complementary copies of DNA Templates in various Recombinant DNA procedures, such as:
    - DNA amplification during Polymerase Chain Reaction (PCR),
    - DNA sequencing,
    - Production of labeled DNA probes using labeled substrates (d NTPs);

- **RNA-dependent DNA Polymerases (**also called **Reverse Transcriptase)** are used:
  - To make **Complementary DNA** (**c-DNA**) copies of RNA Templates;
  - **Mainly in Cloning DNA sequences that are Complementary to m-RNA;**

What is Cloning (Gene cloning)?

- **Cloning: Synthesis of identical copies of a gene;**
- **Gene Cloning:**
  - **The process of manipulating DNA to produce multiple copies of a Single Gene or Segment of DNA or Protein;**
- **Clones are identical host cells that carry identical Recombinant DNA molecule;**
- Cloning requires the use of vector (**Plasmid**, **Bacteriophage**, or **Cosmids**)

What is DNA sequencing?

- DNA sequencing is a laboratory technique used to determine the Sequence of Nucleotide Bases in a molecule of DNA;

What do you understand by Transgenic Plant or Animal?

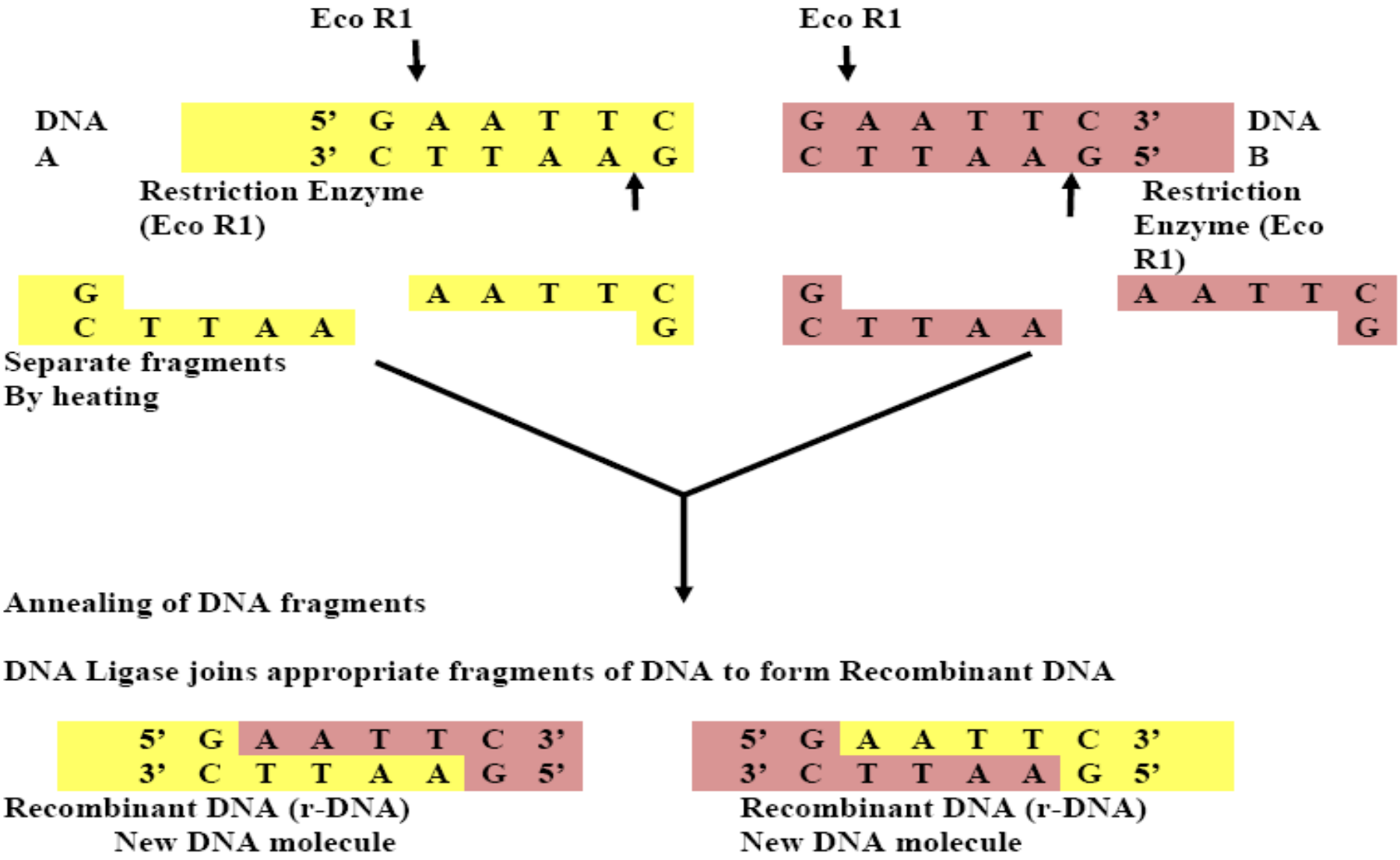
- **Transgenic Plant or Transgenic Animal:**
  - A Plant or Animal that has been genetically engineered,
  - The plant or animal usually contains genetic material from at least one unrelated organism, such as:
    - Virus,
    - Other Plant, or
    - Other Animal;

What are the basic stages in formation of Recombinant DNA? (Use appropriate diagram Fig. 6)

- **BASIC STAGES**:
- DNA from two sources (**DNA A** & **DNA B**) are cut with a Restriction Endonuclease (**Eco -R1**);
- Both fragments formed have either “Sticky” or “Cohesive” ends with complementary bases;
  - Appropriate ends can form Complementary base pairs when mixed;
- DNA fragments are then separated by heating;
- The fragments are then Annealed (allowed to mixed at low temperature);

- Complementary ends form appropriate base pairs
- DNA Ligase then join the appropriate strands by catalyzing formation of Phosphodiester bonds;
- New DNA molecule formed is called Recombinant DNA (r-DNA);
- Recombinant DNA can then be replicated several times as needed;
- **See Fig. 6**

Fig. 6: Schematic diagram of basic stages in the formation of Recombinant DNA using Restriction Endonuclease (Eco-R1)



What are the basic components and steps in Polymerase Chain Reaction (PCR)?

- **Polymerase Chain Reaction (PCR)**:
  - Used for formation of extremely large copies of a DNA fragment or a Gene;
- **Basic components required for PCR are**:
  - Fragment of target DNA (to serve as template),
  - Two appropriate DNA Primers,
  - Four substrates (d-ATP, d-GTP, d-CTP, d-TTP);
  - Heat stable DNA Polymerase (e.g., *Taq* DNA Polymerase);

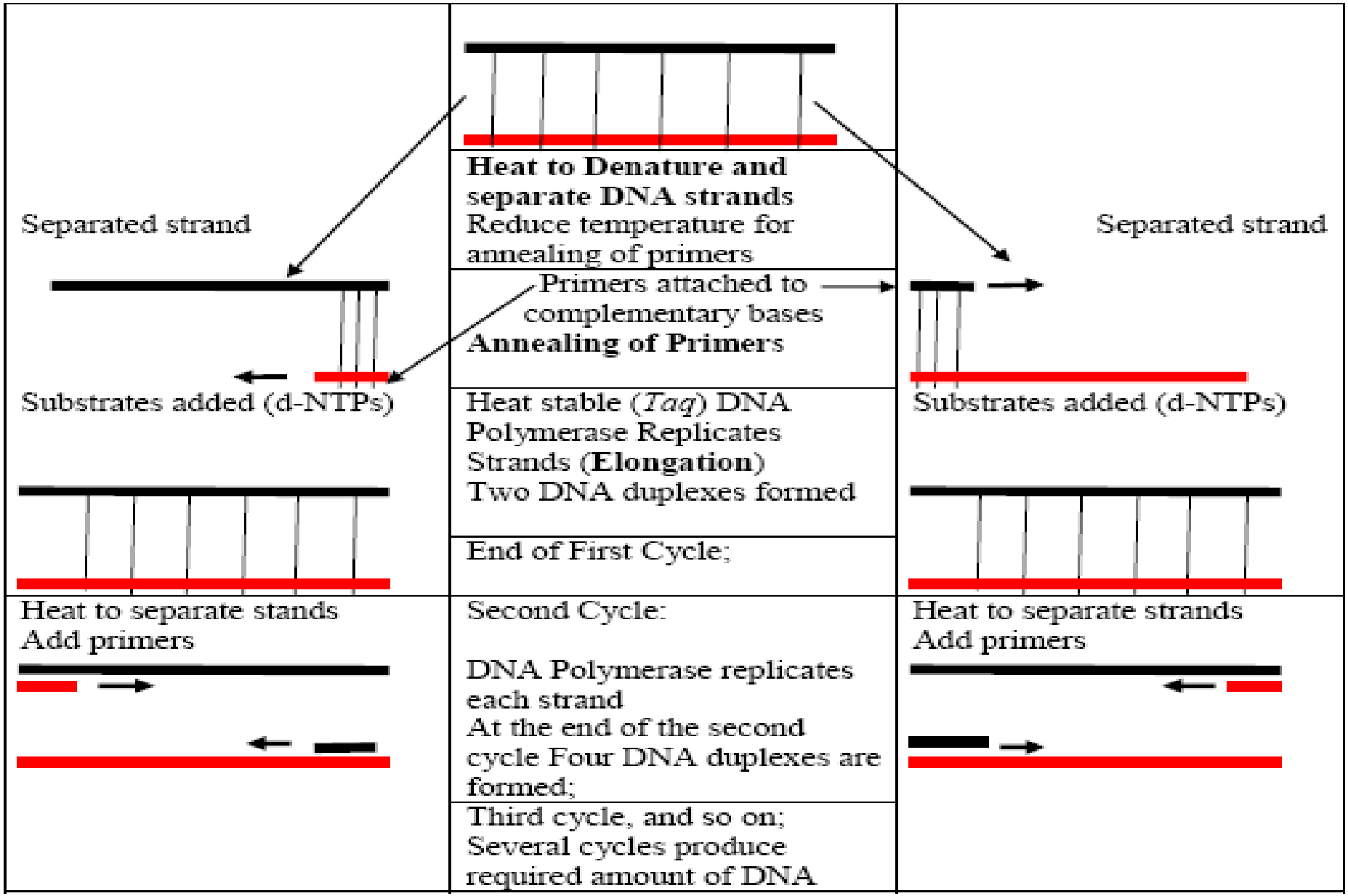
- **One PCR cycle consist of Three Steps**:
  - Denaturing of DNA template;
  - Annealing of Primers with each strand of denatured DNA Template;
  - Elongation (Replication of DNA);

Outline the basic steps in Polymerase Chain Reaction (PCR)

- **Three basic steps** are involved in PCR: **(See Fig. 7**)
- **First Cycle of PCR**:
- **Denaturing of Target DNA (DNA Template):**
  - Heat reaction mixture at 95C for 30 seconds,
  - Target DNA separates into two strands;
  - Each single strand serves as DNA Template;
- **Annealing of Primers**:
  - Primers are added into reaction mixture;
  - Reaction mixture is rapidly cooled causing primers to bind to appropriate templates by base pairing;
  - Primer binds to template according to base sequence;

- **Elongation (Replication step)**:
  - Temperature of reaction mixture increased to 75C;
  - Heat stable DNA polymerase elongates primer by catalyzing replication of the template DNA,
  - Complementary strand to template strand is formed;
  - Formation of 2 Double stranded DNA ends the cycle;
- **Second Cycle of PCR**:
  - Three steps are repeated;
  - Both double stranded DNA are denatured;
  - 4 Single-Strands produced are annealed with primers;
  - Elongation produces 8 double stranded DNA;
- Cycles repeated to produce several DNA copies

Fig. 7: Schematic diagram of basic steps in PCR



What is a Cloning Vector (Give examples)?

- DNA molecule that carry a fragment of foreign DNA into a host cell is called a cloning vector;
- Cloning Vector is usually a Virus or Plasmid DNA that is capable of replicating within the host cell;
- Examples of vectors used to clone DNA are:
  - Bacteriophage,
  - Plasmids,
  - Cosmids;

How useful are Plasmids as cloning vectors?

- Plasmids are small circular double stranded DNA that exist free inside bacterial cells,
  - Extra-chromosomal circular DNA in bacteria;
- Plasmids are capable of self-replicating within the host bacterium, because they carry the origin of replication;
- Plasmids often carry genes that confer Antibiotic resistance on bacteria;
  - This serves as the selectable marker of the plasmid;
- Plasmids are the major cloning vectors in **r-DNA**;
- Plasmids are used to clone for:
  - DNA with less than 10 kb (10 thousand base pairs);
  - Complementary DNA (c-DNA);

How useful are Bacteriophages as cloning vectors?

- **Bacteriophages** (Phages): Viruses that infect bacteria,
- DNA of Bacteriophages are used as vectors, because they can replicate in appropriate host cells;
- Bacteriophages grow in Lytic phase, then cause Lysis of host cells to release phage progeny;
- Bacteriophages are used to:
  - Clone for DNA of about 20kb (20 thousand base pairs),
  - Construct Genomic Libraries,
  - Construct Complementary DNA (c-DNA) Libraries,
  - Produce single-stranded DNA for DNA Sequencing;

What are the basic steps in DNA cloning with Plasmid?

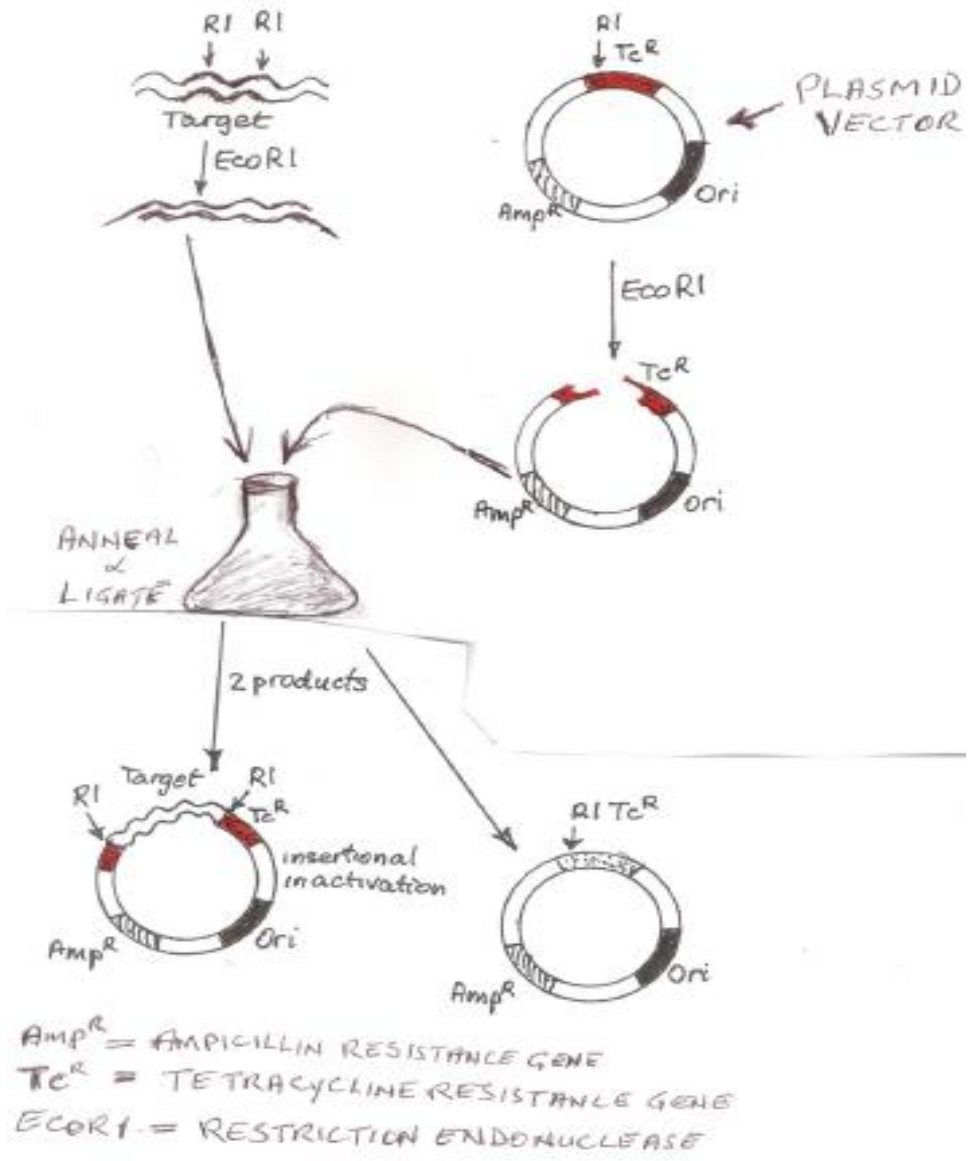
- Process consist of several stages:

❖**Formation of Recombinant Plasmid DNA**:

- Select the appropriate Plasmid to act as vector,
- Plasmid should contain: **Cloning site, Two selectable markers**, e.g.,
  - Ampicillin resistance gene (Amp), and
  - Tetracycline resistance gene (Tc);

- Restriction Enzyme (Eco-R1) is use to cleave Target DNA and Plasmid DNA to create “Sticky ends” on both DNA,
- Mix Target DNA and Plasmid DNA then Anneal,
  - Target DNA will attach to Plasmid DNA (Vector)
  - Add DNA Ligase to join both DNA ends;
- Plasmid DNA becomes a Recombinant Plasmid DNA; (See **Fig. 8)**

Fig. 8: Schematic diagram of basic steps in DNA cloning



❖**Transfection:**

- Incubate Recombinant plasmid DNA with bacteria,
- Bacteria then takes up Recombinant Plasmid DNA and becomes Transfected;

❖**Multiplication**:

- Allow Transfected bacteria to grow and multiply in appropriate growth medium;
- Recombinant Plasmid will replicate within bacteria cells producing Colonies;

❖**Identification and Selection:**

- Identify and select the colonies containing the Recombinant DNA using appropriate probes;
- Isolate and culture those colonies;
- Isolate the Plasmids,
- Cleave r-DNA with Restriction Endonuclease and Isolate the Cloned DNA;
- Isolate and characterize the protein expressed by the Recombinant DNA;

What is DNA Profiling?

- **DNA Profiling:** Technique used to obtain the DNA profile of individuals;
- **DNA Profile** is the pattern of DNA characteristics used to distinguish between individuals;
- Samples for DNA profiling can be taken from various materials that contains nucleated cells, such as:
- Mouth swab, Saliva, Plucked hair roots, Venous blood, Semen or Bones;
- Any samples including mitochondria that contain DNA of the individual whose DNA profile is needed;

What are alleles?

- **Alleles**: variations at particular site on Chromosome
- Each chromosome has similar chromosome partner
  - except for males with their X and Y chromosomes
- **Each locus in chromosome is in pair**;
  - **Homozygous** is when the 2 loci are identical;
  - **Heterozygous** is when the 2 loci are different ;
- **Polymorphic loci** whose alleles are the result of **Short Tandem Repeats (STR)** are the most informative PCR-based genetic markers that can be used to identify and individualize a biological material;

What is Short Tandem Repeat (STR)?

- **Short Tandem Repeat (STR):**
  - **A specific short length of the non-coding segment of DNA that is repeated, end-to-end, within the DNA molecule;**
- Different individuals have different numbers of repeats and hence different lengths of STRs within their DNA molecules;
- Example: DNA sequence **GAGAGAGA** is an **STR** that has a repeating unit of two bases, **G** and **A**, repeated Four times;

- DNA has a variety of STRs scattered along the non-coding segments of the DNA strand;
- Each individual has a unique number of STR loci that varies in frequency;
- **STRs are often variable (polymorphic) and these variations are used to distinguish one individual from another**;
- STRs are located in the non-coding regions of the DNA, thus they do not provide information about the genetic makeup of an individual;

What is STR profiling?

- **STR profiling:** a technique that examines the lengths of STR units within DNA and converts the lengths into digital outputs;
- **STR profiling**: is a technique used by Forensic Science for identification of individuals;
  - It is restricted to looking at only the STR in DNA that vary widely between individuals, which makes them extremely useful for identification purposes;

- STR is more related to the use of Fingerprint for identification of an individual;
- **For each individual there are 2 alleles or markers in each STR, one from the Maternal DNA strand and one from the Paternal DNA strand**;
- Currently, the most popular method of DNA Fingerprinting uses STR;

What is DNA finger printing?

- **DNA fingerprinting:** technique for analyzing DNA content of an individual for the purpose of characterizing the individual;
  - Although the general chemical structure of DNA is the same for all humans, one major difference between individuals is their STR,
- Every individual has different sequences in the non-coding segments (STR) of their DNA;
- STRs have repeat sequences of 2-5 base pairs, thus each STR is short enough to be amplified by PCR;

What are the basic steps in DNA Fingerprinting?

- Basic steps include the following:
- **Isolation of the DNA:**
  - DNA is collected from cells or tissues;
    - Small amount of tissue is needed;
    - Amount of DNA at the root of a strand of hair is sufficient
- **Cutting, Sizing and Sorting**:
  - Restriction enzymes are used to cut the DNA at specific sites in the non-coding segments;

- DNA of individuals are different, so individual DNA will cut at different sites, to produce different number and size of STRs;
- DNA segments (STR) obtained are then sorted according to size by electrophoresis;
- Results can be analyzed using specific standards;
- **See Fig. 9**

Fig. 10: Schematic diagram of STR sorted by electrophoresis

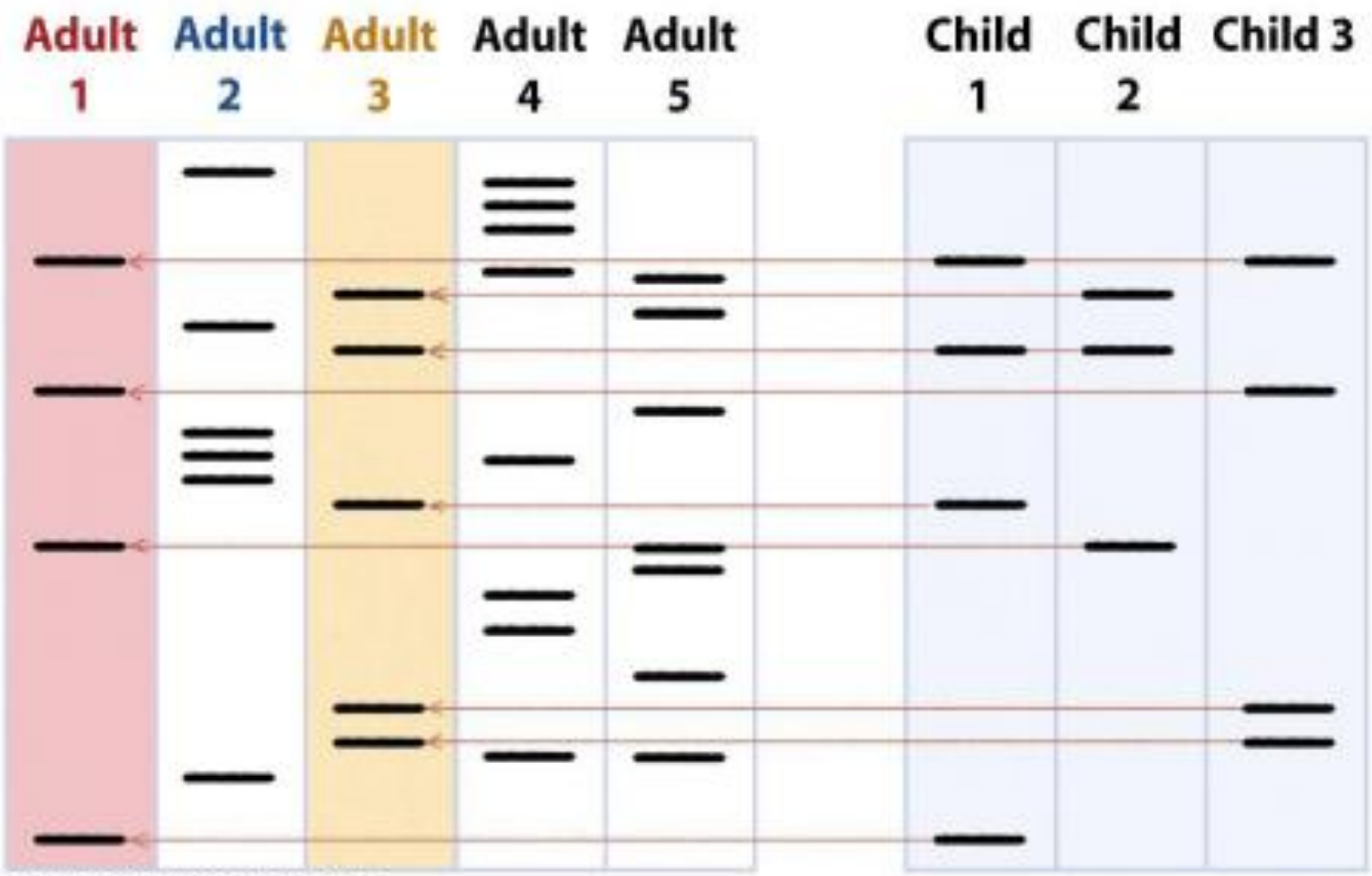


Figure 7-12 Biology: Science for Life, 2/e

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