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STRUCTURE AND DIAGNOSTIC APPLICATIONS OF DNA

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 is a Pyrimidine or Purine base covalently bonded to a sugar
  - Nucleoside = Nitrogenous base + Sugar;
- In DNA, the sugar is **Deoxyribose**, thus DNA contains Deoxy-nucleosides:
  - **Deoxy-nucleoside = 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 is a Nucleoside covalently bonded to a Phosphate group
  - Nucleotide = Nucleoside + Phosphate
- (Note that the Phosphate should be covalently bonded to the Sugar)
- 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 molecule?

- DNA contains deoxy-nucleotides that are covalently linked together by 3'5'-Phosphodiester Bonds;
- It forms a repetitive Sugar-Phosphate Chain, which is the Back-bone to which the Nitrogenous bases are attached;

What base pairs are formed between complementary strands in DNA?

- Purines form base pairs with corresponding Pyrimidines,
- 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

- 1953 Watson and Crick proposed Three-dimensional structure of DNA,
- DNA made up of Two Strands wound round each other to form a Double Helix,
- DNA strands are in an **anti-parallel** arrangement,
    - DNA strands run in opposite directions,
        - One strand is oriented 5' =➔ 3'
        - Other is oriented 3' ==➔ 5'

- 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 the 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 is 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 can be regarded as a Single Letter in an Alphabet that has only Four Letters, A, G, C and T;
- Different Genes have different sequentical arrangements of these Four Letters, thus each gene can code for different biological messages;
- Deoxynucleotides in DNA differ only in the sequence of bases they carry, thus they are recorded simply according to their base sequence;
- 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., it is written in the 5' ==➔ 3' direction;

DNA REPLICATION:

What are the two possible ways of Replication of DNA?

- Replication occurs when the Double-helical DNA strands separate and act as Templates for formation of New Complementary Stands;
- Two possible ways of replication of DNA are:
  - Conservative Replication
  - Semi-Conservative Replication
- Conservative Replication:
  - After replication the parental DNA strands remained together and the newly synthesized DNA strands stay together;
- **Semi-Conservative Replication**:
  - During replication the two DNA strands are separated and each acts as template to its newly synthesized complementary strand;
  - Thus, each new DNA contains one original strand and one newly synthesized 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 the DNA Template by catalyzing the addition of Deoxy-nucleotide units to DNA chain,
- **Substrates** for DNA replication: Four Deoxy-Nucleosides Triphosphates (dNTPs);
  - d ATP, d GTP, d CTP, d TTP;
  - Cleavage of two high-energy phosphate bonds provides energy for forming the Phosphodiester bond;
- **Template**: DNA replication cannot occur without a Template;
  - Template directs the addition of appropriate complementary Deoxy-nucleotide to the newly synthesized DNA strand;
  - Semi-conservative replication, each strand of parent DNA serves as template,
  - Each template and complementary strands serve as DNA in daughter cells;
- **Primer**: DNA replication cannot start without 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 is the RNA Polymerase that synthesizes the Primer (a short piece of RNA) for DNA replication,
- Primase synthesizes RNA directly on the single-stranded DNA template because, like all RNA polymerases, it does not require a Primer to begin synthesis;
- New Deoxy-nucleotides are added to the 3' end of a Primer,
- DNA replication occur in a 5' to 3' direction;

Briefly state how DNA replication occurs in Eukaryotes:

- DNA replication is semi-conservative,
- DNA replication occurs bi-directionally from many origins;

- Use of multiple origins is to ensure that Chromosomal DNA is replicated within the necessary time period,
- At each origin, a replication bubble forms consisting of two replication forks moving in opposite direction,
- DNA replication under the 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 the 5' to 3' direction**; (DNA polymerase reads the parental DNA stand in the 3' to 5' direction)

What are the functions of the DNA Polymerases in Eukaryotic cells?

- Eukaryotic cells contain Five different DNA polymerases;
    - DNA polymerase α:
        - Synthesizes RNA Primer,
        - Involve 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 activity)
    - 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?

- 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?

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

TRANSCRIPTION:

What is Transcription?

- Transcription involves synthesis of RNA (Messenger 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 the Nucleus of eukaryotes;
        - Carries information from Genes (DNA) to Ribosomes where it is translated into proteins;
    - 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 incorporation 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 different 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 the RNA polymerase;
- RNA polymerase moves along the gene, synthesizing a complementary RNA copy of the DNA template using the appropriate substrates;
- DNA strand copied is called the **Antisense (-) strand;**

- **RNA produced has the same sequence as the non-template DNA strand, called the sense (+) strand,**
    - (Note that **RNA contains U instead of T;**

Briefly describe Termination phase of Transcription:

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

What are the basic requirements for Transcription?

- **Template:**
    - One of the strands in a double-helical DNA molecule acts as a template to direct the formation of complementary RNA during transcription;
- **Substrate:**
    - Substrates for RNA transcription are the four Ribonucleoside Triphosphates: ATP, GTP, CTP and UTP;
    - Two high-energy bonds provide the energy needed for the addition of nucleotides to the growing RNA chain;

- **Direction of Transcription:**
    - Transcription proceed in the 5' to 3' direction;

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

- **Promoter sequences:**
    - Initiation of Transcription does not require a Primer,
    - Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a particular point
    - Promoter sequences are not the same in prokaryotes and eukaryotes;

- **Initiation factors:**
    - Needed to initiate transcription
    - Prokaryotes only a single factor called sigma is needed to initiate transcription
    - Eukaryotes multiple factors are needed,

- **Post-transcriptional RNA processing:**
    - Once a gene transcript has been synthesized, numerous post-translational modification or processing events may be needed before the transcript is functional;

GENETIC INFORMATION:

What is the "Gene"?

- Segments of a DNA molecule (Chromosome) that contains the information of how to synthesize a Polypeptide (Protein),
- Genes are located in the DNA of Eukaryotes,

What are Exons?

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

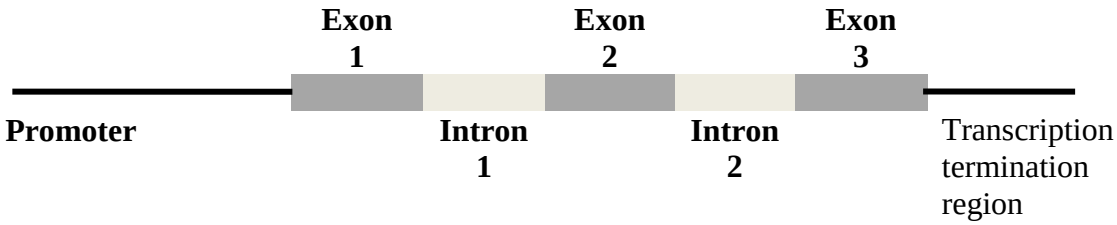
What are Introns?

- 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,

Describe the basic of a protein-coding gene in eukaryotes

- 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,
  (**See Fig. 1**)

Figure 1: shows basic structure of the protein-coding gene



What is the Genetic Information?

- DNA have the ability to direct the construction of polypeptides (proteins),
- Genetic information is the DNA sequence that specifies the exact sequence of amino acids for synthesis of specific protein;
- Genetic information is encoded as the sequence of Nucleotide Bases on One-half of the DNA molecule;
  - Sequence of Nucleotide bases is called the **Genetic Code**;
- 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?

- Genetic information (Genetic code) is located in the genetic material (DNA of eukaryotes and RNA of some Prokaryotes);
- DNA of eukaryotes is located in the Nucleus;

- In Eukaryotes:
    - Genetic information is Transcribed on to Messenger-RNA in the nucleus,
    - Messenger RNA (m-RNA) is then transferred to the Cytosol;
    - Messenger RNA carries the genetic information in eukaryotes;

What is the Genetic Code?

- Genetic Code represents the genetic information;
- Genetic Code is the relationship between the Nucleotide sequence of the m-RNA and the Amino Acid sequence of a Protein (Polypeptide);
- It 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 made up of "Triplet Nucleotides" called Codons,
- There are 64-Triplet Nucleotides in the Genetic Code;

What is a Codon?

- Nucleotide sequence of m-RNA is read in groups of "**Three Nucleotides**" called Codons;
- Codon is the Three-Nucleotide bases (Triplet) set in a particular order that correspond to a protein;
- Each Amino Acid may be specified by 1 – 6 different Codons,
- Synonyms are 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;

TAKE 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 (Protein Synthesis):

What is Translation?

- Translation is the conversion of the information in **m-RN**A to formation of Polypeptide on a Ribosome;
- It is the translation of the Genetic Code in m-RNA into Protein;

    - **Messenger RNA (m-RNA) ======➔ Polypeptide**

- It occurs on m-RNA attached to Ribosome in the Cytoplasm;
- It also involved activated t-RNA molecules with Anti-Codons to the appropriate Codons on the m-RNA;

What are the three basic stages in Translation?

- Three basic stages in Translation are:
  - **Initiation**:
    - m-RNA-Ribosome complex is formed,
    - First Codon (AUG) on m-RNA binds to Anti-codon on Initiator t-RNA;

  - **Elongation**:
    - Anti-codon of t-RNA carrying the appropriate amino acid binds to the 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 the m-RNA signals termination of Transcription;
    - Polypeptide formed is released from the Ribosome;

What is 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;

What is 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 is a DNA molecule that contains DNA originating from two or more sources;
  - Recombinant DNA is a DNA that has been artificially created;
    - It is DNA from two or more sources that is incorporated into a single recombinant molecule;
  - Recombinant DNA is DNA intentionally made from different living sources;
  - Guidelines from the National Institute of Health in USA states that:
    - Recombinant DNA are molecules constructed outside of 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 the techniques that are used to manipulate, move, recombine, and propagate DNA;
- Recombinant DNA technology:
    - Procedures by which DNA from different species can be Isolated, Cut and Spliced together;
    - New "Recombinant DNA" molecules formed are then multiplied in quantity in populations of rapidly dividing cells (e.g. bacteria, yeast)
    - Two important enzymes needed for this process are: Restriction Enzymes and DNA Ligase;

What are Restriction Enzymes (RE)?

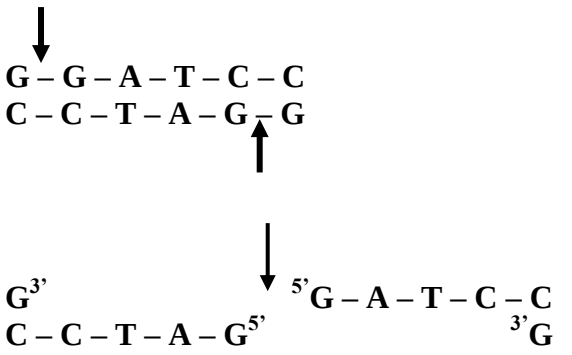
- Restriction enzymes are one of the major tools for formation of Recombinant DNA;
- Restriction enzymes (Restriction Endonucleases) act like scissors, that can cut a DNA double helix at particular sites (Recognition Sites) in the DNA molecule;
- Recognition sites are **Palindromic** in nature;
    - Means that the Nucleotide sequence of each DNA strand is the same when each is read in a 5' to 3' direction;
- Restriction Endonuclease hydrolyses the Phosphodiester bonds on the DNA strands;
- Several Restriction Enzymes have been isolated from bacteria, and named according to the bacterial species from which they were isolated;

Illustrate the three types of cleavages by Restriction Endonucleases

- Restriction Enzymes can cleave DNA strands within the sequence, thus they are called **Restriction Endonucleases**;
- Restriction Endonucleases:
    - Cleave the DNA strands not along the axis of symmetry to produce: "**Cohesive or Sticky ends**" (**Fig. 2**)
        - Two types of Sticky ends:
            - A staggered cut to leave a 5'-end that overhangs the end of the double-stranded DNA;

Fig. 2:

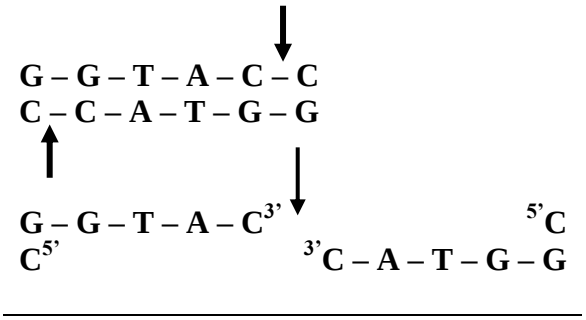
Bam H1	**“Sticky Ends” 5’ Overhang**



- A staggered cut that leaves a 3’-end that overhangs the end of the double-stranded DNA (**Fig. 3**)

Fig. 3:

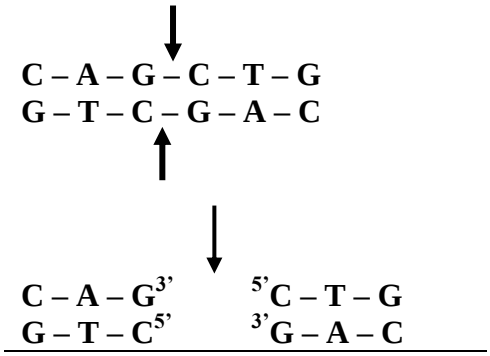
Kpn1	**Sticky Ends 3’Overhang**



- Cleave DNA strands along the axis of symmetry to produce "**Blunt Ends**" (**Fig. 4**)

Fig. 4:

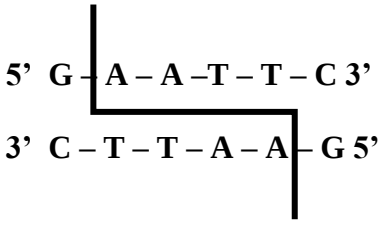
Pvu II	**Blunt End**



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;
- RE then cut both DNA strands at specific locations;
- Example:
  - Restriction Endonuclease Eco-RI cuts DNA 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 the Complementary strand;
  - Resulting in the formation of "**Sticky Ends**" (**Fig. 5**)

Fig. 5:



What is the mode of action of DNA Ligase?

- DNA Ligase is the Glue that holds the ends of the DNAs together;
- DNA Ligase creates a Phosphodiester bond between two DNA ends,
    - if both DNA strands have been cut with the same Restriction Endonuclease, the ends will match up because they are sticky ends;

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

- Recombinant DNA methodology utilizes DNA Polymerases with different activities for various functions;
- DNA-dependent DNA Polymerases: 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 (Reverse Transcriptase):
    - Used to make complementary DNA (c-DNA) copies of RNA Templates;
    - Used in mainly in Cloning DNA sequences that are complementary to m-RNA;

What is Cloning (Gene cloning)?

- Cloning is the synthesis of identical copies of a number of genes;
- Gene cloning is the process of manipulating DNA to produce multiple copies of a single gene or segment of DNA or protein;
- Clone refers to identical host cells that carry an identical Recombinant DNA molecule;
- Cloning requires the use of a vector (Plasmid, Bacteriophage, Cosmid)

What is DNA sequencing?

- DNA sequencing is a lab technique used to determine the sequence of nucleotide bases in a molecule of DNA;

What do you understand by a Transgenic Plant or Animal?

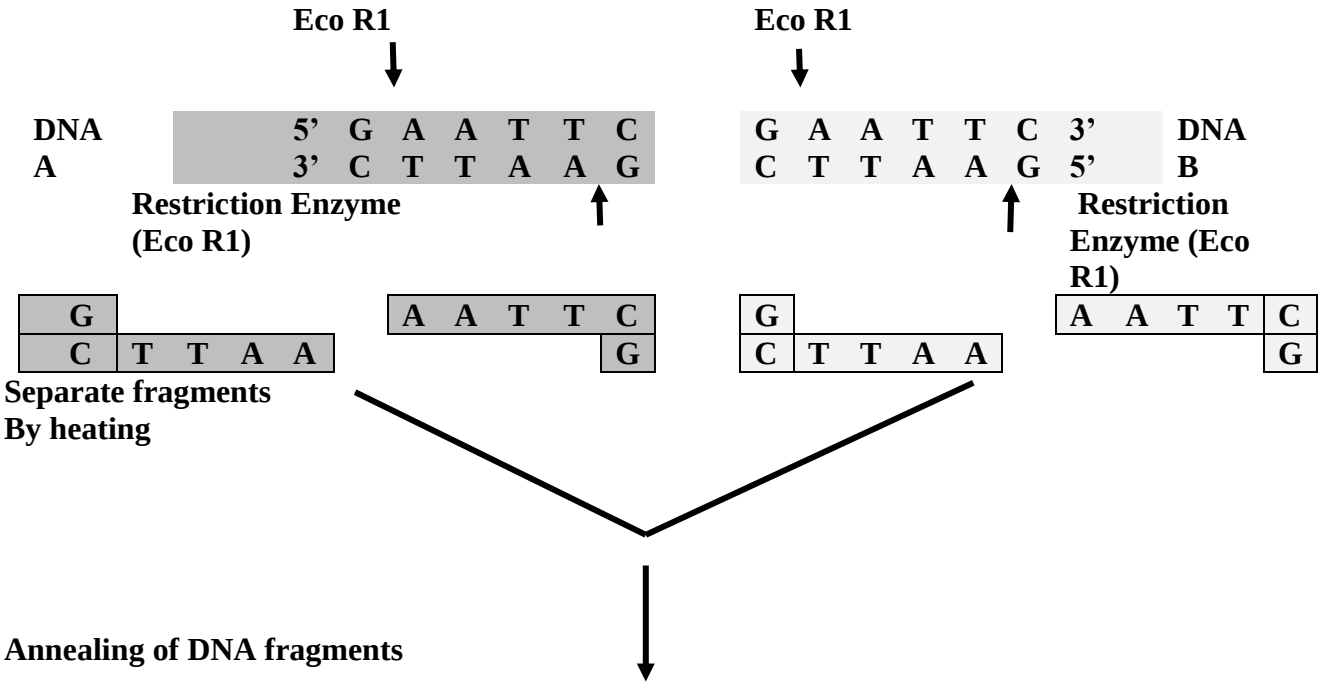
- Transgenic plant or animal is one that has been genetically engineered, and usually contains genetic material from at least one unrelated organism, such as from a virus, other plant, or other animal;

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

- DNA from two sources (e.g., DNA A & DNA B) are cut with the same Restriction Endonuclease (e.g., Eco R1);
- Both fragments formed will have "Sticky" or "Cohesive" ends with complementary bases;
    - Appropriate ends can form Complementary base pairs when mixed;
- DNA fragments are separated by heating;
- Fragments are then Annealed (allowed to mixed at low temperature);
- Complementary ends will form appropriate base pairs;

- DNA Ligase will then join the appropriate strands by catalyzing the formation of Phosphodiester bonds;
- New DNA molecule formed is called Recombinant DNA (r-DNA);
- Recombinant DNA can then be replicated several times as needed;

Fig. 6: **Formation of Recombinant DNA using Restriction Endonuclease (Eco R1)**



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

- 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 is made up 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

- Three basic steps are involved in Polymerase Chain Reaction (PCR):

(See Fig. 7)

First Cycle of PCR:

Denaturing of Target DNA (DNA Template):

- Heating reaction mixture at 95 C for about 30 seconds,
- Double stranded 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;
- One primer will bind to each template according to base sequence;

Elongation (Replication step):

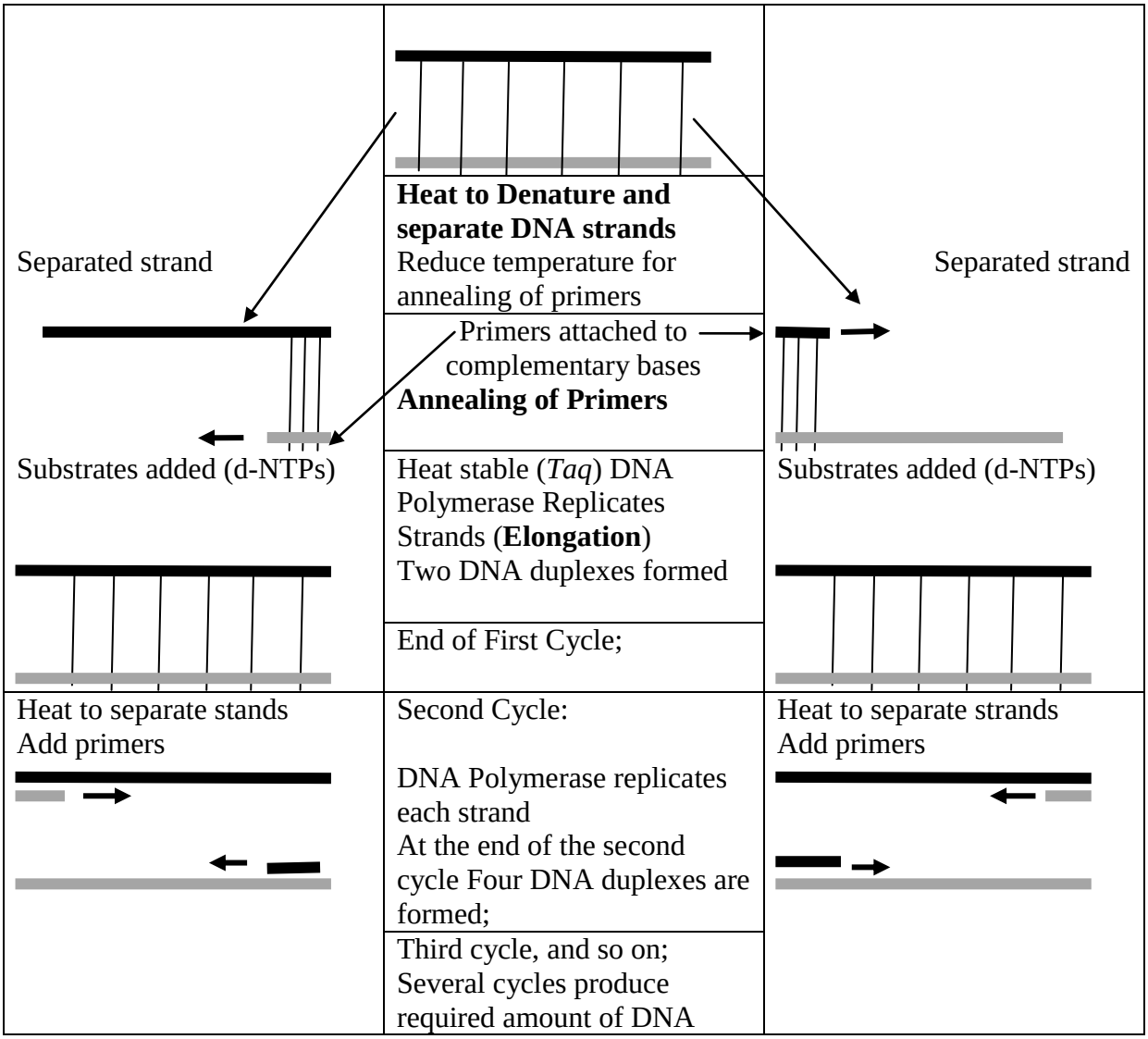
- Temperature of reaction mixture increased to about 75 C;
- Heat stable DNA polymerase elongates primer by catalyzing the replication of the template DNA,
- New strand is formed complementary to the template strand;
- Formation of Two Double Stranded DNA ends the cycle;

Second Cycle of PCR:

- Three steps are repeated;
- Both double stranded DNA are denatured;
- Four Single-Strands produced are annealed with primers;
- Elongation produces Eight double stranded DNA;

Several Cycles repeated to produce several copies of double stranded DNA;

Fig. 7:
Basic steps in Polymerase Chain Reaction (PCR)



What is a Cloning Vector (Give examples)?

- DNA molecule that can 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 and Cosmids;

How useful are plasmids as cloning vectors?

- Plasmids are small circular double stranded DNA molecules 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 their own 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 Recombinant 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) are 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 the host cells to release phage progeny;
- Bacteriophages are used to:
    - Clone for DNA of about 20kb (20 thousand kilo bases),
    - Construct Genomic Libraries,
    - Complementary DNA (c-DNA) Libraries,
    - Produce single-stranded DNA for DNA Sequencing;

What are the basic steps in DNA cloning with Plasmid?

- Process can be separated into following stages: **(Figs 8 & 9)**

- **Formation of Recombinant Plasmid DNA**:
    - Select the appropriate Plasmid to act as vector,
        - Plasmid should contain:
            - Cloning site,
            - Two selectable markers, e.g.,
                - Ampicillin resistant gene, and
                - Tetracycline resistant gene;

    - Use Restriction Enzyme (Eco R1) to cleave the Target DNA and the Plasmid DNA to create the same "Sticky ends" on both DNA,

    - Mix Target DNA and Plasmid DNA together then Anneal,
    - Target DNA will attach to Plasmid DNA (Vector);
    - Add DNA Ligase to join both DNA ends;
    - Plasmid DNA now becomes a Recombinant Plasmid DNA;

- **Transfection:**
    - Incubate the Recombinant plasmid DNA with the bacteria;
    - Bacteria that takes up the Recombinant plasmid DNA and said to have been Transfected;

- **Multiplication**:
    - Allow the Transfected bacteria to grow and multiply in appropriate growth medium;

- Recombinant Plasmid will replicate within the 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 with Restriction Endonuclease and Isolate the Cloned DNA;
  - Isolate and characterize the protein expressed from the Recombinant DNA;

Fig. 8: Basic steps in DNA cloning:

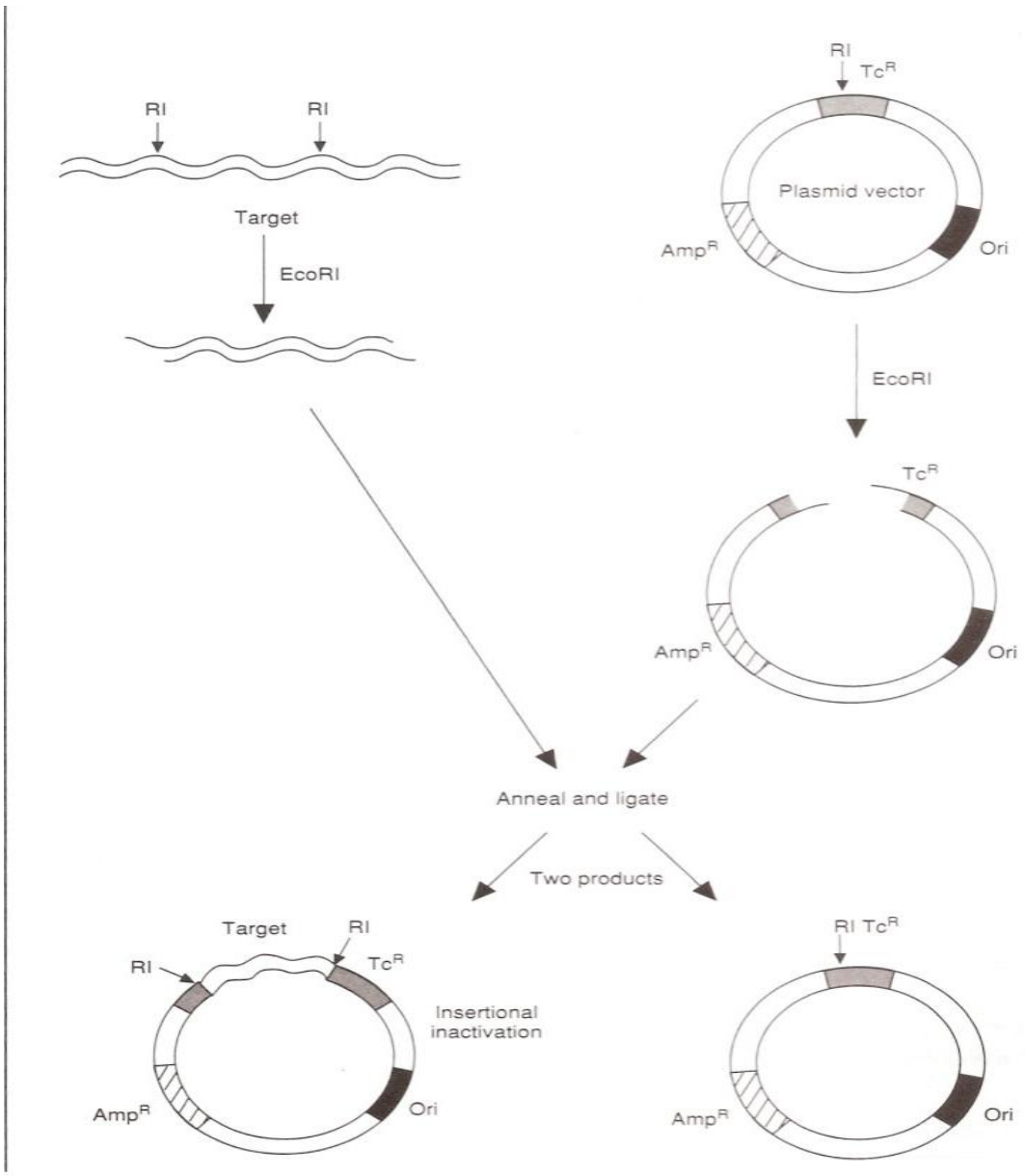
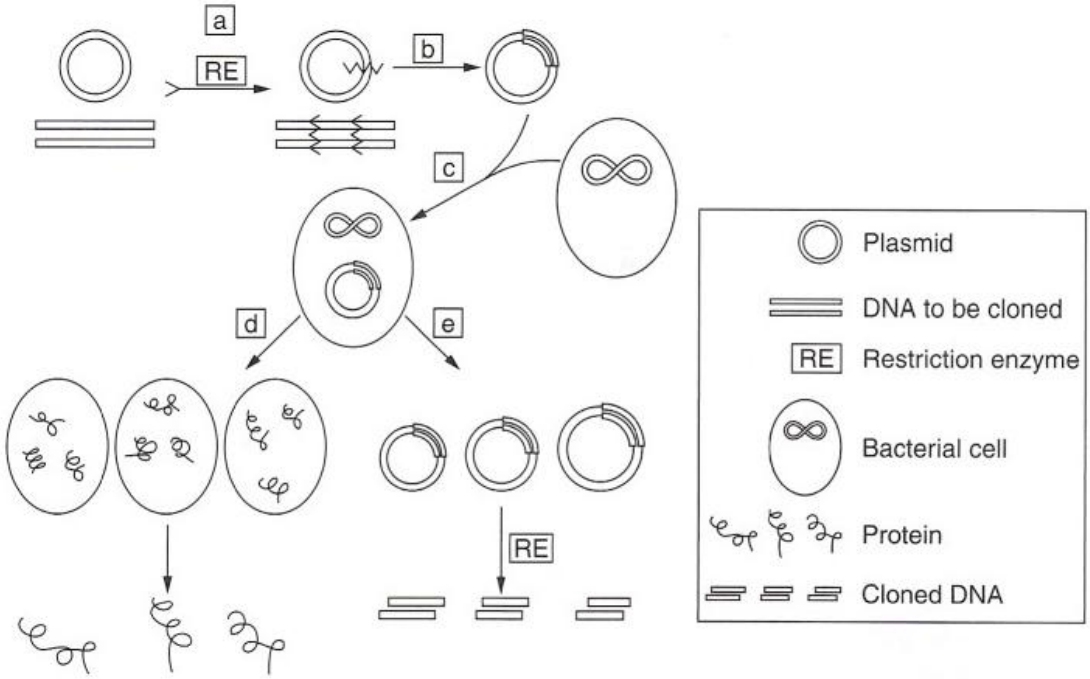


Fig. 9: Basic steps in cloning of DNA and Protein:



What is DNA Profiling?

- DNA Profiling is the 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 or
    - Venous blood or
    - Semen,
    - Bones,
- Any samples including mitochondria that contain DNA of the individual whose DNA profile is needed;

What are alleles?

- Alleles are variations at a particular site on a Chromosome;
- Each chromosome has a similar chromosome partner (except for males with their X and Y chromosomes) each locus is duplicated;
- Individuals with two identical loci said to be homozygous;
- If the two loci are different then they are said to be heterozygous;
- Polymorphic loci whose alleles are the result of short tandem repeats (STR) are the most informative PCR-based genetic markers for attempting to individualize biological material;

What is Short Tandem Repeat (STR)?

- STR is 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 the STRs of the DNA;
    - For example: DNA sequence GAGAGAGA is an STR that has a repeating unit consisting 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 is a technique that examines the lengths of STR units within DNA and converts the lengths into digital outputs;
- STR 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 is a technique for analysing the DNA content of an individual for the purpose of characterizing the individual;
- Although the general chemical structure of the DNA is the same for all humans, one major difference between individuals is the STR,
- Every individual has different sequences in the non-protein-coding segments (STR) of their DNA;
- STRs have repeat sequences of only 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 recovered from the cells or tissues of the body;
    - 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 then be analyzed using specific standards;

See Diagram (**Fig. 10**) below obtained after electrophoresis:

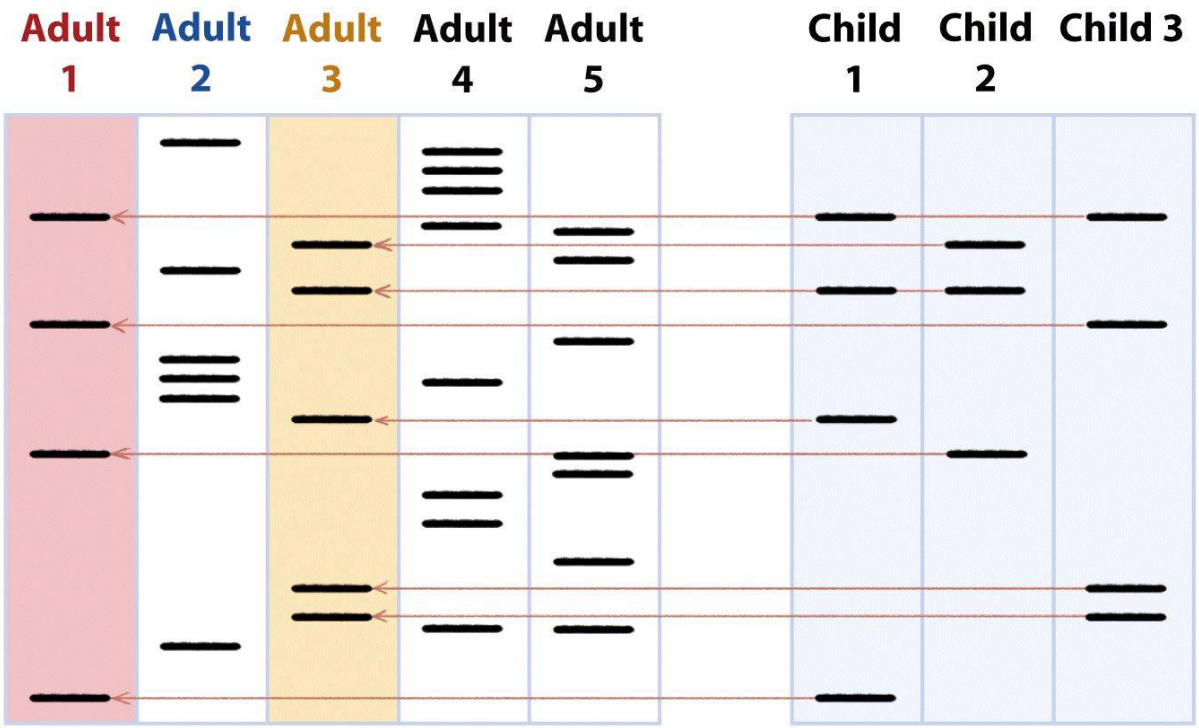


Figure 7-12 Biology: Science for Life, 2/e