Lec.2
Gene cloning

- **Gene** is the basic unit of heredity and is a segment of DNA that contains the instructions for making a specific protein or a functional RNA molecule.
- Gene function
- **Provide instructions:** Genes contain coded information that serves as instructions for building the specific proteins needed for various bodily functions.
- **Determine traits:** They are responsible for traits.
- **Control cell functions**
- Gene Location: Genes are arranged in specific locations on chromosomes(nuclear DNA + mitochondrial DNA).

Gene structure

Promoter:
A DNA sequence at the beginning (5' end) of a gene that acts as a binding site for RNA polymerase, initiating the process of transcription.
Exons:
Segments of a gene that contain the coding information and are expressed in the final functional product (protein).
Introns:
Intervening sequences within a gene that are transcribed into pre-mRNA but are then removed during a process called splicing before the mRNA becomes mature and is ready for translation.
A gene's structure comprises regulatory regions like the promoter and coding regions like the Open Reading Frame (ORF). The promoter is a non-coding DNA sequence upstream of the gene that regulates when and where it's expressed by binding RNA polymerase and other transcription factors. The ORF is the coding sequence starting with a start codon and ending with a stop codon, which is translated into a protein or polypeptide.
ignals the termination of transcription, causing the RNA polymerase to stop and detach from the DNA template.

- Gene expression is the process by which the information encoded in a gene is used to create a functional product, most often a protein or an RNA molecule. This regulated process begins with the transcription of the gene's DNA into a messenger RNA (mRNA) molecule, which then leaves the nucleus to be translated into a specific protein that performs a function in the cell. Cells control gene expression to produce necessary products at the right time and in the right amounts.
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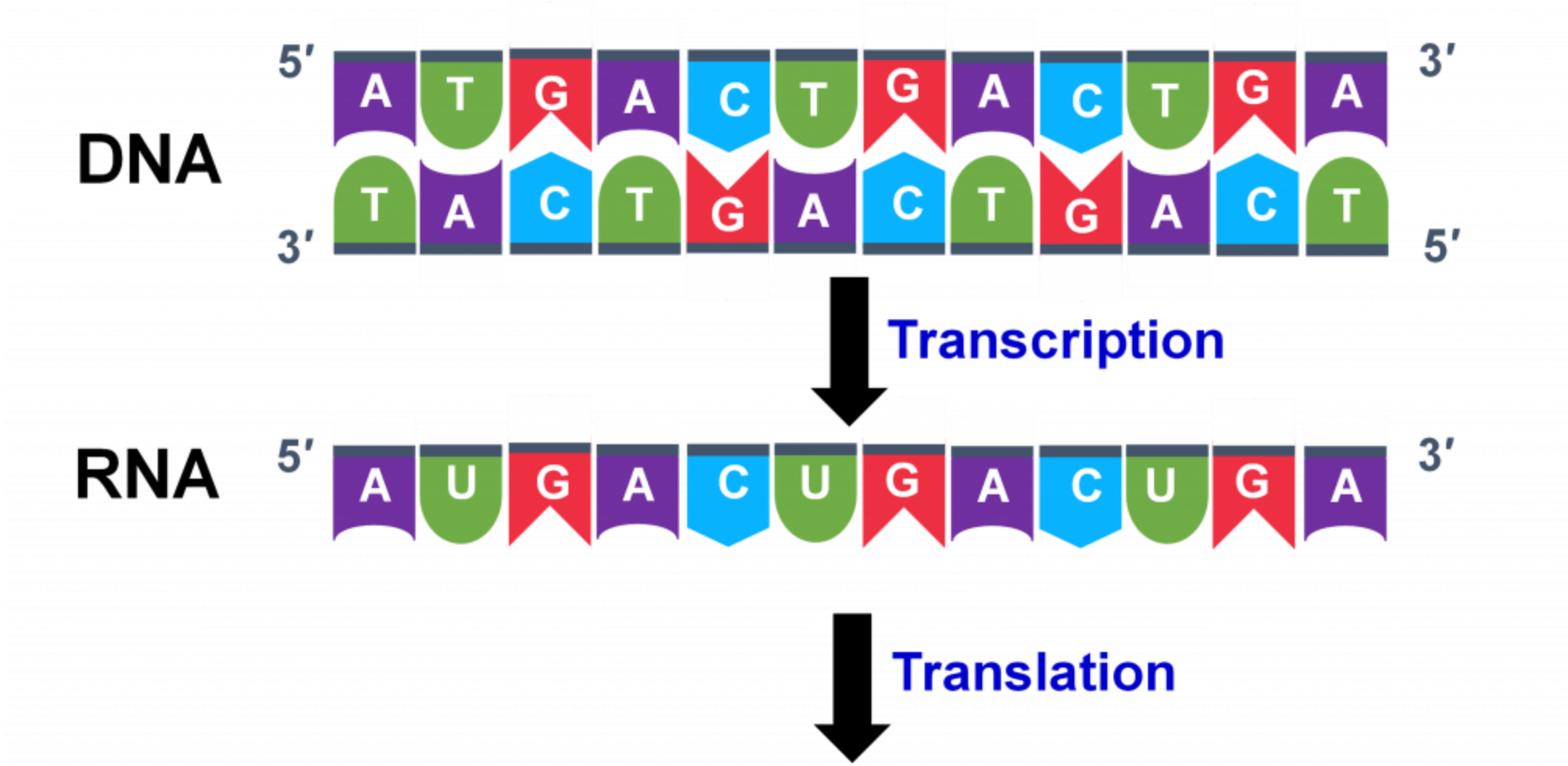
The Two Main Stages

1.Transcription:

2.The cell makes a copy of the DNA sequence from a gene into a messenger RNA (mRNA) molecule. This process occurs in the nucleus, and the mRNA then travels to the cytoplasm.

3.Translation:

4.The mRNA molecule is "read" by cellular machinery to produce a specific protein. This protein then carries out a function within the cell.


DNA
5′
A T G A C T G A C T G A
T A C T G A C T G A C T
3′
3′
5′
Transcription
RNA
5′
A U G A C U G A C U G A
3′
Translation
Protein
Methionine (Met)-Threonine (Thr)-Aspartate (Asp)

- Gene cloning
- The term ´´DNA cloning`` refers to the insertion of a DNA fragment (foreign DNA) into the host genome, resulting in the generation of new strains with acquired traits that are not present in the wild type. The foreign DNA is isolated from an external source and inserted into the host genome through transformation.

- Hamilton Smith and co-workers demonstrated unequivocally that restriction endoncleases cleave a specific DNA sequence.
- Later, Daniel Nathans used restriction endonucleases to map the simian virus 40 (SV40) genome and to locate the origin of replication. These major breakthroughs underscored the great potential of restriction endonucleases for DNA work.
- Building on their discoveries, the cloning experiments of Herbert Boyer, Stanley Cohen, Paul Berg, and their colleagues in the early 1970s ushered in the era of recombinant DNA technology.
- One of the first recombinant DNA molecules to be engineered was a hybrid of phage λ and the SV40 mammalian DNA virus genome

- In 1974 the first eukaryotic gene was cloned. Amplified ribosomal RNA (rRNA) genes or “ribosomal DNA” (rDNA) from the South African clawed frog *Xenopus laevis* were digested with a restriction endonuclease and linked to a bacterial plasmid.

- **The requirements for gene cloning are as follows:**
- 1. Target DNA fragment.
- 2. Restriction enzymes (traditional method), Ligase.
- 3. Cloning vector.
- 4. Host cells.

- To become familiar with gene cloning, the following are the principle steps illustrated in the figure of gene cloning:
- 1. Determine the **targeted gene sequences (foreign DNA fragment)** to design primers and amplify the target gene, taking into consideration gene elements, such as promoter, open reading frame and terminator, for successful gene expression.
- 2. Select an appropriate cloning **vector** that can bind the foreign DNA fragment and ensure the generation of a recombinant DNA molecule.
- 3. Transfer the **recombinant DNA** molecule into the **host** cell, such as bacterial or fungal cells.
- 4. The vector replicates inside host cells, allowing multiple copies of the foreign DNA to be produced along with the vectors.
- 5. The new generation (progeny) of host cells is generated through cell division, passing on the recombinant DNA molecule (vector with foreign DNA) to the new cells.
- 6. Selection of transformed host cells and identification of the clone containing the gene of interest.

- **Fate of inserted gene**
- 1. Multiplication and Expression of the introduced Gene inside host.
- Consequently:
- * Isolation of multiple gene copies/Protein expressed by the gene.

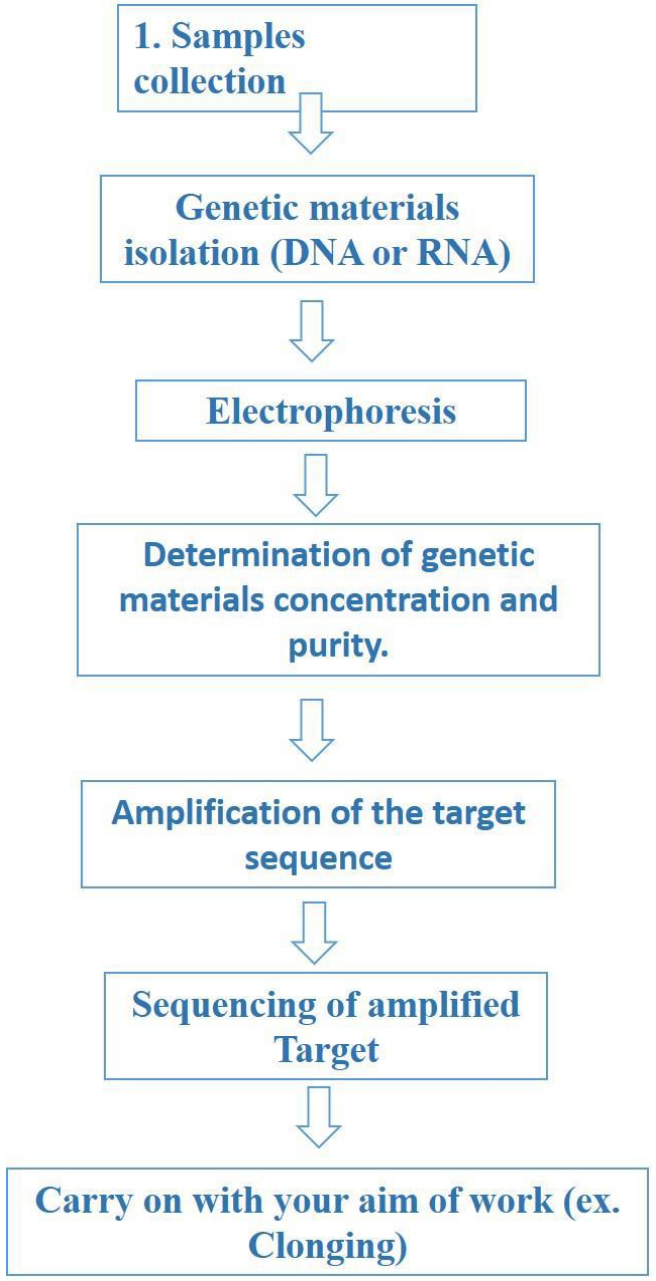
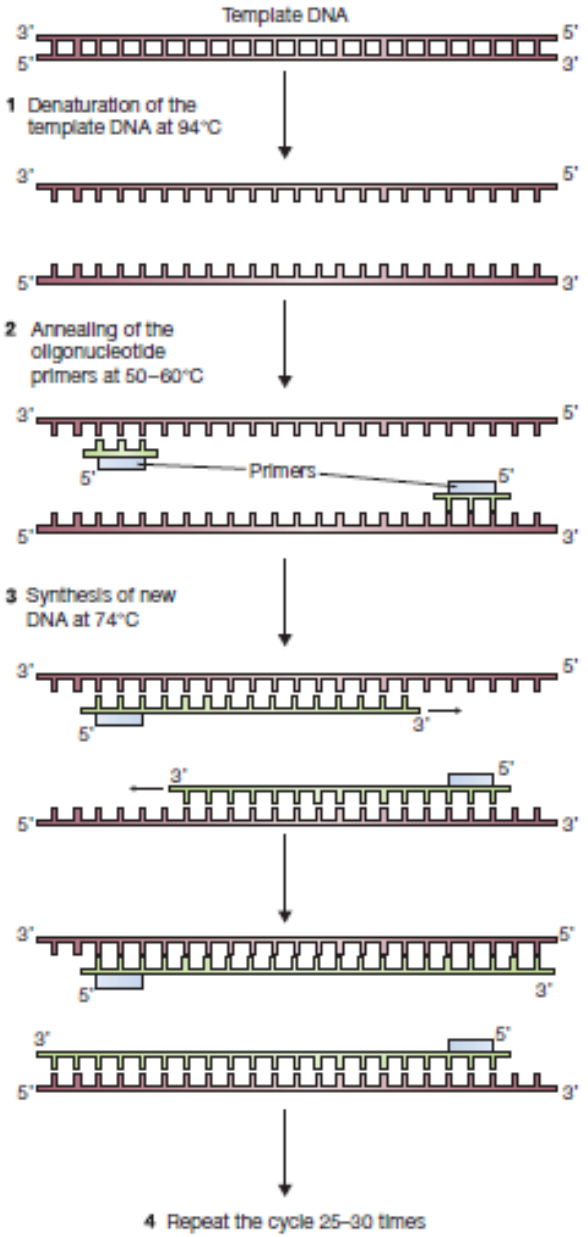


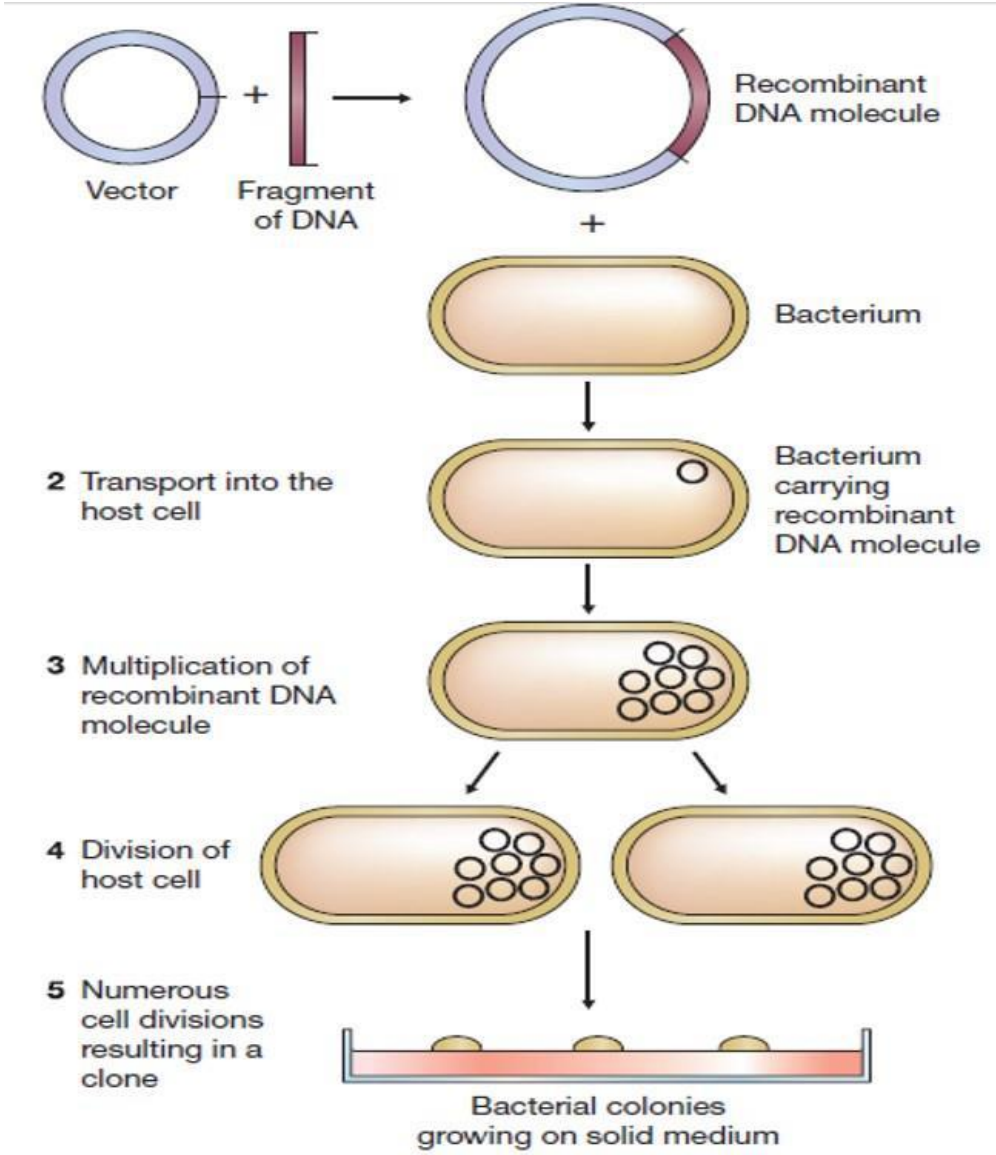
Figure.3 highlights the principle steps necessary to initiate genetic engineering work.



PCR is carried out in a single test tube simply by mixing DNA with a set of reagents and placing the tube in a thermal cycler, a piece of equipment that enables the mixture to be incubated at a series of temperatures that are varied in a preprogrammed manner. The basic steps in a PCR experiment are as follows

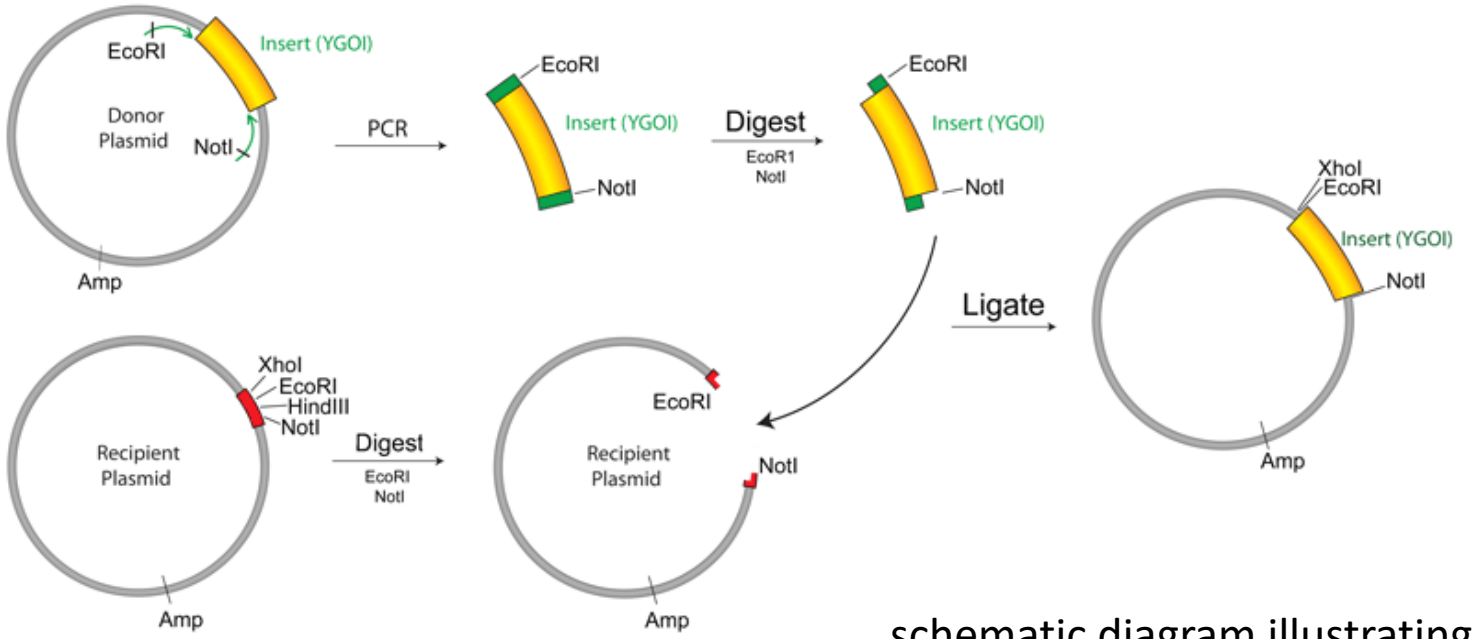
Taq DNA polymerase

- There are two methods utilized in the gene cloning:
- **1. Traditional gene cloning**
- In this methods, a recombinant DNA molecule is generated before transformation. The DNA fragments and circular vectors are subjected to restriction enzymes to generate specific complementary ends sequences. The aim of creating complementary ends is to bind the fragments and generate a recombinant DNA molecule (a vector with foreign DNA) using ligase. Following, this step, transformation is carried out.



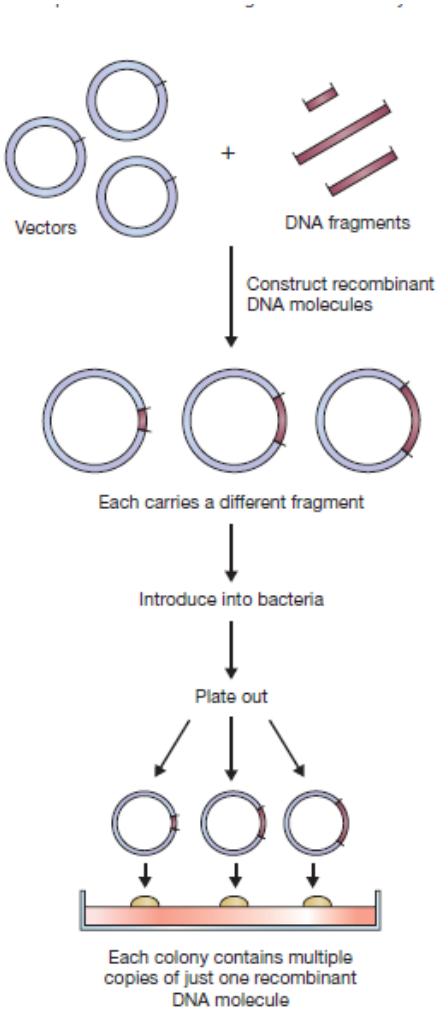
- **2. PCR Cloning Method**
- A new method has been developed that saves time, effort and cost. The procedure involves ligating foreign DNA and a cloning vector using he PCR technique, without the use of restriction enzymes (RE). The ligation of fragments can be carried out using blunt or single-base overhang ligation.

- During the amplification of the fragments, restriction sites are added to the ends of the fragments by incorporating *EcoR*I and *Not*I sites into the designed primer pairs. As shown in the **Figure 2.2**, the restriction site sequences of the vector are derived from (MRS) and are added to the primer pair sequences, which are used to amplify foreign DNA.



schematic diagram illustrating two methods of gene cloning.

- **Why gene cloning and PCR are so important**
- Gene cloning and Polymerase Chain Reaction (PCR) are crucial because they allow scientists to obtain and amplify pure DNA samples, which is fundamental for various applications in medicine, forensics, and industry. Cloning provides access to desired genes and large amounts of proteins for study, while PCR quickly generates many copies of a specific DNA fragment from even a tiny starting amount, enabling rapid detection of diseases, mutations, and unique genetic profiles.



- Why is Gene Cloning Important?
- **Access to Specific Genes:**
- Cloning allows scientists to isolate and purify a single, specific gene from the complex mixture of an organism's DNA.
- **Production of Proteins:**
- Isolated genes can be inserted into expression vectors to produce large quantities of specific proteins, which is vital for research, drug development, and disease treatment.
- **Fundamental Research:**
- Cloning provides the foundation for studying gene structure and function, helping to understand how genes are regulated and how they contribute to various biological processes.

- Why is PCR Important?
- **DNA Amplification:**
- PCR creates billions of copies of a specific DNA segment, even if the starting sample contains only a few molecules.
- **Speed and Sensitivity:**
- It is a fast and highly sensitive method, allowing for quick detection of even minute amounts of DNA or RNA.
- **Diverse Applications:**
- **Medical Diagnostics:** Used to detect infectious agents (viruses, bacteria) early in disease, and to identify genetic mutations that predispose individuals to certain conditions.
- **Forensics:** Amplifies DNA from small evidence samples to create unique genetic profiles for criminal investigations.
- **Research:** Generates enough DNA for sequencing and other experiments, and is essential for studying gene expression and cloning PCR products.

- How They Work Together
- PCR often works in conjunction with gene cloning. A specific gene can be amplified using PCR, and then the resulting fragments can be cloned into a plasmid for further propagation, study, or protein production.