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Programme : M.Sc Biotechnology
Course Title : Genetic Engineering
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Unit -3
Expression Vectors

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EXPRESSION VECTORS

AGENDA

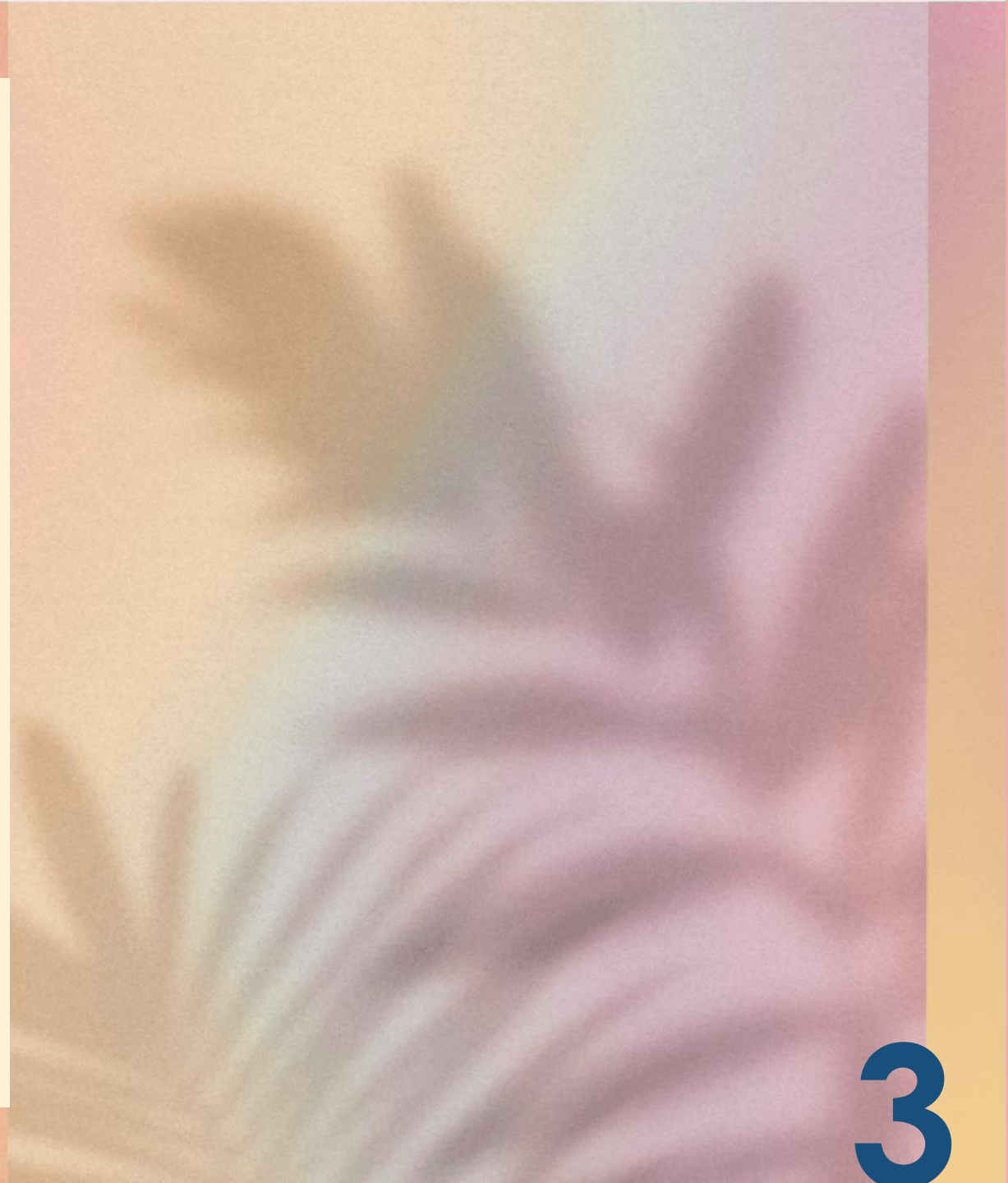
Growth strategy

Market analysis

Financial overview

Innovative solutions

Future initiatives



EXPRESSION VECTORS

- Expression vectors are specialized plasmids or DNA constructs used to introduce a gene of interest into a host cell for the purpose of producing large amounts of a specific protein.
- These vectors are engineered with additional regulatory elements that enable the gene to be expressed in the host cell, allowing for the synthesis, isolation, and purification of proteins for research, therapeutic, and industrial applications.

EXPRESSION VECTORS

- **Basic Components of Expression Vectors**
- Expression vectors contain several key elements to facilitate the successful expression of the gene of interest. These components include:
- **Promoter**: The promoter is a DNA sequence that drives the transcription of the gene of interest. It must be compatible with the host's transcriptional machinery. Common promoters include:
  - **Constitutive Promoters**: Continuously active, such as the *CMV (Cytomegalovirus)* promoter for mammalian cells or *lacZ* for bacteria.
  - **Inducible Promoters**: Activated under specific conditions (e.g., IPTG-induced *lac* promoter in bacteria or tetracycline-regulated systems in mammalian cells).
- **Gene of Interest (GOI)**: This is the DNA sequence that encodes the protein to be expressed. The gene is inserted downstream of the promoter.

EXPRESSION VECTORS

- **Basic Components of Expression Vectors**
- **Ribosome Binding Site (RBS)**: In prokaryotic expression vectors (such as those used in bacteria), an RBS helps the ribosome recognize where translation should begin. In eukaryotic systems, this may not be needed, but translation initiation sites are required.
- **Terminator Sequence**: A sequence that signals the end of transcription, ensuring proper termination of the gene expression process.
- **Multiple Cloning Site (MCS)**: This region contains a series of unique restriction enzyme recognition sites that allow for easy insertion of the gene of interest. It facilitates cloning by providing flexibility for different cloning strategies.
- **Selectability Markers**: These genes provide a way to select cells that contain the expression vector. Common markers include antibiotic resistance genes such as *ampicillin* or *kanamycin* resistance for bacterial cells, or GFP (green fluorescent protein) for eukaryotic cells.

Types of Expression Vectors

- There are different types of expression vectors designed to be used in various host organisms. The choice of expression vector depends on the host system and the intended application.
- **a) Bacterial Expression Vectors**
- **Host Organism**: *Escherichia coli* (E. coli) is the most commonly used host.
- **Features**: These vectors often contain the *lac* operon, which allows for inducible expression (e.g., in the presence of IPTG). They also typically have a small MCS and a selectable marker for antibiotic resistance.
- **Advantages**:
  - High expression levels.
  - Fast and cost-effective protein production.
- **Limitations**:
  - May lead to improper folding of proteins, especially complex eukaryotic proteins.
  - Lack of post-translational modifications like glycosylation.

TYPES OF EXPRESSION VECTORS

- **Types of Expression Vectors**
- **b) Yeast Expression Vectors**
- **Host Organism**: *Saccharomyces cerevisiae* or other yeast species.
- **Features**: These vectors may contain strong yeast promoters (e.g., *ADH1* or *GAL1*), which can be inducible, and integrate with the yeast's protein secretion machinery.
- **Advantages**:
  - Can perform some eukaryotic post-translational modifications.
  - Easier to scale up than mammalian systems.
- **Limitations**:
  - May have limitations in terms of glycosylation and folding compared to mammalian systems.

TYPES OF EXPRESSION VECTORS

- **Types of Expression Vectors**
- **Mammalian Expression Vectors**
- **Host Organism**: Mammalian cells (e.g., CHO cells, HEK293).
- **Features**: These vectors are typically more complex, containing mammalian-specific regulatory elements (e.g., CMV promoter, polyadenylation signal) and selection markers such as *neomycin* or *hygromycin* resistance.
- **Advantages**:
  - Capable of correct protein folding and complex post-translational modifications, including glycosylation.
  - Ideal for producing therapeutic proteins.
- **Limitations**:
  - Slower growth and production compared to bacterial or yeast systems.
  - More expensive to maintain and scale.

- **Types of Expression Vectors**
- **d) Insect Cell Expression Vectors**
- **Host Organism**: *Spodoptera frugiperda* (Sf9) cells or other insect cells.
- **Features**: Often used in combination with the baculovirus system, which allows for efficient gene delivery and high-level expression in insect cells.
- **Advantages**:
  - Good for producing large amounts of protein, including those requiring complex folding and modifications.
- **Limitations**:
  - Requires specialized equipment for handling viruses.
  - Post-translational modifications may differ from those in mammalian cells.

EXPRESSION VECTORS

- **Applications of Expression Vectors**
- Expression vectors are widely used in various fields of biotechnology and medicine:
- **a) Recombinant Protein Production**
- Expression vectors are used to produce proteins for research, including enzymes, antibodies, and hormones. For instance, *insulin*, *growth factors*, and *monoclonal antibodies* can be expressed in bacterial or mammalian systems and purified for therapeutic use.
- **b) Gene Therapy**
- In gene therapy, expression vectors are used to deliver therapeutic genes into patients' cells. These vectors can be used to produce proteins that correct genetic defects or treat diseases by introducing beneficial genes into the body.
- **c) Vaccines**
- Expression vectors can be used to express antigens that are then used to produce vaccines. The antigens stimulate an immune response that protects against specific pathogens.
- **d) Agricultural Biotechnology**
- Expression vectors can be used to produce transgenic plants and animals that express desirable traits such as resistance to pests or enhanced nutritional content.

EXPRESSION VECTORS

- **Challenges and Considerations**
- While expression vectors are powerful tools, there are several challenges and considerations when using them:
- **Codon Optimization**: In some systems, the codons in the gene of interest may not be efficient for the host cell's translational machinery. Codon optimization involves modifying the sequence to match the preferred codons of the host without altering the protein's amino acid sequence.
- **Protein Folding and Solubility**: Some proteins, especially large or complex eukaryotic proteins, may not fold correctly in non-native environments, leading to inclusion body formation in bacteria or misfolded proteins in yeast. Fusion tags (e.g., GST, His-tag) or co-expression systems may help alleviate this problem.
- **Post-translational Modifications**: In bacterial systems, post-translational modifications like glycosylation may not occur, which is essential for some eukaryotic proteins to function properly. This limitation is why mammalian or insect cell systems are often preferred for complex proteins.
- **Scale-up and Purification**: Once the protein is produced, the ability to purify it at a large scale for research or therapeutic use is a significant factor. Proper purification systems need to be in place for successful product development.

THANK YOU

VECTORS NAVIGATING THE FUTURE