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Programme : M.Sc Biotechnology
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Unit -3
Expression Vectors

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GENE FUSION VECTORS

GENE FUSION VECTORS

- A **gene fusion vector** is a specialized plasmid or viral vector used in molecular biology for the purpose of fusing a gene of interest to a reporter or another gene.
- This allows researchers to study the expression, localization, activity, or function of proteins, as well as facilitate the production of recombinant proteins.
- Gene fusion vectors are critical tools in recombinant DNA technology, enabling various applications such as gene expression studies, protein purification, and functional analysis.

GENE FUSION VECTORS

- **What is Gene Fusion?**
- Gene fusion refers to the combination of two or more genes (or gene fragments) into a single continuous DNA sequence, which typically results in the expression of a hybrid protein.
- In the context of a gene fusion vector, one gene is typically fused to a reporter gene, affinity tag, or another protein, allowing for easier tracking, purification, or analysis.
- **The fusion can occur at different levels, such as:**
- **Gene fusion**: A hybrid gene is constructed from the coding sequences of two genes.
- **Protein fusion**: The resulting hybrid gene codes for a single, chimeric protein made from both gene products.

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GENE FUSION VECTORS

- **2. Components of Gene Fusion Vectors**
- Gene fusion vectors are typically plasmids or viruses that contain:
- **Promoter region**: A regulatory sequence that controls the expression of the fused gene. Common promoters used in gene fusion vectors include bacterial (e.g., lac, T7), yeast (e.g., GAL1), and mammalian (e.g., CMV).
- **Gene of interest**: The gene or coding sequence that will be fused to another gene. This is the target gene that researchers want to study or express.
- **Fusion partner**: This could be a reporter gene or tag that will be fused to the gene of interest. Examples include:
  - **Reporter genes**: GFP (Green Fluorescent Protein), luciferase, β-galactosidase, etc.
  - **Affinity tags**: His-tag (poly-histidine), FLAG tag, GST (glutathione S-transferase), MBP (maltose-binding protein), etc.
- **Cloning site**: A region in the vector designed for inserting the gene of interest, often containing restriction enzyme sites.
- **Selectable marker**: A gene that confers resistance to a selective agent, allowing for the identification of cells that have successfully taken up the vector (e.g., antibiotic resistance genes like ampicillin resistance in bacteria).

GENE FUSION VECTORS

- **Types of Gene Fusion Vectors**
- Gene fusion vectors can be classified into different types based on the application and the nature of the fusion:
- **Reporter gene vectors**: These vectors are used to study gene expression, protein localization, or cellular activity. Reporter genes such as GFP or luciferase are fused with the gene of interest. The expression of the reporter gene is used to track or visualize the activity of the fusion protein in cells.
- **Affinity tag vectors**: These vectors fuse a tag (like a His-tag or FLAG tag) to the gene of interest to facilitate protein purification or detection. The tag allows for the protein of interest to be easily purified using affinity chromatography (e.g., using nickel columns for His-tags).
- **Expression vectors for protein production**: In these vectors, a gene of interest is fused with another gene or tag to enable the overproduction of the protein in a host cell, often for the purposes of purification or functional studies. These vectors usually include regulatory sequences to enhance protein expression, such as inducible promoters.
- **Two-hybrid vectors**: Used in yeast two-hybrid systems, these vectors are designed to study protein-protein interactions by fusing one protein with a DNA-binding domain and the other with a transcriptional activation domain.

GENE FUSION VECTORS

- **Applications of Gene Fusion Vectors**
- Gene fusion vectors have a wide range of applications, including:
- **Protein expression and purification**: By tagging the protein with an affinity tag (like His-tag), researchers can easily isolate the protein from a complex mixture using techniques such as affinity chromatography.
- **Studying protein localization**: Reporter genes like GFP are often used to monitor the location of a protein within the cell. When fused with the gene of interest, the resulting fusion protein emits fluorescence, allowing researchers to visualize its subcellular localization in live cells.
- **Functional studies**: By fusing a gene of interest to a reporter or another protein, researchers can analyze the function, activity, and interactions of the protein in vitro or in vivo.
- **Gene expression analysis**: Gene fusion vectors can be used to examine the expression levels of genes, especially in controlled systems (e.g., bacterial, yeast, or mammalian cell cultures).
- **Protein-protein interaction studies**: Fusion vectors are instrumental in studying protein interactions through techniques like the yeast two-hybrid system, co-immunoprecipitation, or FRET (Fluorescence Resonance Energy Transfer).

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- **Examples of Popular Gene Fusion Vectors**
- **pET vectors**: These are popular vectors for protein expression in E. coli, often used with affinity tags like His-tags.
- **pGEX vectors**: These vectors contain the gene for glutathione-S-transferase (GST), which is fused to the gene of interest for easy purification.
- **pCMV-based vectors**: Commonly used for mammalian cell expression, these vectors often include strong promoters like CMV and may also have tags like FLAG or HA for protein detection.

GENE FUSION VECTORS

- **Advantages of Gene Fusion Vectors**
- **Easier detection**: The use of reporter genes or affinity tags makes it easier to detect and quantify the protein of interest.
- **Simplified protein purification**: Fusion tags allow for one-step purification, making protein isolation more efficient.
- **Enhanced expression**: Fusion with tags can sometimes enhance the stability or expression levels of the protein of interest in a host system.
- **Functional analysis**: They enable detailed studies of protein functions, including localization, interactions, and post-translational modifications.

GENE FUSION VECTORS

- **Challenges and Considerations**
- While gene fusion vectors are incredibly useful, there are some challenges and considerations when using them:
- **Impact on protein function**: Fusion tags or reporter genes can potentially alter the conformation, stability, or function of the target protein. Researchers must assess whether the fusion affects the protein's native properties.
- **Size of fusion construct**: Large fusion tags or genes may interfere with the ability of the fusion protein to fold correctly or be expressed in high quantities.
- **Incompatibility with host systems**: Some fusion tags or vectors may not be compatible with all host cells, especially if they require specific machinery for processing or localization.
- **Protease cleavage**: Some fusion proteins may need to be cleaved to remove the fusion tag after purification or analysis, and researchers must choose vectors that allow for controlled cleavage (using proteases like thrombin or TEV protease).

THANK YOU

VECTORS NAVIGATING THE FUTURE