MARUDHAR KESARI JAIN COLLEGE FOR WOMEN (AUTONOMOUS)

VANIYAMBADI

PG and Research Department of Biotechnology

III B.Sc. Biotechnology – Semester - v

E-Notes (Study Material)

Core Course -1: Genetic Engineering	**Code: FBT 51**

Unit: 5 - DNA finger printing, Production of recombinant proteins – insulin and HGH. Genelibraries – Establishing a library, screening the gene library, cDNA library**.**

Learning Objectives: The learning outcomes for DNA fingerprinting, recombinant protein production (such as insulin and HGH), and gene libraries focus on understanding the molecular techniques used in genetic analysis and biotechnology. Students will gain knowledge in DNA fingerprinting methods for identifying genetic variation, the process of producing recombinant proteins by incorporating genes like insulin and human growth hormone into host cells for therapeutic use, and the creation of gene libraries. This includes learning how to establish gene libraries, screen them to identify specific genes, and construct cDNA libraries for studying gene expression and function. These techniques are essential in fields like genetic research, medicine, and biotechnology.

Course Outcome: Upon completing the course, students will be proficient in understanding and applying key techniques in molecular biology, including DNA fingerprinting for genetic identification, the production of recombinant proteins like insulin and human growth hormone (HGH), and the creation and utilization of gene libraries. They will gain hands-on experience in establishing gene libraries, screening for specific genes, and constructing cDNA libraries for gene expression analysis. The course will equip students with practical skills and theoretical knowledge applicable to genetic research, biotechnology, and medical applications.

Overview:

- To study more about DNA fingerprinting .
- To get more knowledge about the gene library.

DNA FINGERPRINTING

DNA fingerprinting, also known as DNA profiling, is a technique used to identify individuals based on their unique DNA characteristics. It is a”powerful tool used in forensic science, paternity testing, and genetic research.

Principle of DNA Fingerprinting

DNA fingerprinting is based on the principle that every individual has a unique DNA sequence, except for identical twins. The technique involves analyzing specific regions of an individual's DNA, known as variable number tandem repeats (VNTRs), which are short sequences of DNA that are repeated multiple times.

Process of DNA Fingerprinting

The process of DNA fingerprinting involves the following steps:

1. DNA extraction: A DNA sample is extracted from a biological fluid, such as blood, saliva, or tissue.

2. _DNA amplification_: The extracted DNA is amplified using the polymerase chain reaction (PCR) technique.

3. _Restriction enzyme digestion_: The amplified DNA is then cut into smaller fragments using restriction enzymes.

4. _Electrophoresis_: The DNA fragments are separated based on their size using electrophoresis.

5. _Hybridization_: The separated DNA fragments are then hybridized with probes that bind to specific VNTRs.

6. _Autoradiography_: The hybridized DNA fragments are visualized using autoradiography.

Interpretation of DNA Fingerprinting Results:

The resulting DNA fingerprint is a unique pattern of bands that represents an individual's DNA characteristics. The pattern is compared to a known DNA sample or a DNA database to identify a match.

Applications of DNA Fingerprinting

DNA fingerprinting has several applications, including:

1. _Forensic science_: DNA fingerprinting is used to identify suspects in crimes, such as murder, rape, and burglary.

2. _Paternity testing_: DNA fingerprinting is used to determine paternity in disputed cases.

3. _Genetic research_: DNA fingerprinting is used to study genetic disorders and identify genetic markers for diseases.

Advantages of DNA Fingerprinting

DNA fingerprinting has several advantages, including:

1. _High accuracy_: DNA fingerprinting is a highly accurate technique, with a low risk of false positives or false negatives.

2. _Unique identification_: DNA fingerprinting provides a unique identification of an individual, making it a powerful tool for forensic science and paternity testing.

3. _Non-invasive_: DNA fingerprinting can be performed using non-invasive samples, such as saliva or buccal cells.

Limitations of DNA Fingerprinting

DNA fingerprinting has several limitations, including:

1. _Contamination_: DNA samples can be contaminated with foreign DNA, which can affect the accuracy of the results.

2. _Degradation_: DNA samples can degrade over time, making it difficult to obtain accurate results.

3. _Cost_: DNA fingerprinting can be a costly technique, making it inaccessible to some individuals or organizations.

RECOMBINANT PROTEIN- INSULIN

Production of Recombinant Protein-Insulin

Insulin is a protein hormone produced by the pancreas that regulates blood sugar levels. Recombinant insulin is produced using genetic engineering techniques, where the human insulin gene is inserted into a bacterial or yeast cell, which then produces the insulin protein.

Steps involved in the production of recombinant insulin:_

1. _Isolation of the human insulin gene_: The human insulin gene is isolated from a human DNA library and cloned into a plasmid vector.

2. _Insertion of the insulin gene into a bacterial or yeast cell_: The plasmid vector containing the insulin gene is inserted into a bacterial or yeast cell, such as E. coli or Saccharomyces cerevisiae.

3. _Expression of the insulin gene_: The bacterial or yeast cell is induced to express the insulin gene, resulting in the production of insulin protein.

4. _Fermentation_: The bacterial or yeast cells are grown in large quantities using fermentation techniques, resulting in the production of large amounts of insulin protein.

5. _Purification_: The insulin protein is purified from the bacterial or yeast cells using various techniques, such as chromatography and centrifugation.

6. _Formulation_: The purified insulin protein is formulated into a pharmaceutical product, such as a solution or suspension, and packaged for distribution.

Techniques used in the production of recombinant insulin:_

1. _Recombinant DNA technology_: This involves the use of genetic engineering techniques to insert the human insulin gene into a bacterial or yeast cell.

2. _Gene expression_: This involves the use of various techniques, such as induction and repression, to control the expression of the insulin gene.

3. _Fermentation technology_: This involves the use of large-scale fermentation techniques to grow bacterial or yeast cells and produce large amounts of insulin protein.

4. _Protein purification techniques_: This involves the use of various techniques, such as chromatography and centrifugation, to purify the insulin protein from the bacterial or yeast cells.

Advantages of recombinant insulin:_

1. _Improved purity_: Recombinant insulin is produced using genetic engineering techniques, resulting in a highly pure product.

2. _Increased availability_: Recombinant insulin can be produced in large quantities, making it more widely available than traditional insulin.

3. _Reduced cost_: Recombinant insulin is often less expensive to produce than traditional insulin, making it more accessible to patients.

4. _Improved safety_: Recombinant insulin is produced using bacterial or yeast cells, which reduces the risk of contamination with animal-derived products.

Disadvantages of recombinant insulin:_

1. _High production costs_: While recombinant insulin is often less expensive to produce than traditional insulin, the initial investment in genetic engineering and fermentation technology can be high.

2. _Complex production process_: The production of recombinant insulin involves a complex process that requires specialized equipment and expertise.

3. _Potential for contamination_: While recombinant insulin is produced using bacterial or yeast cells, there is still a risk of contamination with other substances.

In conclusion, the production of recombinant insulin involves the use of genetic engineering techniques to insert the human insulin gene into a bacterial or yeast cell, which then produces the insulin protein. The resulting product is highly pure, widely available, and often less expensive than traditional insulin. However, the production process is complex and requires specialized equipment and expertise.

PRODUCTION OF HUMAN GROWTH HORMONE

Human Growth Hormone (HGH) is a protein hormone produced by the pituitary gland that regulates growth and development. Recombinant HGH is produced using genetic engineering techniques, where the HGH gene Is inserted into a bacterial or mammalian cell, which then produces the HGH protein.

Steps involved in the production of recombinant HGH:_

1. _Isolation of the HGH gene_: The HGH gene is isolated from a human DNA library and cloned into a plasmid vector.

2. _Insertion of the HGH gene into a bacterial or mammalian cell_: The plasmid vector containing the HGH gene is inserted into a bacterial cell, such as E. coli, or a mammalian cell, such as Chinese Hamster Ovary (CHO) cells.

3. _Expression of the HGH gene_: The bacterial or mammalian cell is induced to express the HGH gene, resulting in the production of HGH protein.

4. _Fermentation_: The bacterial cells are grown in large quantities using fermentation techniques, resulting in the production of large amounts of HGH protein.

5. _Purification_: The HGH protein is purified from the bacterial or mammalian cells using various techniques, such as chromatography and centrifugation.

6. _Formulation_: The purified HGH protein is formulated into a pharmaceutical product, such as a solution or lyophilized powder, and packaged for distribution.

Techniques used in the production of recombinant HGH:

1. _Recombinant DNA technology_: This involves the use of genetic engineering techniques to insert the HGH gene into a bacterial or mammalian cell.

2. _Gene expression_: This involves the use of various techniques, such as induction and repression, to control the expression of the HGH gene.

3. _Fermentation technology_: This involves the use of large-scale fermentation techniques to grow bacterial cells and produce large amounts of HGH protein.

4. _Protein purification techniques_: This involves the use of various techniques, such as chromatography and centrifugation, to purify the HGH protein from the bacterial or mammalian cells.

Advantages of recombinant HGH:_

1. _Improved purity_: Recombinant HGH is produced using genetic engineering techniques, resulting in a highly pure product.

2. _Increased availability_: Recombinant HGH can be produced in large quantities, making it more widely available than traditional HGH.

3. _Reduced cost_: Recombinant HGH is often less expensive to produce than traditional HGH, making it more accessible to patients.

4. _Improved safety_: Recombinant HGH is produced using bacterial or mammalian cells, which reduces the risk of contamination with animal-derived products.

Disadvantages of recombinant HGH:_

1. _High production costs_: While recombinant HGH is often less expensive to produce than traditional HGH, the initial investment in genetic engineering and fermentation technology can be high.

2. _Complex production process_: The production of recombinant HGH involves a complex process that requires specialized equipment and expertise.

3. _Potential for contamination_: While recombinant HGH is produced using bacterial or mammalian cells, there is still a risk of contamination with other substances.

GENE LIBRARIES

A gene library, also known as a DNA library or gene bank, is a collection of cloned DNA fragments that represent the entire genetic material of an organism. It is a powerful tool used in molecular biology to study the

structure, function, and evolution of genes. Gene libraries are used to identify and isolate specific genes, study gene expression, and understand the genetic basis of diseases.

Types of Gene Libraries

There are several types of gene libraries, including:

1. _Genomic DNA library_: A collection of cloned DNA fragments that represent the entire genome of an organism.
2. _cDNA library_: A collection of cloned complementary DNA (cDNA) fragments that represent the messenger RNA (mRNA) molecules expressed in a particular cell or tissue.
3. _Expression library_: A collection of cloned DNA fragments that are expressed in a particular host organism, such as bacteria or yeast.
4. _Subtractive library_: A collection of cloned DNA fragments that are enriched for specific genes or sequences.

Construction of Gene Libraries

The construction of a gene library involves several steps, including:

1. _DNA isolation_: Isolation of DNA from a particular organism or cell type.
2. _DNA fragmentation_: Breaking the DNA into smaller fragments using restriction enzymes or other methods.
3. _Vector preparation_: Preparation of a cloning vector, such as a plasmid or bacteriophage, to accept the DNA fragments.
4. _Ligation_: Joining the DNA fragments to the cloning vector using DNA ligase.
5. _Transformation_: Introducing the recombinant DNA molecules into a host organism, such as bacteria or yeast.
6. _Selection_: Selecting the transformed cells that contain the desired DNA fragments.

Characteristics of Gene Libraries

Gene libraries have several characteristics, including:

1. Representation: The library should represent the entire genetic material of the organism.
2. Completeness: The library should contain all the genes or sequences of interest.
3. Redundancy: The library should contain multiple copies of each gene or sequence.
4. Cloning efficiency: The library should have a high cloning efficiency, meaning that a large proportion of the DNA fragments should be successfully cloned.

Applications of Gene Libraries

Gene libraries have several applications, including:

1. _Gene identification_: Gene libraries can be used to identify specific genes or sequences.
2. _Gene expression studies_: Gene libraries can be used to study gene expression in different cells or tissues.
3. _Gene function studies_: Gene libraries can be used to study the function of specific genes or sequences.
4. _Disease diagnosis_: Gene libraries can be used to diagnose genetic diseases.
5. _Gene therapy_: Gene libraries can be used to develop gene therapy strategies.

Advantages of Gene Libraries

Gene libraries have several advantages, including:

1. _Comprehensive representation_: Gene libraries provide a comprehensive representation of the genetic material of an organism.
2. _High-throughput screening_: Gene libraries can be screened using high-throughput methods, such as DNA sequencing or microarray analysis.
3. _Cost-effective_: Gene libraries can be constructed at a relatively low cost compared to other methods.
4. _Versatile_: Gene libraries can be used for a wide range of applications.

Limitations of Gene Libraries

Gene libraries have several limitations, including:

1. _Complexity_: Gene libraries can be complex and difficult to construct.
2. _Bias_: Gene libraries can be biased towards certain genes or sequences.
3. _Limited representation_: Gene libraries may not represent the entire genetic material of an organism.
4. _Instability_: Gene libraries can be unstable and prone to degradation.

ESHTABLISHING A GENE LIBRARY

Establishing a gene library is a complex process that involves several steps, from planning and designing to constructing and characterizing the library. A gene library is a collection of cloned DNA fragments that represent the entire genetic material of an organism or a specific gene family. It is a valuable resource for researchers, allowing them to study gene function, regulation, and evolution.

Before starting the construction of a gene library, it is essential to plan and design the project carefully. This involves several steps:

1. _Define the scope and goals of the project_: Determine the purpose of the gene library and the types of genes or gene families to be included.

2. _Choose the organism or gene family_: Select the organism or gene family to be targeted for the gene library.

3. _Determine the type of gene library_: Decide on the type of gene library to be constructed, such as a genomic DNA library, a cDNA library, or a subtractive library.

4. _Plan the library construction strategy_: Determine the methods and techniques to be used for constructing the gene library, including the choice of vectors, cloning strategies, and screening methods.

The construction of a gene library involves several steps:

1. _Isolation of DNA or RNA_: Isolate DNA or RNA from the organism or cell type of interest.

2. _Fragmentation of DNA_: Fragment the DNA into smaller pieces using restriction enzymes or other methods.

3. _Cloning of DNA fragments_: Clone the DNA fragments into a vector, such as a plasmid or bacteriophage.

4. _Transformation of host cells_: Transform the recombinant vector into a host cell, such as bacteria or yeast.

5. _Selection and screening of clones_: Select and screen the clones for the presence of the desired gene or gene fragment.

After constructing the gene library, it is essential to characterize it to ensure that it is representative and comprehensive. This involves several steps:

1. _Determination of library complexity_: Determine the complexity of the gene library by estimating the number of independent clones.

2. _Assessment of library representation_: Assess the representation of the gene library by analyzing the distribution of clones and the presence of specific genes or gene families.

3. _Verification of clone identity_: Verify the identity of individual clones by sequencing or other methods.

Once the gene library is constructed and characterized, it is essential to maintain and store it properly to ensure its stability and integrity. This involves several steps:

1. _Storage of clones_: Store the clones in a suitable medium, such as agar plates or frozen stocks.

2. _Maintenance of library records_: Maintain accurate records of the gene library, including information on clone identity, location, and availability.

3. _Quality control and monitoring_: Regularly monitor the gene library for contamination, degradation, or other problems.

Applications of Gene Libraries

Gene libraries have several applications in molecular biology and genetics, including:

1. _Gene discovery and identification_: Gene libraries can be used to discover and identify new genes or gene families.

2. _Gene expression analysis_: Gene libraries can be used to study gene expression and regulation.

3. _Gene function analysis_: Gene libraries can be used to study gene function and interaction.

4. _Genetic engineering and biotechnology_: Gene libraries can be used to develop new genetic engineering and biotechnology applications.

Advantages of Gene Libraries

Gene libraries have several advantages, including:

1. _Comprehensive representation_: Gene libraries can provide a comprehensive representation of an organism's genome or a specific gene family.

2. _High-throughput screening_: Gene libraries can be screened using high-throughput methods, allowing for the rapid identification of specific genes or gene fragments.

3. _Flexibility and versatility_: Gene libraries can be used for a variety of applications, including gene discovery, gene expression analysis, and genetic engineering.

Limitations of Gene Libraries

Gene libraries have several limitations, including:

1. _Complexity and redundancy_: Gene libraries can be complex and redundant, making it difficult to identify specific genes or gene fragments.

2. _Bias and representation_: Gene libraries can be biased towards certain genes or gene families, and may not provide a comprehensive representation of an organism's genome.

3. _Maintenance and storage_: Gene libraries require proper maintenance and storage to ensure their stability and integrity.

SCREENING A GENE LIBRARY

Screening a gene library is the process of identifying and isolating specific genes or clones from a large collection of recombinant DNA molecules. This is typically done using a variety of techniques, including hybridization, immunological screening, and functional screening.

Hybridization Screening*

Hybridization screening is a technique used to identify clones that contain a specific DNA sequence. This is typically done using a labeled probe that is complementary to the target sequence.

1. _Preparation of the probe_: The probe is prepared by labeling a DNA fragment that is complementary to the target sequence.

2. _Denaturation of the library_: The gene library is denatured to create single-stranded DNA molecules.

3. _Hybridization_: The labeled probe is hybridized to the denatured library, allowing the probe to bind to any complementary sequences.

4. _Detection_: The hybridized probe is detected using autoradiography or other methods, allowing the identification of clones that contain the target sequence.

Immunological Screening

Immunological screening is a technique used to identify clones that express a specific protein. This is typically done using antibodies that are specific to the target protein.

1. _Expression of the library_: The gene library is expressed in a host organism, such as bacteria or yeast.

2. _Preparation of the antibodies_: Antibodies that are specific to the target protein are prepared.

3. _Screening_: The expressed library is screened using the antibodies, allowing the identification of clones that express the target protein.

4. _Detection_: The bound antibodies are detected using techniques such as immunoblotting or immunoprecipitation.

Functional Screening

Functional screening is a technique used to identify clones that have a specific function or activity. This is typically done using a variety of assays that measure the activity of the expressed protein.

1. _Expression of the library_: The gene library is expressed in a host organism, such as bacteria or yeast.

2. _Assay development_: An assay is developed to measure the activity of the expressed protein.

3. _Screening_: The expressed library is screened using the assay, allowing the identification of clones that have the desired activity.

4. _Detection_: The activity is detected using techniques such as spectroscopy or chromatography.

Other Screening Techniques

Other screening techniques that can be used to screen a gene library include:

1. _PCR screening_: This involves using PCR to amplify specific sequences from the library.

2. _Sequencing screening_: This involves sequencing the library to identify clones that contain specific sequences.

3. _Bioinformatics screening_: This involves using bioinformatics tools to analyze the library and identify clones that contain specific sequences or motifs.

Advantages and Disadvantages of Screening Techniques

Each screening technique has its own advantages and disadvantages. For example:

1. _Hybridization screening_: This technique is sensitive and specific, but can be time-consuming and labor-intensive.

2. _Immunological screening_: This technique is specific and can be used to identify clones that express specific proteins, but can be affected by protein folding and modification.

3. _Functional screening_: This technique can be used to identify clones that have specific activities, but can be affected by the expression level and stability of the protein.

cDNA LIBRARY

cDNA Library: A Comprehensive Overview

A cDNA (complementary DNA) library is a collection of cloned DNA fragments that represent the messenger RNA (mRNA) molecules expressed in a particular cell or tissue. It is a powerful tool used in molecular biology to study gene expression, identify new genes, and understand the genetic basis of diseases.

Construction of a cDNA Library:

The construction of a cDNA library involves several steps:

1. _Isolation of mRNA_: mRNA is isolated from a particular cell or tissue using techniques such as oligo(dT) cellulose chromatography or magnetic bead-based methods.

2. _Reverse transcription_: The isolated mRNA is converted into cDNA using reverse transcriptase, an enzyme that synthesizes DNA from RNA.

3. _Second-strand synthesis_: The first-strand cDNA is converted into double-stranded cDNA using DNA polymerase.

4. _Cloning_: The double-stranded cDNA is cloned into a vector, such as a plasmid or bacteriophage, using restriction enzymes and DNA ligase.

5. _Transformation_: The recombinant vector is transformed into a host organism, such as bacteria or yeast.

Characteristics of a cDNA Library

A cDNA library has several characteristics:

1. _Representation_: A cDNA library represents the mRNA molecules expressed in a particular cell or tissue.

2. _Completeness_: A cDNA library is considered complete if it contains all the mRNA molecules expressed in a particular cell or tissue.

3. _Redundancy_: A cDNA library may contain multiple copies of the same cDNA clone.

4. _Directionality_: A cDNA library can be directional, meaning that the cDNA clones are oriented in the same direction as the original mRNA molecules.

Types of cDNA Libraries

There are several types of cDNA libraries, including:

1. _Unidirectional cDNA library_: A unidirectional cDNA library is constructed using a directional cloning strategy, where the cDNA clones are oriented in the same direction as the original mRNA molecules.

2. _Bidirectional cDNA library_: A bidirectional cDNA library is constructed using a non-directional cloning strategy, where the cDNA clones are not oriented in a specific direction.

3. _Normalized cDNA library_: A normalized cDNA library is constructed using a normalization strategy, where the abundance of each cDNA clone is normalized to a uniform level.

4. _Subtractive cDNA library_: A subtractive cDNA library is constructed using a subtractive hybridization strategy, where cDNA clones that are common to two or more samples are removed.

Applications of cDNA Libraries

cDNA libraries have several applications:

1. _Gene expression analysis_: cDNA libraries can be used to study gene expression in different cells or tissues.

2. _Gene discovery_: cDNA libraries can be used to identify new genes and their functions.

3. _Disease diagnosis_: cDNA libraries can be used to identify genes that are differentially expressed in diseased versus healthy tissues.

4. _Therapeutic target identification_: cDNA libraries can be used to identify genes that are potential therapeutic targets for diseases.

Advantages of cDNA Libraries

cDNA libraries have several advantages:

1. _High sensitivity_: cDNA libraries can detect low-abundance mRNA molecules.

2. _High specificity_: cDNA libraries can distinguish between closely related mRNA molecules.

3. _Flexibility_: cDNA libraries can be used for a variety of applications, including gene expression analysis and gene discovery.

Limitations of cDNA Libraries

cDNA libraries have several limitations:

1. _Complexity_: cDNA libraries can be complex and difficult to analyze.

2. _Redundancy_: cDNA libraries may contain multiple copies of the same cDNA clone.

3. _Bias_: cDNA libraries may be biased towards certain mRNA molecules.

Reference

- Molecular Cloning: A Laboratory Manual" by Michael R. Green and Joseph Sambrook
- "Biotechnology: A Textbook of Industrial Microbiology" by S. N. Jogdand
- "Molecular Biology of the Cell" by Alberts et al.
- "Genomes" by T.A. Brown
- "Recombinant DNA: Genes and Genomes – A Short Course" by James D. Watson et al.

Additional Link

DNA Library (Genomic, cDNA): Types, Preparation, Uses

cDNA Libraries and Expression Libraries | Fundamentals of Biology | Biology | MIT OpenCourseWare

Practice Question

1. What is DNA fingerprinting?
2. What are recombinant proteins? Give an example.
3. Define gene library.
4. What is the role of a cDNA library?
5. Explain the purpose of screening a gene library.
6. Describe the process of DNA fingerprinting and its applications.
7. Explain the steps involved in the production of recombinant insulin.
8. Discuss the types of gene libraries and their significance in genetic research.
9. Explain how a cDNA library is established and its importance in gene expression studies.
10. Describe the process of screening a gene library to identify a specific gene.
11. Explain the technique of DNA fingerprinting, its methods, and applications in forensic science, paternity testing, and genetic diversity studies.
12. Discuss in detail the process of recombinant protein production, using insulin and human growth hormone (HGH) as examples, including the use of bacterial or yeast expression systems.
13. Define gene libraries and explain the process of establishing both genomic and cDNA libraries. Discuss their applications in biotechnology and gene discovery.
14. Explain the steps involved in constructing and screening a gene library to isolate a gene of interest. Discuss the importance of this process in functional genomics.
15. Elucidate the significance of cDNA libraries in understanding gene expression, including their role in cloning and identifying eukaryotic genes.