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III B.Sc. Biotechnology – Semester - v

E-Notes (Study Material)

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

Unit: 2 - Cloning Vectors – Plasmid vectors – PBR 322, PUC, Ti plasmid, Phage vectors – Lambda, M13, Cosmids, Phagemid. Yeast Vector – Expression vector , shuttle vector. Plant and animal vector – caMv, sv40. Artificial chromosomes – BAC and YAC.

Learning Objectives: The objective of learning about cloning vectors is to understand their role in genetic engineering, focusing on their essential features such as origin of replication, selectable markers, and cloning sites. This includes studying plasmid vectors like pBR322, pUC, and Ti plasmid, as well as phage vectors such as Lambda and M13, along with hybrid vectors like cosmids and phagemids. It also covers yeast vectors, including expression and shuttle vectors, which facilitate gene expression in different hosts. Additionally, the study extends to plant (CaMV) and animal (SV40) viral vectors, which enable genetic modifications in eukaryotic cells. Finally, learners will explore artificial chromosomes like BAC and YAC, which are used for cloning large DNA fragments in genomic studies and biotechnology applications.

Course Outcome: Upon completing this course, students will understand the fundamental role of cloning vectors in genetic engineering, including their key features such as origin of replication, selectable markers, and multiple cloning sites. They will be able to differentiate between plasmid vectors (pBR322, pUC, Ti plasmid) and phage vectors (Lambda, M13), as well as hybrid vectors like cosmids and phagemids. Additionally, they will analyze yeast vectors (expression and shuttle vectors) and evaluate the use of plant (CaMV) and animal (SV40) viral vectors in genetic transformation. Furthermore, students will explore artificial chromosomes like BAC and YAC, gaining insights into their applications in cloning large DNA fragments for genomic studies and biotechnology research. This knowledge will enable them to apply vector-based systems in genetic modification, recombinant protein production, gene therapy, and transgenic research.

Overview:

Plasmid Vectors

Phage Vectors

Cosmids and Phagemids

Yeast Vectors

CLONING VECTORS

Cloning vectors are small DNA molecules that can replicates autonomously in a host cell and carry foreign DNA fragments for cloning purposes. They are essential tools in molecular biology and biotechnology.

Types of cloning vectors:

Plasmids

Bacteriophages

Cosmids

YACs (yeast artificial chromosomes)

BACs (bacterial artificial chromosomes)

Plasmid Vectors

Definition:

Plasmid vectors are small, self-replicating circular DNA molecules used to carry foreign DNA fragments in host organisms.

Characteristics:

1. Origin of replication (ori)
2. Selectable marker (e.g., antibiotic resistance)
3. Multiple cloning site (MCS)
4. Small size (typically 1-10 kb)

Types:

1. High-copy number plasmids (e.g., pUC19)
2. Low-copy number plasmids (e.g., pBR322)
3. Shuttle vectors (can replicate in multiple hosts)
4. Expression vectors (optimized for protein production)

Applications:

1. Gene cloning and expression
2. DNA sequencing
3. Gene therapy
4. Vaccine development
5. Biotechnology and genetic engineering

Advantages:

1. Easy to manipulate and propagate
2. Can carry small to medium-sized DNA inserts

3. Wide range of selectable markers available

Limitations:

1. Limited insert capacity

2. May be unstable in certain hosts

3. Can be lost during cell division

pBR322 Vector

pBR322 :

pBR322 is a high-copy-number, 4.3-kb plasmid vector commonly used in molecular biology for gene cloning, sequencing, and expression.

Key Features:

1. Origin of Replication (ori): pBR322 contains the pMB1 origin of replication, allowing for high-copy-number replication in E. coli.

2. Selectable Markers:

- Ampicillin Resistance (ampR): pBR322 carries the β-lactamase gene, conferring resistance to ampicillin.

- Tetracycline Resistance (tetR): pBR322 also carries the tetR gene, providing resistance to tetracycline.

3. Multiple Cloning Site (MCS): pBR322 has a 54-bp MCS containing 13 unique restriction sites, allowing for easy insertion of DNA fragments.

4. Transcriptional Terminators: pBR322 contains transcriptional terminators to prevent read-through transcription.

Advantages:

1. High-copy-number replication

2. Dual selectable markers (ampR and tetR)

3. Multiple cloning site with 13 unique restriction sites

4. Well-established and widely used

Disadvantages:

1. Limited insert capacity (up to 10 kb)

2. May be unstable in certain E. coli strains

3. Not suitable for large-scale protein production

Applications:

1. Gene cloning and sequencing

2. Gene expression and protein production

3. Subcloning and plasmid construction

4. Molecular biology research and development

pBR322 Derivatives:

Several pBR322 derivatives have been developed, including pBR325, pBR322- derivative with a modified MCS, and pBR322-GFP, a derivative with a GFP reporter gene.

pUC Vector

pUC Overview:

pUC vectors are a family of high-copy-number plasmid vectors commonly used in molecular biology for gene cloning, sequencing, and expression.

Key Features:

1. Origin of Replication (ori): pUC vectors contain the pMB1 origin of replication, allowing for high-copy-number replication in E. coli.

2. Selectable Marker: pUC vectors carry the β-lactamase gene, conferring resistance to ampicillin.

3. Multiple Cloning Site (MCS): pUC vectors have a 54-bp MCS containing 13 unique restriction sites.

4. LacZ' Gene Fragment: pUC vectors contain a fragment of the lacZ gene, allowing for blue/white screening.

Advantages:

1. High-copy-number replication

2. Easy to manipulate and propagate

3. Wide range of restriction sites in the MCS

4. Allows for blue/white screening

Disadvantages:

1. Limited insert capacity (up to 10 kb)

2. May be unstable in certain E. coli strains

Applications:

1. Gene cloning and sequencing

2. Gene expression and protein production

3. Subcloning and plasmid construction

4. Molecular biology research and development.

Phage Vector

A phage vector is a type of vector used in molecular biology for cloning and gene transfer. It is a modified bacteriophage (a virus that infects bacteria) that carries foreign DNA into a host bacterium. Phage vectors are commonly used in recombinant DNA technology to insert genetic material into bacteria for research purposes, such as gene expression or protein production.

In this process, the phage vector can infect a bacterial host, replicate, and express the inserted foreign DNA. Examples of phage vectors include lambda phage vectors and M13 phage vectors, which have been engineered to be useful in genetic studies.

A lambda vector is a type of phage vector derived from the lambda bacteriophage (a virus that infects E. coli). Lambda vectors are engineered to carry foreign DNA into a bacterial host. They can accommodate larger inserts of DNA compared to plasmid vectors, making them particularly useful for cloning large genes or genomic libraries.

Structure:

Lambda vectors are modified versions of the lambda phage genome, which typically include:

Cos sites: Required for the packaging of the DNA into the phage particle.

Cloning sites: Areas where foreign DNA can be inserted.

Selectable markers: To identify successful transformations.

Applications:

Gene Cloning: Lambda vectors are used to clone large pieces of DNA (up to ~20 kb) into E. coli. This is helpful when plasmid vectors are too small to handle larger inserts.

Genomic Libraries: Researchers use lambda vectors to create genomic libraries, where the entire genome of an organism is inserted into a collection of lambda phages.

Protein Production: They are also used for expressing recombinant proteins in bacteria by inserting the gene encoding the protein of interest.

Phage Display: Lambda vectors can be used for displaying peptides or proteins on the surface of phage particles for screening purposes, such as antibody discovery.

Lambda vectors are valuable tools in genetic research, gene expression studies, and biotechnology applications.

M13 Vector:

The M13 vector is a type of phage vector derived from the M13 bacteriophage, a virus that infects Escherichia coli (E. coli) cells. Unlike lambda vectors, which are used for cloning large DNA fragments, M13 vectors are mainly used for cloning smaller DNA inserts and for applications that require the production of single-stranded DNA.

Structure:

The M13 phage genome is circular and single-stranded. M13 vectors are engineered to carry a foreign DNA insert into the phage's genome. These vectors typically include:

Cloning sites for inserting foreign DNA.

Origin of replication: To allow replication within the host.

Selectable markers: To identify cells that have successfully incorporated the vector.

Packaging signals: For efficient packaging of the recombinant DNA into new phage particles.

Applications:

DNA Sequencing: M13 vectors are widely used in Sanger sequencing, where single-stranded DNA is required for the sequencing process. The M13 vector allows easy production of single-stranded templates.

Gene Cloning: M13 vectors are used to clone small to medium-sized DNA fragments (typically up to 10 kb).

Phage Display: M13 vectors are employed in phage display systems, where peptides or proteins are displayed on the surface of the phage particle for applications like screening for binding affinity, antibody discovery, or protein interaction studies.

Mutagenesis: M13 vectors can be used for site-directed mutagenesis, as the single-stranded nature of the phage allows for precise manipulation of the inserted DNA.

M13 vectors are useful in research areas like DNA manipulation, protein engineering, and diagnostics.

Cosmids

Cosmids are a type of vector used in molecular biology that combine features of both plasmids and bacteriophages (specifically, the lambda phage). They are designed to carry larger fragments of DNA than traditional plasmid vectors, typically in the range of 35-45 kb.

Structure:

Cosmids contain:

Plasmid origin of replication: Allows replication in bacterial cells.

Cos sites: These are sequences derived from the lambda phage that enable the DNA to be packaged into a phage particle for infection into host bacteria.

Cloning sites: Where foreign DNA can be inserted.

Selectable markers: Used to identify transformed cells.

Applications:

Cloning Large DNA Fragments: Cosmids are used to clone larger DNA fragments (35-45 kb), which is much more than typical plasmid vectors can accommodate. This is particularly useful for creating genomic libraries.

Genomic Library Construction: Due to their capacity to hold large fragments of DNA, cosmids are ideal for constructing genomic libraries where the entire genome of an organism is represented in fragments.

Gene Expression: Cosmids can also be used for expressing larger genes or multiple genes, especially when the inserts are too large for conventional plasmid vectors.

Cosmids are powerful tools for applications that require the manipulation and analysis of large DNA fragments, such as genomic studies and large-scale cloning projects.

Phagemid:

A phagemid is a hybrid vector that combines elements of both plasmids and bacteriophage vectors, specifically the M13 phage. Phagemids are used in molecular biology for cloning and displaying foreign DNA, particularly when researchers want the advantages of both plasmid replication and phage-mediated packaging.

Structure:

A typical phagemid contains:

Plasmid origin of replication: Allows the vector to replicate like a regular plasmid in bacterial cells.

Phage origin of replication: Allows the production of single-stranded DNA, similar to the M13 phage, which can be packaged into phage particles.

Cloning sites: Where foreign DNA can be inserted.

Selectable markers: To identify bacteria that have successfully incorporated the phagemid.

Applications:

Phage Display: Phagemids are widely used in phage display systems, where foreign peptides or proteins are expressed on the surface of the phage particles. This is useful for screening protein interactions, discovering antibodies, or identifying ligands.

Single-Stranded DNA Production: Phagemids can generate single-stranded DNA for applications like mutagenesis or DNA sequencing. The M13-based replication system allows easy production of single-stranded templates.

Cloning and Transformation: Phagemids can be used for cloning small DNA fragments, and they offer the flexibility of both plasmid replication and phage packaging.

Phagemids are versatile tools, commonly used for cloning, protein expression, and in applications requiring single-strand DNA.

Expression Vector

An expression vector is a type of plasmid or viral vector designed to facilitate the expression of a specific gene within a host organism. Unlike cloning vectors, which are used mainly for the storage or amplification of genetic material, expression vectors contain additional regulatory elements that enable the transcription and translation of the inserted gene into a functional protein.

Key Features of Expression Vectors:

Promoter: A strong promoter is included to drive the transcription of the inserted gene. This promoter can be inducible (turned on/off under specific conditions) or constitutive (always active).

Ribosome Binding Site (RBS): In bacteria, this sequence helps initiate translation by ensuring the ribosome can bind to the mRNA and start protein synthesis.

Selectable Markers: These markers allow the identification of cells that have successfully taken up the expression vector (e.g., antibiotic resistance genes).

Terminator Sequence: Ensures proper termination of transcription.

Tag Sequences: Sometimes included to aid in protein purification or detection (e.g., His-tag, GFP).

Applications:

Protein Expression: Expression vectors are widely used in biotechnology for producing recombinant proteins in host cells, such as bacteria, yeast, or mammalian cells.

Gene Therapy: They are also used in gene therapy, where they deliver therapeutic genes into patients' cells.

Functional Studies: Expression vectors enable researchers to study the function of a gene by producing the corresponding protein and observing its effects in cells or organisms.

Vaccine Production: Recombinant vaccines often use expression vectors to produce antigens.

Expression vectors are essential tools in genetic engineering, enabling high-yield production of proteins for research, medicine, and industrial applications.

Shuttle Vector

A shuttle vector is a type of vector used in molecular biology that is designed to replicate in two different host organisms, typically a bacterial cell (such as E. coli) and a eukaryotic cell (such as yeast or mammalian cells). This ability allows for the easy transfer of genetic material between organisms, making shuttle vectors useful in a variety of genetic engineering and cloning applications.

Key Features of Shuttle Vectors:

Dual Origin of Replication: A shuttle vector contains two separate origins of replication—one that works in a bacterial system (e.g., E. coli) and another that works in a eukaryotic system (e.g., yeast or mammalian cells).

Selectable Markers: Shuttle vectors typically include selectable markers that function in both host organisms, allowing researchers to select for successful transformations in each host.

Cloning Sites: Similar to other vectors, shuttle vectors contain cloning sites where foreign DNA can be inserted.

Promoter Regions: Often, shuttle vectors include promoters compatible with both prokaryotic and eukaryotic systems to drive expression in both types of cells.

Applications:

Gene Cloning and Expression: Shuttle vectors allow researchers to clone genes in bacteria (for amplification and easy handling) and then transfer those genes into eukaryotic cells for further study or protein expression.

Functional Studies: They are useful for expressing and studying genes in different organisms, including studying gene function in yeast or mammalian systems.

Protein Production: Shuttle vectors enable the production of recombinant proteins in a variety of host cells, including yeast or mammalian cells, which may be more suitable for certain types of proteins (e.g., those requiring post-translational modifications).

Gene Therapy: Shuttle vectors are used in gene therapy applications to deliver genes to eukaryotic cells after cloning and amplification in bacterial cells.

Shuttle vectors are valuable tools in genetic engineering, providing flexibility for working with both prokaryotic and eukaryotic systems.

Plant and animal vector

In molecular biology, plant vectors and animal vectors are tools used for transferring foreign genes into plant or animal cells, respectively, for research, breeding, or therapeutic purposes.

Plant Vectors

Plant vectors are used to introduce foreign DNA into plant cells. These vectors are crucial for genetic engineering in plants, such as for developing genetically modified (GM) crops.

Key Types

Agrobacterium Tumefaciens Vectors

The most commonly used plant vectors are based on the Agrobacterium tumefaciens bacterium, which naturally transfers DNA into plants through a process known as transformation.

The Ti plasmid (tumor-inducing plasmid) in Agrobacterium is often modified to carry the desired gene instead of causing tumors.

Applications: Used for introducing genes into dicot plants (e.g., tobacco, tomatoes, and potatoes).

Viral Vectors

Plant viruses, like Tobacco Mosaic Virus (TMV) and Cauliflower Mosaic Virus (CaMV), can also be engineered to carry foreign genes and infect plant cells.

Applications: Primarily used for transient expression in plants (short-term gene expression).

Applications of Plant Vectors

Crop Improvement: Introducing genes for pest resistance, drought tolerance, or improved nutrition.

Gene Function Studies: Investigating the role of specific genes in plant growth or stress responses.

Production of Biopharmaceuticals: Some plants are engineered to produce vaccines, antibodies, or other therapeutic proteins.

Animal Vectors:

Animal vectors are used to introduce foreign DNA into animal cells or organisms. These vectors are important in research, gene therapy, and the production of recombinant proteins.

Key Types:

Viral Vectors:

Retroviruses: These are commonly used for gene transfer in mammalian cells, as they integrate their genetic material into the host cell's genome.

Adenoviruses: These are used for gene transfer into mammalian cells but do not integrate into the host genome, making them useful for transient expression.

Adeno-associated viruses (AAVs): These are used in gene therapy, as they have a low immune response and can integrate into the host genome.

Plasmid Vectors:

Plasmids can be introduced into animal cells via electroporation or lipofection, where the plasmid DNA is taken up by the cells and expresses the foreign gene.

Applications: Common in gene editing and transfection studies.

Lentiviral Vectors:

A subclass of retroviruses, lentiviral vectors are used for gene transfer into both dividing and non-dividing cells. They are often used in gene therapy and research involving stem cells.

Applications: Used in genetic modification of mammalian cells and in the creation of transgenic animals.

Applications of Animal Vectors:

Gene Therapy: Used to treat genetic disorders by introducing therapeutic genes into a patient's cells.

Research: Creation of transgenic animals for studying gene function, disease models, or protein expression.

Recombinant Protein Production: Animal cells (e.g., Chinese hamster ovary cells) are used to produce proteins for pharmaceuticals, such as insulin or growth hormones.

Vaccine Development: Used to create genetically modified animals that can produce antigens for vaccine development.

Conclusion:

Plant vectors are used to transfer genes into plants for genetic engineering, crop improvement, and pharmaceutical production.

Animal vectors are used for gene therapy, research, and recombinant protein production in animals, using viral or plasmid-based systems.

CaMV:

The CaMV vector refers to a type of plant expression vector based on the Cauliflower Mosaic Virus (CaMV), a plant virus that infects members of the Brassicaceae family, including cabbage and cauliflower. CaMV vectors are commonly used in plant genetic engineering for introducing foreign genes into plants, thanks to their ability to infect plant cells and propagate their genetic material.

Key Features:

Viral Origin: CaMV vectors are derived from the genome of the Cauliflower Mosaic Virus, which has been modified for use in plant transformation.

Promoter: One of the critical components of the CaMV vector is the CaMV 35S promoter, which is a strong, constitutive promoter that drives high-level gene expression in a wide variety of plants. This promoter is widely used to ensure continuous expression of inserted genes in the plant.

Packaging Signal: The vector contains sequences from the virus that allow the foreign gene to be packaged into new virus particles and transmitted between plant cells.

Selectable Markers: These vectors often carry genes that confer resistance to antibiotics or herbicides (such as kanamycin or hygromycin), allowing researchers to select transformed plants successfully.

Applications:

Gene Expression: CaMV vectors are used for the stable expression of foreign genes in plants. The 35S promoter drives strong and constitutive expression, which is useful for expressing genes of interest, including genes for pest resistance, drought tolerance, or nutritional enhancement.

Transgenic Plant Development: Used in the development of genetically modified (GM) plants, such as Bt cotton or herbicide-resistant crops, by transferring specific genes into plant genomes.

Protein Production: Some transgenic plants are engineered using CaMV vectors to produce recombinant proteins, including pharmaceutical proteins or vaccines (e.g., edible vaccines).

Gene Function Studies: Used to study the function of particular genes in plants by inserting genes and observing their effects on growth, development, or stress responses.

Advantages:

High Expression Levels: The 35S promoter provides strong and reliable expression of transgenes in many plant species.

Wide Host Range: CaMV vectors can be used in a variety of plant species, making them versatile tools for plant biotechnology.

Limitations:

Viral Nature: Since the vector is derived from a virus, it could potentially cause infection in plants, though the modified vectors typically have their pathogenic potential removed.

Not Suitable for All Plants: The efficiency of transformation using CaMV vectors can vary between plant species.

In summary, CaMV vectors, particularly those carrying the CaMV 35S promoter, are essential tools in plant genetic engineering, enabling the expression of foreign genes in plants for research, agriculture, and biotechnology applications.

SV40

SV40 (Simian Virus 40) is a polyomavirus originally discovered in 1960 as a contaminant in polio vaccines produced using monkey kidney cells. It is a small, circular, double-stranded DNA virus that infects both monkeys and humans. SV40 has been extensively studied due to its implications in virology, cell biology, and cancer research.

Key Characteristics of SV40

Structure:

The virus is about 45 nm in diameter.

Its genome is approximately 5,243 base pairs in length.

The genome encodes early and late proteins:

Early region: Produces large T-antigen and small T-antigen, which are critical for viral replication and can affect cell cycle regulation.

Late region: Encodes structural proteins VP1, VP2, and VP3, which are essential for forming the viral capsid.

Lifecycle:

SV40 infects host cells by binding to specific receptors, entering the cell, and delivering its DNA to the nucleus.

The large T-antigen plays a key role in replication by unwinding the viral DNA and hijacking the host's replication machinery.

Oncogenic Potential:

SV40 is classified as a potentially oncogenic virus. The large T-antigen can interfere with tumor suppressor proteins like p53 and Rb (retinoblastoma protein), leading to uncontrolled cell proliferation.

In laboratory settings, SV40 has been shown to induce tumors in rodents and transform human and animal cells in vitro.

Role in Research:

SV40 is widely used in molecular biology as a model for studying DNA replication, transcription, and tumorigenesis.

It helped elucidate key mechanisms of cell cycle control and oncogenesis.

Human Health Concerns:

The presence of SV40 in early polio vaccines raised concerns about potential links to human cancers. While some studies have detected SV40 DNA in tumors like mesotheliomas, brain tumors, and lymphomas, other research has failed to establish a causal relationship.

Current evidence remains inconclusive regarding its role in human disease, but most vaccines are now screened rigorously for contaminants.

Applications of SV40 in Research

Gene Delivery: Modified SV40 has been used as a vector for delivering genes in research and potential gene therapy.

Cancer Studies: SV40-transformed cell lines are common models for understanding cancer biology.

While SV40 no longer contaminates vaccines, its discovery significantly impacted scientific understanding of viruses and cellular mechanisms.

Artificial chromosomes

Artificial Chromosomes are engineered DNA molecules that can replicate and segregate alongside natural chromosomes within a host organism. They serve as tools for studying genes, delivering therapeutic genes, or producing proteins. A key type of artificial chromosome is the BAC (Bacterial Artificial Chromosome).

Bacterial Artificial Chromosome (BAC)

Definition:

BACs are circular, double-stranded DNA molecules derived from the F-factor plasmid of Escherichia coli. They are designed to clone and propagate large fragments of DNA (up to 300 kilobases) in bacterial cells.

Key Features:

Origin of Replication (OriS):

Ensures the BAC replicates in E. coli.

Controlled by the replication genes from the F-factor plasmid.

Selectable Marker:

Typically includes an antibiotic resistance gene (e.g., kanamycin or chloramphenicol resistance) to allow selection of bacteria carrying the BAC.

Cloning Site:

A multiple cloning site (MCS) enables the insertion of large DNA fragments.

Partitioning System (par genes):

Ensures stable maintenance of the BAC during cell division by ensuring proper segregation into daughter cells.

Low Copy Number:

BACs are maintained in E. coli in low copy numbers (usually 1–2 copies per cell) to minimize the metabolic burden on the host and reduce recombination errors.

Steps in BAC Construction and Use:

Insertion of Foreign DNA:

A large DNA fragment (e.g., a human gene or genomic region) is inserted into the MCS of the BAC.

Transformation:

The recombinant BAC is introduced into E. coli cells via electroporation or chemical transformation.

Replication and Selection:

Only bacteria with the BAC are selected on antibiotic-containing media.

DNA Extraction:

The BAC is isolated from bacterial cultures for downstream applications.

Applications of BACs:

Genome Sequencing:

BACs were essential for projects like the Human Genome Project to sequence large regions of DNA systematically.

DNA fragments in BACs were sequenced and assembled into entire chromosomes.

Functional Genomics:

Used to study gene function and regulation by delivering specific genomic regions into model organisms.

Modeling Genetic Diseases:

BACs carrying human genes are used to create transgenic animal models for studying genetic disorders.

Therapeutics:

In some experimental contexts, BACs have been explored for delivering large therapeutic genes.

Biotechnology:

Used in synthetic biology to clone and express entire metabolic pathways or complex traits.

Advantages of BACs:

Can clone large DNA fragments (up to 300 kb), unlike plasmids that handle smaller inserts.

High stability due to the partitioning system and low copy number.

Reliable maintenance in bacterial cells.

Limitations:

Labor-intensive compared to smaller cloning vectors.

Low copy number can make DNA extraction yield lower quantities compared to high-copy plasmids.

Limited to prokaryotic hosts for propagation.

BAC technology is a powerful tool in genetics, genomics, and biotechnology, particularly for manipulating and studying large genomic regions.

YAC:

YAC (Yeast Artificial Chromosome) is an artificial chromosome designed to clone large fragments of DNA (up to 1 megabase) in yeast cells, typically in the yeast species Saccharomyces cerevisiae. YACs are used to study large genomic regions, express eukaryotic genes, and map genomes.

Structure of a YAC

YACs are linear DNA constructs that mimic eukaryotic chromosomes. They include the following key elements:

Telomeres (TEL):

Protect the ends of the artificial chromosome and maintain its stability within the yeast cell.

Centromere (CEN):

Ensures correct segregation of the YAC during yeast cell division by binding to spindle fibers.

Autonomously Replicating Sequence (ARS):

Acts as an origin of replication in yeast, allowing the YAC to replicate independently of the yeast genome.

Selectable Markers:

Genes such as URA3 and TRP1 enable selection of yeast cells that have successfully taken up the YAC. These markers allow yeast to grow in media lacking specific nutrients.

Cloning Site:

A multiple cloning site (MCS) and restriction enzyme sites are used to insert the DNA fragment of interest.

Vector Backbone:

Contains bacterial sequences such as an origin of replication (ori) and antibiotic resistance genes to propagate and manipulate the YAC in bacterial cells before introducing it into yeast.

Steps in YAC Construction and Use

Preparation of the YAC Vector:

The YAC is linearized by restriction enzymes to create a gap for inserting the target DNA.

Insertion of Target DNA:

Large DNA fragments (up to 1 megabase) are ligated into the YAC vector.

Transformation into Yeast:

The recombinant YAC is introduced into yeast cells through transformation.

Selection:

Transformed yeast cells are selected on media that lack nutrients corresponding to the selectable markers (e.g., uracil or tryptophan).

Replication and Stability:

The YAC replicates in yeast, and its segregation is maintained through mitosis and meiosis, mimicking a natural chromosome.

Applications of YACs

Genome Mapping:

YACs were pivotal in early genomic projects, such as the Human Genome Project, for mapping large regions of DNA and assembling genomes.

Cloning Large DNA Fragments:

Useful for cloning entire genes, regulatory regions, and large genomic loci that are too big for other vectors like BACs.

Functional Genomics:

YACs can introduce human genes into yeast or other organisms to study gene expression, regulation, and function.

Transgenic Models:

YACs carrying large DNA regions are used to create transgenic animals for studying diseases or genetic traits.

Advantages of YACs

Can clone very large DNA fragments (up to 1 Mb), which is beyond the capacity of BACs or plasmids.

Supports eukaryotic modifications, such as splicing and post-translational modifications, making it ideal for studying eukaryotic genes.

Limitations of YACs

Instability:

Large inserts may undergo rearrangements, deletions, or loss during yeast replication.

Technical Challenges:

Working with large DNA fragments is labor-intensive and prone to breakage.

Lower Yield:

Compared to bacterial systems, yields of YAC DNA are lower.

YAC technology played a crucial role in genomics before being partially replaced by BACs, which are more stable and easier to manipulate. However, YACs remain valuable for certain specialized applications requiring large DNA fragments or eukaryotic host systems.

Reference

1. "Molecular Cloning: A Laboratory Manual" by Michael R. Green and Joseph Sambrook
2. "Principles of Gene Manipulation and Genomics" by Sandy B. Primrose and Richard Twyman
3. "Molecular Biology of the Gene" by James D. Watson et al.

4. "Recombinant DNA: Genes and Genomes - A Short Course" by James D. Watson, Alan W. Weiner, and Richard Myers
5. "Genomes 3" by T.A. Brown

Additional Link

pBR322 Vector -Definition, Structure, Sites, Applications

https://www.brainkart.com/article/Vectors---Types-and-Characteristics_41067/?utm_source=chatgpt.com

Practices Question:

1. What is a cloning vector and why is it important in genetic engineering?
2. Explain the difference between a plasmid and a phage vector?
3. Describe the function of the origin of replication (ori) in a plasmid vector.
4. Why are restriction enzymes sites important in cloning vectors?
5. Describe the structure of Pbr322 and explain its use as a cloning vector?
6. Distinguish between YACs and BACs and define artificial chromosomes?
7. What are the advantages of bacterial artificial chromosomes (BACs)?
8. Explain briefly on lambda phage?
9. Discuss about the ti plasmid ?
10. Discuss briefly on expression vector?