Quadrant II – Transcript and Related Materials

Programme : Bachelor of Science (Third Year)

Subject : Zoology

Semester : IV

Course code : ZOD 104

Course title : Animal Biotechnology

Unit : 3

Title of the Unit : Molecular Techniques (Enzymes and vectors) in Gene manipulation.

Module Name : Lambda Bacteriophage vector

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Notes:

LAMBDA BACTERIOPHAGE VECTOR

Introduction:

What are Bacteriophages?

Bacteriophages are the viruses that infect bacterial cells after injecting their genetic material and kill them. The viral DNA or RNA replicates and expressed inside the bacterial cells, and produce a number of phage particles released after bursting the bacterial cell. Bacteriophages are used as a phage vector in gene cloning. Phages are very simple in structure, consisting merely of a DNA (or occasionally RNA) molecule carrying a number of genes, surrounded by a protective coat or capsid made up of protein molecules.

They can undergo two modes of life cycle

- Lytic cycle
- Lysogenic cycle

Why Bacteriophage used as a vector?

- It can accept very large pieces of foreign DNA.
- Genetic engineers have constructed numerous derivatives of phage vectors that contain only one or two sites for a variety of restriction enzymes.
- Phage that have a stuffer fragment are called substitution vectors because they are designed to have a piece removed and substituted with something else.
- Examples are Lambda phage, M13 phage, T4,T7 phage, P1 phage etc.

LAMBDA BACTERIOPHAGE

- This is a DNA virus.
- Ori present for Replication .
- Lambda genome size is 48.5kb (kilobase pairs).
- Phage lambda contains a proteinaceous head and a long tail attached to the head.
- There is a gene for the required enzymes for DNA replication.

CHARACTERISTIC FEATURES OF LAMBDA PHAGE

- Nearly 50 nm diameter
- Icosahedral head
- A flexible tubular protein tail
- Connector serves as a site for attachment of preformed head to tail
- Cos sites on ends
- Has double stranded linear DNA molecule
- Because of presence of cos sites DNA adopts circular structure when injected into host cell.

THE PHAGE INFECTION CYCLE

The general pattern of infection, which is the same for all types of phage, is a three-step process.

1. The phage particle attaches to the outside of the bacterium and injects its DNA chromosome into the cell.

2. The phage DNA molecule is replicated, usually by specific phage enzymes coded by genes in the phage chromosome.

3. Other phage genes direct synthesis of the protein components of the capsid, and new phage particles are assembled and released from the bacterium.

Lysogenic Cycle

In contrast to a lytic cycle, lysogenic infection is characterized by retention of the phage DNA molecule in the host bacterium. With many lysogenic phages the phage DNA (called the prophage) is inserted into the bacterial genome. However, the prophage is eventually released from the host genome and the phage reverts to the lytic mode and lyses the cell.

λ BACTERIOPHAGE AS A CLONING VEHICLE

- Large genome size and low insert capacity.
- Head can accommodate certain amount of DNA (48.5 kbp).
- Presence of multiple sites for a commonly used restriction enzymes.
- Size diminution and increasing cloning capacity.
- Only 50% of genes of wild type phage are essential for its replication and lysis of host cell.

CLONING IN λ VECTORS

Steps are as follows:

1. Restriction digestion of λ vector as well as genomic DNA.
2. Ligation of the DNA fragments into λ vector to form recombinants.
3. Introduction into host by transfection of competent E. coli host or in vitro packaging of recombinant DNA by addition of packaging extract followed by natural infection of the host.
4. Selection and screening of recombinants.

CLASSES OF λ VECTORS

1.Insertional vectors

- Vectors containing at least one unique restriction enzyme site for the insertion of the foreign DNA are insertional vectors
- Size of insertional vectors should not be below 37kbp and that of foreign DNA should be such that it should not increase the size of vector above 52kbp
- E.g. :λ gt10, λ gt11, λ gt18, λ gt23

2. Replacement or substitution vectors

- Substituting the non essential part of vector with gene of interest
- Lowest possible size of replacement vector should be 37kbp
- E.g.: Charon series, λ EMBL Series, λ gt. λ C, λ GEM 11, λ GEM 12, λ DASH, λ FIX