جامعة الانبار
كلية العلوم
قسم التقنيات الأحيائية

اسم المادة: التقنيات الاحيائية
عنوان المحاضرة : Molecular cloning vectors
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MOLECULAR CLONING VECTORS

CLONING VECTORS

DNA molecules that can carry a foreign DNA fragment when inserted into it

Share four common properties:

1) Ability to promote autonomous replication.
2) Contain a genetic marker (usually dominant) for selection.
3) Unique restriction sites to facilitate cloning of insert DNA.
4) Minimum amount of nonessential DNA to optimize cloning.

DIFFERENT TYPES

• **PLASMIDS** • **PHAGES** • **HYBRID VECTORS** • **ARTIFICIAL CHROMOSOMES**

PLASMIDS

Extra-chromosomal,self-replicating ,double stranded, closed circular DNA molecules present in the bacterial cell

- Copy Number: High copy number and low copy number
- Small size
- No of genes is limited
- Episomes

Chang and Cohen first proved the use of plasmid as gene cloning vectors.

CHARACTERISTCS OF IDEAL PLASMID VECTORS

1. Small size: 1.2-3kb
2. Copy number
3. Genetic marker
4. Origin of replication
5. Unique Restriction Site
6. No pathogenicity
7. Multiple Cloning Site
8. Promoter Sequence

NATURAL AND ARTIFICIAL PLAMIDS

- Plasmids isolated from bacteria&directly used for gene cloning without any modification :-Natural Plasmid
- But not used in gene cloning due to large size,confer pathogenicity etc
- Artificial/derived plasmids constructed by cutting out unwanted portions from wild type plasmids.Eg:pBR322
- These are of much use in gene transfer

ADVANTAGES AND DISADVANTAGES

ADVANTAGES:

1. Readily isolated from cells
2. Can be reintroduced into a bacterial cell
3. Possess a single restriction site for 1 or more restriction enzyme
4. MCS 5. Introduction of a linear molecule does not alter its replication

DISADVANTAGES

1) Cannot accept large fragments

LAMBDA PHAGE VECTORS

- Lambda phage is a bacterial virus that infects E.coli.
- Consists of an icosahedral head and flexible tail.
- Phage DNA packed inside the head
- Lambda DNA is a linear DNA duplex with cohesive single strand extensions
- The single stranded extensions of the DNA are COMPLEMENTARY to each other and consists of 12 nucleotides:: Cos Sites
- Free end of the cos site has a 5’ phosphate group
- Lambda Phage DNA can carry large DNA fragments and integrate into host chromosome
- Wild type Lambda DNA can carry only small fragments;The unwanted seq are cut out:- CONSTRUCTED LAMBDA DNA VECTOR
- It is of 2 types:

1) Insertion Vectors- one restriction site; fragments up to 18kb
2) Replacement Vectors-two restriction sites; Removal of stuffer seq

ADVANTAGES & DISADVANTAGES

ADVANTAGES

1. Large DNA fragments upto 23kb can be carried
2. Efficiency of gene transfer is high

DISADVANTAGES

1) Rec. DNA may enter lytic cycle
2) Lambda phage has narrow host range

M13 BACTERIOPHAGE VECTOR

- A single stranded DNA contaning phage virus;Can infect F+ cells
- Consists of about 6402 b.p
- The Intergenic Seq (IS) of M13 modified so as to introduce additional restriction site
- The gene LacZ is integrated and then introduced into the IS to get M13 Vector

HYBRID VECTORS: Cosmids and Phagemids

COSMIDS

- ✓ Artificial plasmid containing Cos-sites of lambda DNA
- ✓ Formed by joining ends of a linearised plasmid DNA with cossites
- ✓ Has an ori,selectable marker,cloning sites of plasmid DNA

Features of COSMID

- ➢ Circular,ds DNA
- ➢ 2 complementary ss regions- cos sites
- ➢ Cosmid DNA does not code for phage protein and host cell lysis
- ➢ Does not involve in multiplication of phage particles
- ➢ Has an ori from plasmid- for independent repl.
- ➢ Has selectable marker gene & gene cloning site of plasmid DNA

ADVANTAGES AND DISADVANTAGES

ADVANTAGES

- Large DNA fragments
- Used to establish gene libraries of lower &higher organisms
- Gene cloning through cosmid helps in the study of nonsense seq in the genome of organism

DISADVANTAGES

1. The packaging enzyme fails to pack rec cosmid into phage heads if 1 of cos-sites is missing;

PHAGEMIDS

- A.k.a Phasmids
- A hybrid vector that has origin of repl from a a plasmid and a lambda phage DNA
- It is constructed by inserting a linearised plasmid DNA into a cleaved lambda DNA

ADVANTAGES

- PHAGEMIDS can be maintained as plamids or phage particles in E.coli
- The desired gene can be integrated into chromosomal DNA of E.coli using phagemids
- Plasmid portion can be released free from a phagemid after rec.phagemid is introduced into an E.coli Strain
- The phages having rec.phagemids can be stored easily for a long time

ARTIFICIAL CHROMOSOMES

YEAST ARTIFICIAL CHROMOSOME(YAC)

Purpose:

- Cloning vehicles that propogate in eukaryotic cell hosts as eukaryotic Chromosomes
- Clone very large inserts of DNA: 100 kb - 10 Mb

Features:

- YAC cloning vehicles are plasmids Final chimeric DNA is a linear DNA molecule with telomeric ends: Artificial Chromosome
- Often have a selection for an insert
- YAC cloning vehicles often have a bacterial origin of DNA replication (ori) and a selection marker for propogation of the YAC through bacteria.
- The YAC can use both yeast and bacteria as a host

Human Artificial Chromosome(HAC)

- Synthetically produced vector DNA possessing the characteristics of human chromosome.
- Discovered in 1997 by H.Williard

Advantages:

1. Can carry Human genes that are too long
2. Can carry genes to be introduced into the cells via gene therapy

Other types

- ✓ Expression vectors
- ✓ Shuttle vectors
- ✓ Integration Vectors
- ✓ Transposons, Retroviruses, Adenoviruses, Baculo viruses

References

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