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التقانة الحيوية
Biotechnology

الكلونة الجزيئية و نواقل الكلونة
MOLECULAR CLONING & CLONING VECTORS

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MOLECULAR CLONING & CLONING VECTORS

MOLECULAR CLONING

- Molecular cloning refers to the process by which recombinant DNA molecules are produced and transformed into a host organism, where they are replicated without the need to cultivate the original organisms.
- A molecular cloning reaction is usually comprised of two components:
  1. The DNA fragment of interest to be replicated.
  2. A vector that contains all the components for replication in the host.
- DNA of interest, such as a gene, regulatory element(s), operon, etc., can be obtained as a PCR product, digested genome or as cDNA.

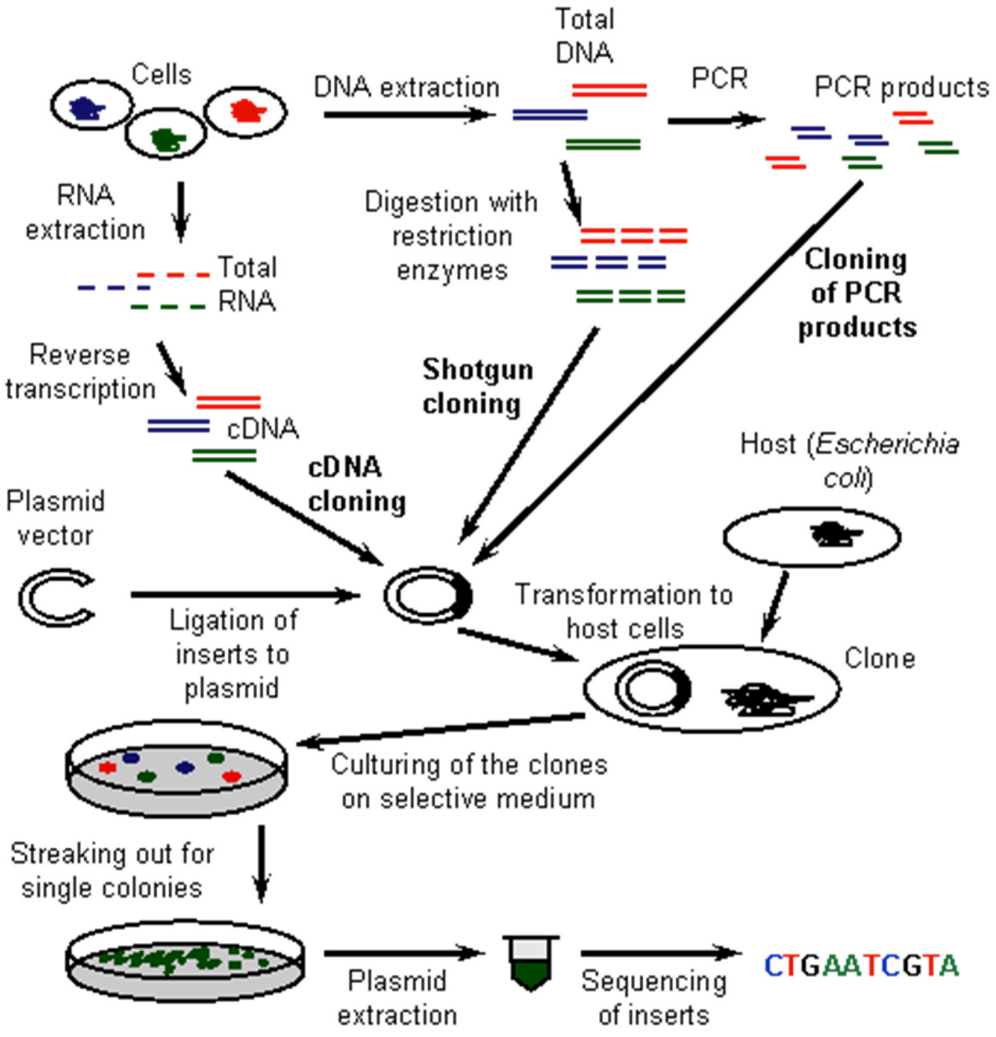


Figure: Strategies and steps in cloning

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: low or high copy number
- Small size
- Number of genes is limited

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. High copy number
3. Contains a 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 modifications are called Natural Plasmid
- Natural plasmids are 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

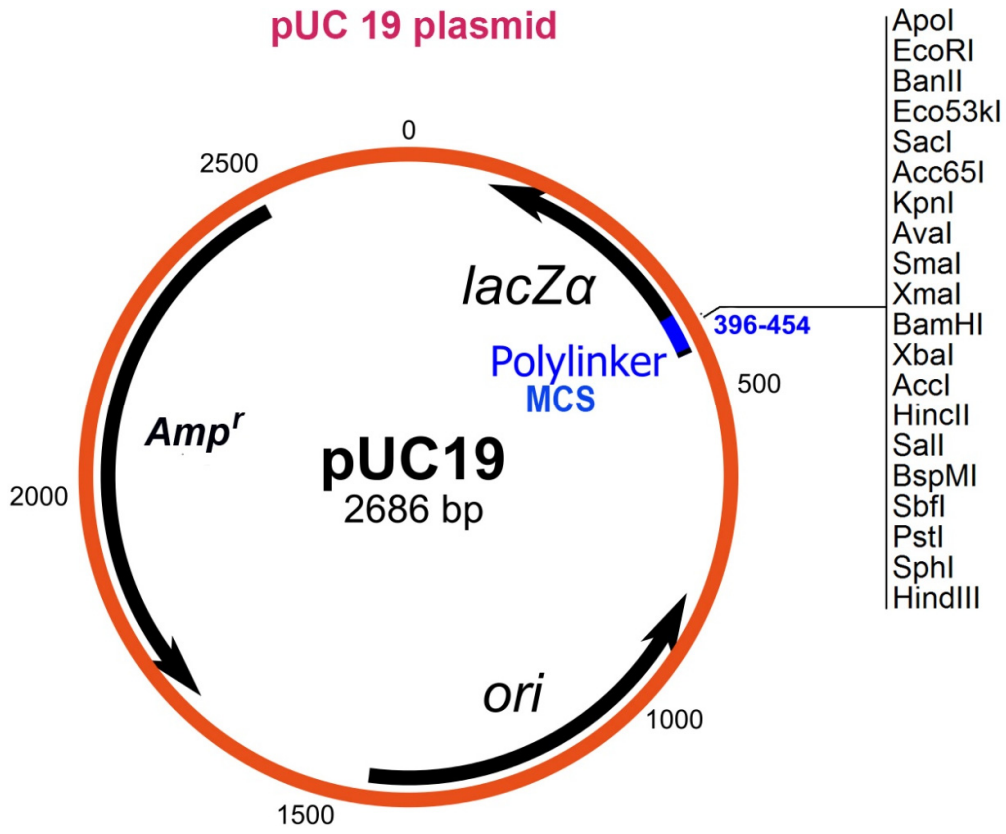
ADVANTAGES AND DISADVANTAGES

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 (Multiple Cloning Site)
5. Introduction of a linear molecule does not alter its replication

DISADVANTAGES

1) Cannot accept large fragments

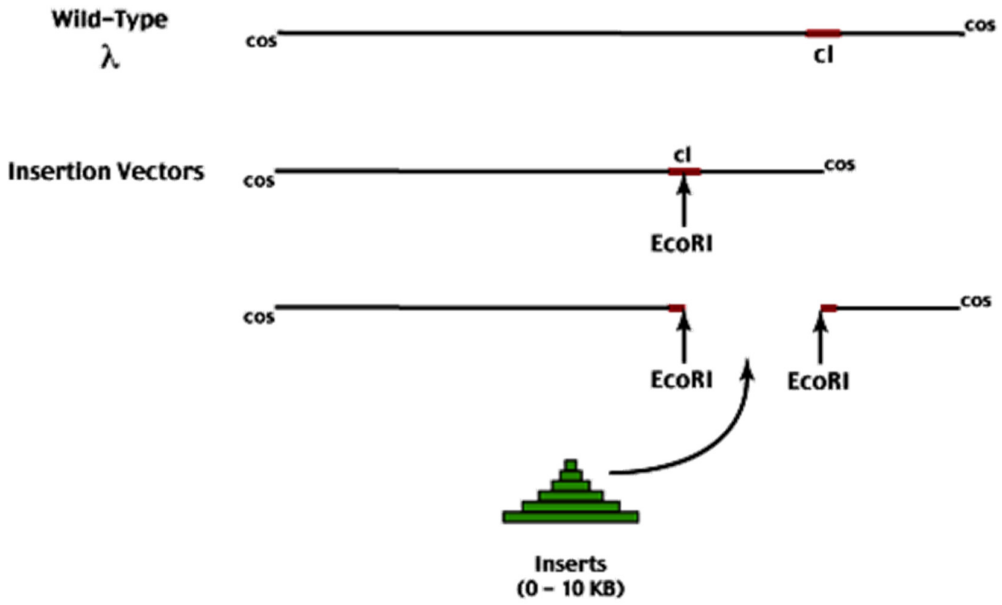


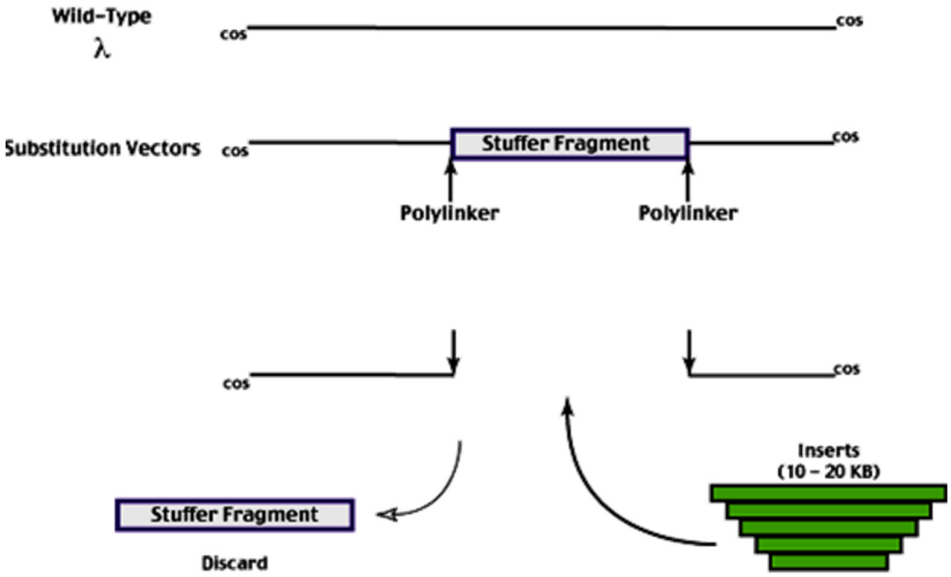
LAMBDA PHAGE VECTORS

- 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: **Co**he**s**ive: **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 of cloned DNA (2.5 kb).
- Approximately 40% of the wild-type lambda's genome is not required for replication by the lytic life cycle therefore, it can be replaced with foreign DNA without affecting the ability of the phage to form plaques.
- The unwanted sequences are cut out to make constructed lambda DNA vector
- It is of 2 types:

1) Insertion Vectors- one restriction site; fragments (0-10 kb)
2) Replacement Vectors-two restriction sites; Removal of stuffer sequence; fragments (10 -20 kb).





ADVANTAGES & DISADVANTAGES

ADVANTAGES

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

DISADVANTAGES

1) Lambda phage has narrow host range
2) Large-scale DNA preps are more time-consuming and yields are lower than for plasmids
3) Lambda clones include a much larger proportion of vector DNA
4) DNA sequencing from lambda clones is more difficult than from plasmids

HYBRID VECTORS: Cosmids and Phagemids

COSMIDS

- ✓ Artificial plasmid containing Cos-sites of lambda DNA
- ✓ Formed by joining ends of a linearized plasmid DNA with Cos sites
- ✓ 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 replication therefore, they can replicate in the cell like a plasmid.
- Has selectable marker gene & gene cloning site of plasmid DNA

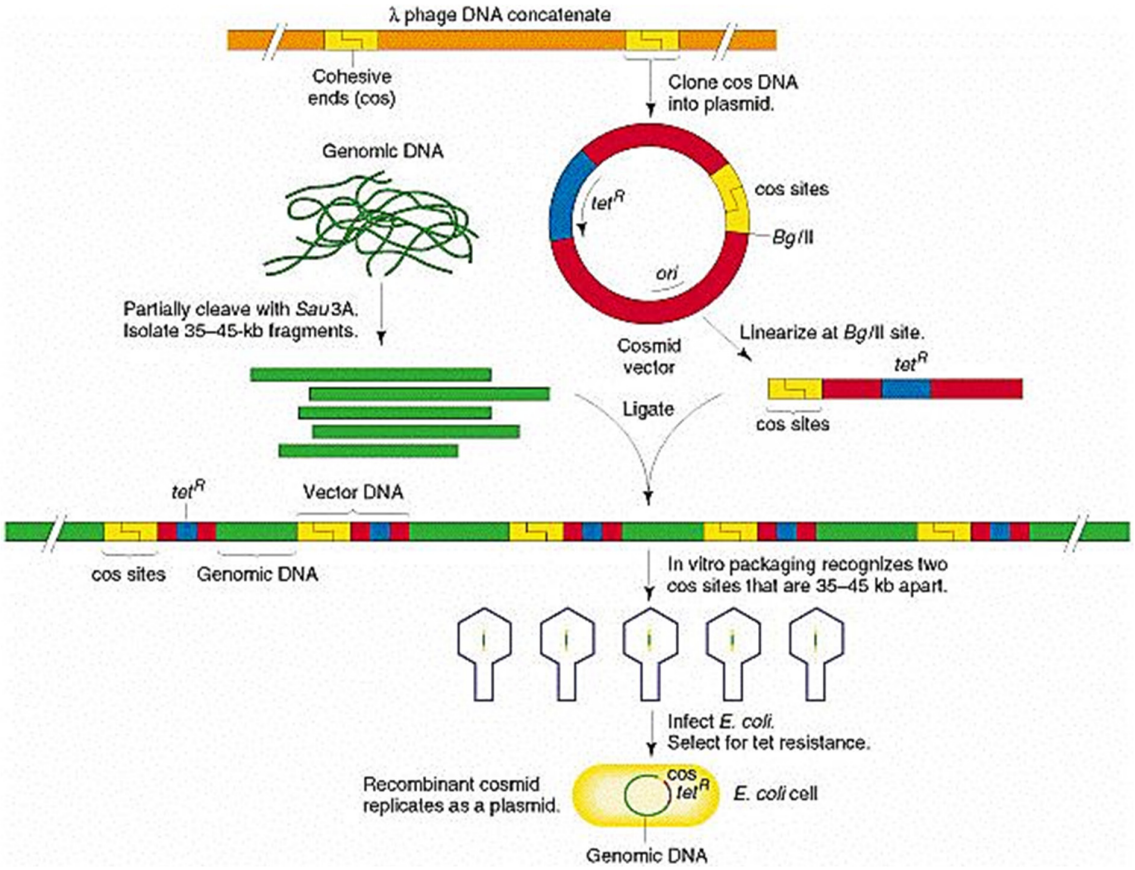
ADVANTAGES AND DISADVANTAGES

ADVANTAGES

- The cosmid vector can carry up to 45 kb whereas plasmid and λ phage vectors are limited to 25 kb.
- Used to establish gene libraries of lower &higher organisms
- High transformation efficiency

DISADVANTAGES

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



PHAGEMIDS

- A.K.A Phasmids
- A hybrid vector that has origin of replication of a plasmid and *ori* of f1 phage or M13 phage DNA
- The basic components of a phagemid mainly include the replication origin of a plasmid, the selective marker, restriction enzyme recognition sites, a promoter and a molecular tag.

ADVANTAGES

- PHAGEMIDS can be maintained as plasmids or phage particles in *E. coli*
- The desired gene can be integrated into chromosomal DNA of *E. coli* using phagemids
- Phagemids can accommodate a larger foreign DNA fragment (up to 10 kb)
- The phages having recombinant phagemids can be stored easily for a long time.

ARTIFICIAL CHROMOSOMES

YEAST ARTIFICIAL CHROMOSOME(YAC)

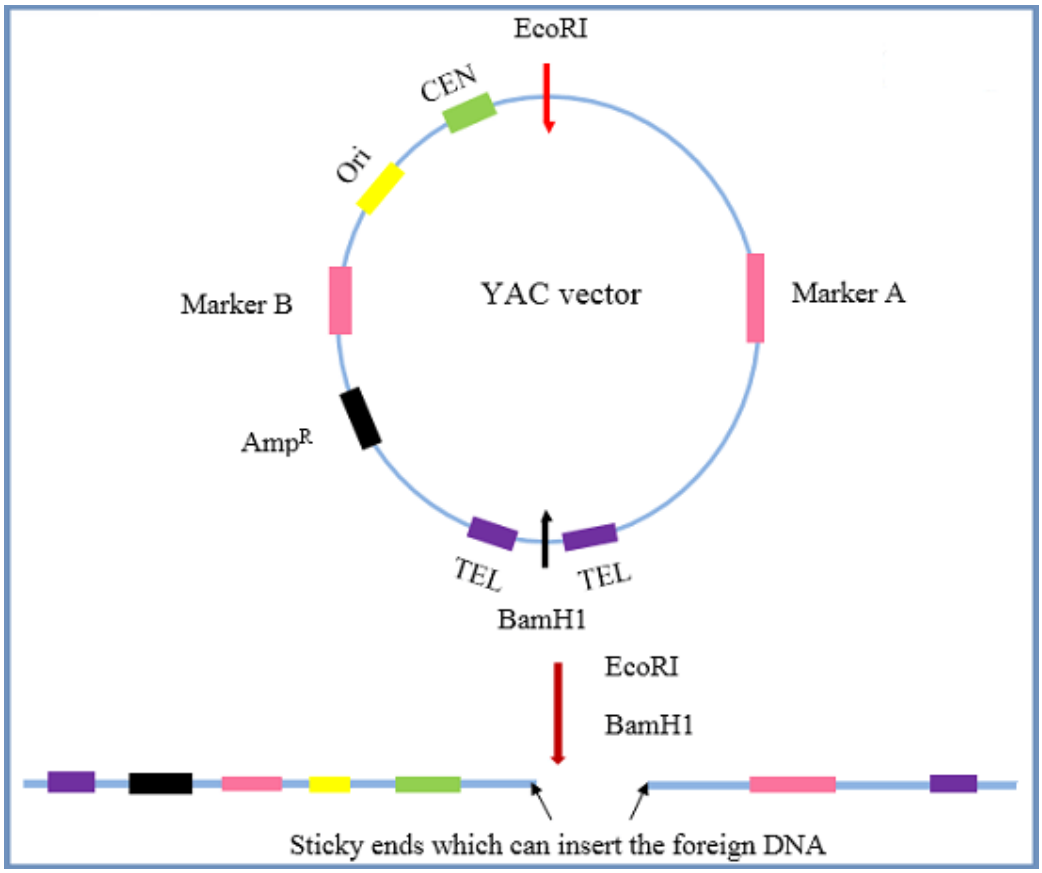
Purpose:

- Shuttle-vectors that propagate in eukaryotic cell hosts as eukaryotic chromosomes
- Constructed in order to study chromosome behaviour in mitosis and meiosis
- Clone very large inserts of DNA: 100 kb - 10 Mb

Features:

- YAC is a linear DNA molecule with telomeric ends: Artificial Chromosome
- Often have a selection for an insert
- They consist of two arms between which large DNA fragments can be cloned
- Each arm contains a telomere (TEL) at the end for stabilization like a normal chromosome

- One of the arms contains an autonomous replication sequence (ARS) required for yeast chromosome replication, a centromere (CEN), and a marker for selection of recombinant yeast (usually a gene for the synthesis of an amino acid)
- TEL, CEN and ARS are essential for maintenance and stability of YAC.



Human Artificial Chromosome (HAC)

- Synthetically produced vector DNA possessing the characteristics of human chromosome.
- Constructed 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

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