

BIOTECHNOLOGY
TOPIC: ARTIFICIAL CHROMOSOME
VECTORS

FOURTH SEMESTER M.SC ZOOLOGY

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ARTIFICIAL CHROMOSOME VECTORS

**BAC- BACTERIAL ARTIFICIAL
CHROMOSOME**

Artificial Chromosome Vectors

- Artificial chromosomes are DNA molecules assembled *in vitro* from defined constituents, which guarantee stable maintenance of large DNA fragments with the properties of natural chromosomes.
- Artificial chromosomes are useful for genome sequencing programmes, for functional characterization of entire genomic regions and for the transduction of large DNA segments into human and nonhuman mammalian cells.
- Types: BAC, YAC, PAC, HAC

BAC

- A bacterial artificial chromosome (BAC) is an engineered DNA molecule used to clone DNA sequences in bacterial cells (for example, *E. coli*).

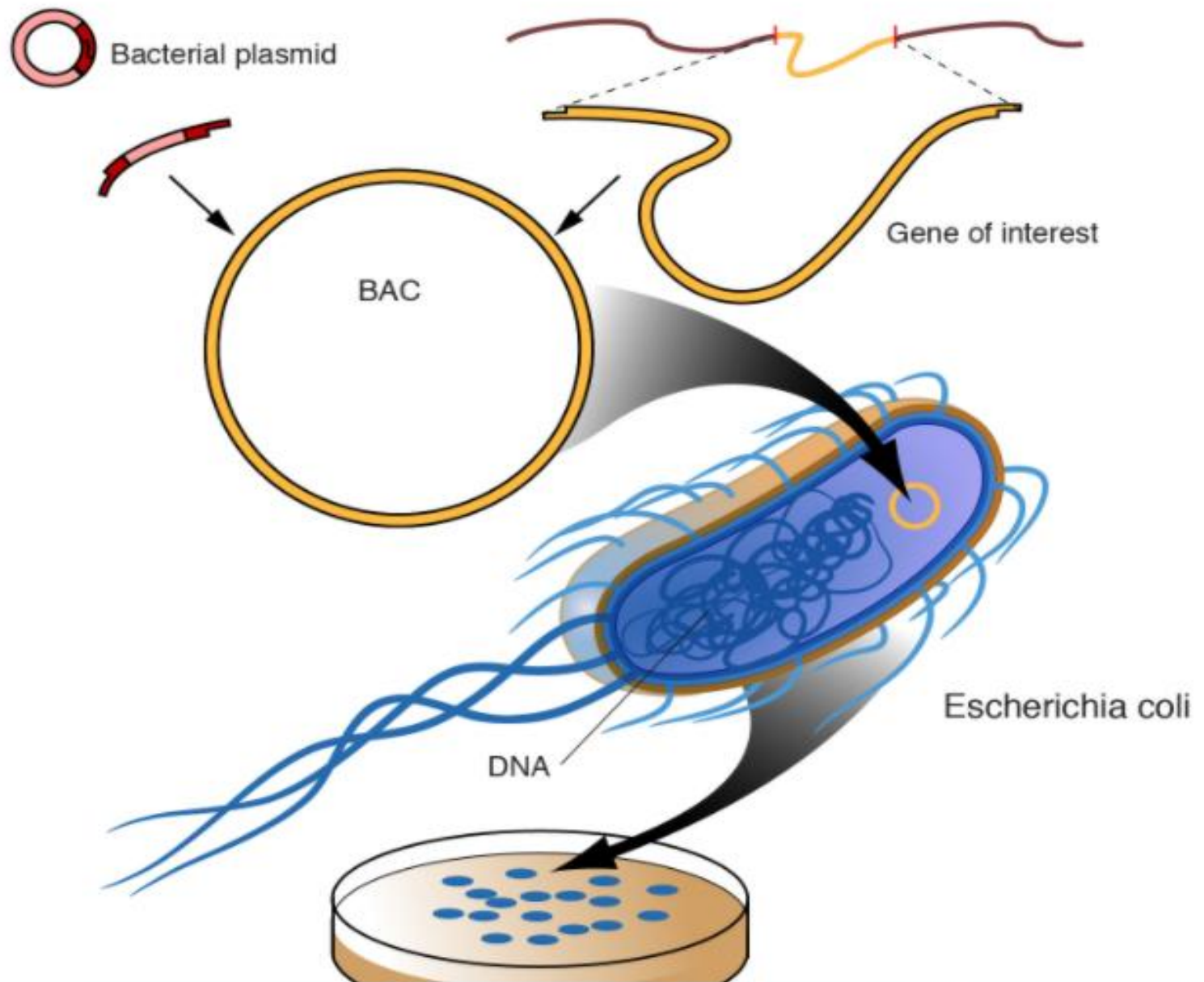
- BACs are often used in connection with DNA sequencing.
- Segments of an organism's DNA, ranging from 100,000 to about 300,000 base pairs, can be inserted into BACs.

- The BACs, with their inserted DNA, are then taken up by bacterial cells. As the bacterial cells grow and divide, they amplify the BAC DNA, which can then be isolated and used in sequencing DNA.

Bacterial Artificial Chromosome (BAC)

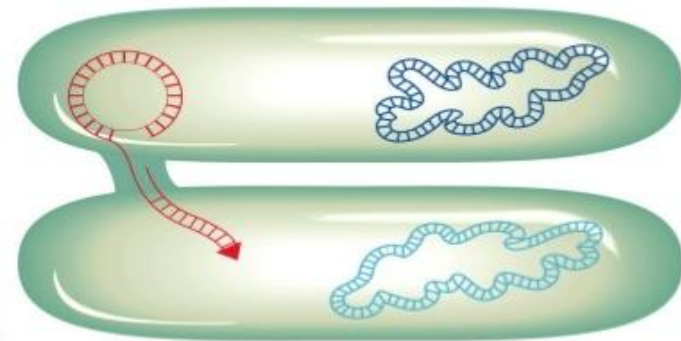
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- BAC vectors are similar to standard *E. coli* plasmid vectors.
- Contain the origin and genes encoding the ori binding proteins required for plasmid replication.
- Derived from a naturally occurring large plasmid, **the F' plasmid**.
- **Low copy number** (1-2 copies per cell)
- The bacterial artificial chromosome's usual insert size is **150-350 kb**.
- BACs are preferred for different kind of genetic studies of inherited or infectious diseases because **they accommodate much larger sequences without the risk of rearrangement**, and are therefore more stable than other types of cloning vectors.



Bacterial artificial chromosome

- A bacterial artificial chromosome (BAC) is a DNA construct, based on a functional fertility plasmid (or F-plasmid), used for transforming and cloning in bacteria, usually *E. Coli*.
- They are capable of carrying approximately upto 300 kbp of inserted DNA sequence.



Common gene components in BAC

- **RepE:** for plasmid replication and regulation of copy number.
- **parA and parB:** for partitioning F plasmid DNA to daughter cells during division and ensures stable maintenance of the BAC.
- **Selectable marker:** for antibiotic resistance; some BACs also have lacZ at the cloning site for blue/white selection.
- **T7 & Sp6:** phage promoters for transcription of inserted genes.

Applications of BAC

- **Contribution to models of disease: Inherited disease**
 - BACs are now being utilized in modeling genetic diseases, often alongside transgenic mice.
 - BACs have been useful in this field as complex genes may have several regulatory sequences upstream of the encoding sequence, including various promoter sequences that will govern a gene's expression level.
 - BACs have been used to study neurological diseases such as Alzheimer's disease or as in the case of aneuploidy associated with Down syndrome. There have also been instances when they have been used to study specific oncogenes associated with cancers.

Applications of BAC [contd.]

- **Contribution to models of disease: Infectious disease**
 - The genomes of several large DNA viruses and RNA viruses have been cloned as BACs.
 - These constructs are referred to as "infectious clones".
 - The infectious property of these BACs has made the study of many viruses such as the herpesviruses, poxviruses and coronaviruses more accessible.

Applications of BAC [contd.]

- **Sequencing:** BACs are often used to sequence the genome of organisms in genome projects, for example the Human Genome Project. A short piece of the organism's DNA is amplified as an insert in BACs, and then sequenced. Finally, the sequenced parts are rearranged *in silico*, resulting in the genomic sequence of the organism.

Yeast artificial chromosome

- Yeast artificial chromosomes (YACs) are genetically engineered chromosomes derived from the DNA of the yeast, *Saccharomyces cerevisiae*
- Yeast artificial chromosome (YAC) vectors allow the cloning, within yeast cells, of fragments of foreign genomic DNA that can approach 500kbp in size.

Cloning vectors and their insert capacities

Vector system	Host cell	Insert capacity (kb)
Plasmid	<i>E. coli</i>	0.1-10
Bacteriophage λ	<i>E. coli</i>	10-20
Cosmid Fosmid	<i>E. coli</i>	35-45
Bacteriophage P1	<i>E. coli</i>	80-100
BAC (bacterial artificial chromosome)	<i>E. coli</i>	50-300
P1 bacteriophage- derived AC (PAC)	<i>E. coli</i>	100-300
YAC	Yeast	100-2,000
Human AC	Cultured human cells	>2,000