Unit 2: Recombinant DNA Technology

Lecture: Artificial vectors – Part 2
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Yeast artificial chromosome

- **Yeast** artificial chromosomes (YACs) are genetically engineered chromosomes derived from the DNA of the yeast.
- It is a human-engineered DNA molecule used to clone DNA sequences in yeast cells.
- They are the products of a recombinant DNA cloning methodology to isolate and propagate very large segments of **DNA** in a yeast host.

Refer to the YAC property figure diagram provided to you on your Whatsapp/ emailgroup
A yeast artificial chromosome cloning vector consists of two copies of a yeast telomeric sequence (telomeres are the sequences at the ends of chromosomes), a yeast centromere, a yeast ars (an autonomously replicating sequence where DNA replication begins), and appropriate selectable markers.

Refer to the YAC vector figure diagram provided to you on your Whatsapp/ e-mailgroup.
The yeast artificial chromosome, which is often shortened to YAC, is an artificially constructed system that can undergo replication. The design of a YAC allows extremely large segments of genetic material to be inserted. Subsequent rounds of replication produce many copies of the inserted sequence, in a genetic procedure known as cloning.

Refer to the YAC principal of construction diagram provided to you on your Whatsapp/ e-mailgroup
• YAC vector is initially propagated as circular plasmid inside bacterial host utilizing bacterial ori sequence.
• The circular plasmid is cut at a specific site using restriction enzymes to generate a linear chromosome with two telomere sites at terminals.

Refer to the YAC property of linearity, genomic insertion, and vector transformation schem figure diagram provided to you on your Whatsapp/e-mailgroup
Advantages:

- Yeast artificial chromosomes (YACs) provide the largest insert capacity of any cloning system.
- Yeast expression vectors, such as YACs, YIPs (yeast integrating plasmids), and YEPs (yeast episomal plasmids), have advantageous over bacterial artificial chromosomes (BACs). They can be used to express eukaryotic proteins that require post-translational modification.

Refer to the YAC cloning advantage figure diagram provided to you on your Whatsapp/ e-mailgroup
**Uses:**

- Yeast artificial chromosomes (YACs) were originally constructed in order to study chromosome behavior in mitosis and meiosis without the complications of manipulating and destabilizing native chromosomes.

Refer to the YAC application figure diagram provided to you on your Whatsapp/e-mailgroup.
Limitations:

- A problem encountered in constructing and using YAC libraries is that they typically contain clones that are chimeric, i.e., contain DNA in a single clone from different locations in the genome.
- YAC clones frequently contain deletions, rearrangements, or noncontiguous pieces of the cloned DNA. As a result, each YAC clone must be carefully analyzed to be sure that no rearrangements of the DNA have occurred.

Refer to the YAC limitation figure diagram provided to you on your Whatsapp/e-mailgroup
Human artificial chromosome

- A human artificial chromosome (HAC) is a microchromosome that can act as a new chromosome in a population of human cells.
- That is, instead of 46 chromosomes, the cell could have 47 with the 47th being very small, roughly 6-10 megabases (Mb) in size instead of 50-250 Mb for natural chromosomes, and able to carry new genes introduced by human researchers.
- Ideally, researchers could integrate different genes that perform a variety of functions, including disease defense.
A human artificial chromosome (HAC) is a mini-chromosome that is constructed artificially in human cells. Using its own self-replicating and segregating systems, a HAC can behave as a stable chromosome that is independent from the chromosomes of host cells. The essential elements for chromosome maintenance and transmission are the following three regions:
(1) the “replication origin,” from which the duplication of DNA begins,
(2) the “centromere,” which functions in proper chromosome segregation during cell division, and
(3) the “telomere,” which protects the ends of linear chromosomes.

Refer to the HAC planning diagram provided to you on your Whatsapp/ e-mailgroup
Advantage of HAC

- Alternative methods of creating transgenes, such as utilizing YACs and BACs, lead to unpredictable problems. The genetic material introduced by these vectors not only leads to different expression levels, but the inserts also disrupt the original genome. HACs differ in this regard, as they are entirely separate chromosomes. This separation from existing genetic material assumes that no insertional mutants would arise. This stability and accuracy makes HACs preferable to other methods such as viral vectors, YACs and BACs.

- HACs allow for delivery of more DNA (including promoters and copy-number variation) than is possible with viral vectors.
Applications of HAC

- HACs are useful in expression studies as gene transfer vectors, as a tool for elucidating human chromosome function, and as a method for actively annotating the human genome.
- HACs have been used to create transgenic animals for use as animal models of human disease and for production of therapeutic products.
- HAC can carry genes to be introduced into the cells in gene therapy.
Mammalian Artificial Chromosomes: MAC

- MACs or mammalian artificial chromosomes, like YACs, rely on the presence of centromeric and telomeric sequences and origin of DNA replication.
- They involve autonomous replication and segregation in mammalian cells, as opposed to random integration into chromosomes (as for other vectors).

Refer to the MAC vector planning diagram provided to you on your Whatsapp/e-mailgroup
Mammalian Artificial Chromosomes: MAC

- MAC vectors are difficult to assemble as compared to YAC vectors. Mammalian DNA has a higher degree of repetition and larger centromere and telomere regions. Also, the sequences necessary for chromosome replication in mammalian systems are not well defined till now. MAC vectors have application in the field of gene therapy and eukaryotic protein expression and production.

Refer to the MAC vector cloning and advantage diagram provided to you on your Whatsapp/ e-mailgroup.
Next lecture will be about Lambda phage, M13 Phagemid, Cosmid and shuttle vector.