Quadrant II – Transcript and Related Materials

Programme : Bachelor of Science (Third Year)

Subject : Zoology

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Course title : Animal Biotechnology

Unit : 3

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

Module Name : Yeast Artificial Chromosome and (YAC)

Mammalian Artificial Chromosome (MAC).

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Notes:

YEAST ARTIFICIAL CHROMOSOME (YAC)

Introduction:

Yeast Artificial Chromosomes (YACs) are genetically engineered chromosomes derived from the DNA of the yeast, *Saccharomyces cerevisiae,* which is then ligated into a bacterial plasmid. It is human-engineered DNA molecule used to clone DNA sequences in yeast cells. First described in 1983 by Murray and Szostak. YACs are the largest capacity Yeast vectors, accommodating 0.2 to 2 megabase range foreign DNA fragments within Yeast Cell.

STRUCTURE OF YAC VECTOR

YAC consists of

- two copies of Yeast telomeric sequence (TEL), for maintenance.
- Yeast centromere (CEN4), for segregation at cell division.
- Yeast ARS (an autonomously replicating sequence where DNA replication begins), and
- Appropriate selectable markers

CONSTRUCTION OF YAC VECTOR

Several YAC vectors have been developed but each one is constructed along the same lines, with pYAC3 being a typical example.

YAC is built using an initial circular DNA plasmid, which is typically cut into a linear DNA molecule using restriction enzymes.

DNA ligase is then used to ligate a DNA sequence or gene of interest into the linearized DNA, forming a single large, circular piece of DNA.

The recombinant vectors are transformed into yeast cells and screened for the selection markers to obtain recombinant colonies.

APPLICATIONS OF YAC

- YAC provide the largest insert capacity of any cloning system, i.e., up to 2 Mb.
- Yeast expression vectors, such as YAC's, YIPs(Yeast Integrating Plasmids), and YEP's(Yeast Episomal Plasmids), have advantageous over BAC'S. They can be used to express eukaryotic proteins that require post-translational modification.
- A major advantage of cloning in yeast, a eukaryote, is that many sequences that are unable, underrepresented or absent when cloned into prokaryotic systems, remain stable and intact in YAC clones.
- Generating whole DNA libraries of the genomes of higher organisms.
- To identifying essential mammalian chromosomal sequences necessary for the future construction of specialized mammalian artificial chromosomes (MACs).
- To study the regulation of gene expression by cis-acting, controlling DNA elements.

LIMITATIONS OF YAC

- Though the principle of cloning is similar to plasmid or cosmid, but the process of cloning is too complicated to carry out.
- It is difficult to isolate YAC DNA from host cell because of its smaller size to host chromosome. Even higher yields cannot be obtained.
- YACs are significantly less stable than BACs, producing "chimeric effects".
- YACs harbour very large sized insert which is prone to breakage resulting in rearrangement and recombination with other DNA in host cell.

MAMMALIAN ARTIFICIAL CHROMOSOME (MAC)

INTRODUCTION

Mammalian artificial chromosomes (MACs) are conceptually similar to YACs, but instead of yeast sequences they contain mammalian or human ones.

In this case the telomeric sequences are multimers (multiple copies) of the sequence TTAGGG, and the commonly used centromeric sequence is composed of another repeated DNA sequence found at the natural centromeres of human chromosomes and called alphoid DNA.

Chromosomes in eukaryotes have evolved as vehicles for nuclear genes and have developed specialized nucleoprotein structures for this purpose, some of them are centromeres, telomeres and origin of replication. Alphoid arrays are found at all human centromeres and consist of a 171 bp monomer organized in higher order repeats encompassing 0.5-5 Mb. They have been considered the best candidate for the specific DNA requirement for centromere function.

HUMAN ARTIFICIAL CHROMOSOME (HAC)

- MACs require a functional centromere essential to maintain their nuclear location and to ensure their correct mitotic and meiotic segregation; they can be linear or circular depending on the method used for their generation.
- Most of the known MACs have been built starting from human chromosome elements therefore they are named as human artificial chromosomes (HACs).
- Such HACs are mitotically stable in human cells, in mouse and in chicken cells as well; very large genomic DNA fragments have been introduced into mammalian cells using HACs
- 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- 10megabases (Mb) in size instead of 50-250 Mb for natural chromosomes, and able to carry new genes introduced by human researchers.
- HACs were first constructed de novo in 1997 by adding alpha-satellite DNA to telomeric and genomic DNA in humanHT1080 cells.

CONSTRUCTION OF HAC VECTOR

- There are currently two accepted models for the creation of human artificial chromosome vectors;
  - Top - down approach
  - Bottom - up approach

TOP - DOWN APPROACH

The top-down approach starts with a natural human chromosome and seeks to strip it down and reengineer large regions. The chromosome is repeatedly truncated into mini-chromosome through telomere-directed chromosome truncation. The resulting HAC, which is a modified natural chromosome rather than a completely de novo synthetic chromosome, can then be transferred into other cell lines by microcell fusion. The most advanced top-down HAC is the 21HAC.

BOTTOM-UP APPROACH.

In the bottom-up approach, a de novo HAC is generated transfecting eukaryotic cells with synthetic alpha-satellite DNA. This alpha-satellite DNA has the potential for the de novo centromere establishment in HACs. These modifications will lead to the generation of a fully active centromere, thus converting the vector into an artificial chromosome. The resulting HAC will be circular if the input DNA is cloned into a BAC or linear when a YAC carrying telomeric sequences is used as a vector to introduce the alphoid DNA in the target cells.

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.
- A 2009 study has shown additional benefits of HACs, namely their ability to stably contain extremely large genomic fragments.
- HACs have been used to create transgenic animals for use as animal models of human disease and for production of therapeutic products.

LIMITATIONS OF MAC

- Despite some of the advances, the engineering of MACs is still quite difficult.
- The “bottom up” approach relies on the quite tedious protocol.
- The HAC formation is always accompanied by multimerization of the input alphoid DNA. These rearrangement events result in structurally uncharacterized artificial chromosomes and raise the issue on how to get better control on MAC formation to avoid this problem.