Artificial Chromosome Constructions And Its Uses

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Abstract

Artificial chromosomes are constructed in laboratory and it contain DNA sequences assembled *in vitro* from defined constituents, which assures steady maintenance of big DNA pieces. These artificial chromosomes performs all the main functions like natural chromosomes and are useful for genomic sequencing. These artificial chromosome mainly considered of bacterial artificial chromosome (BAC), P-1 derived artificial chromosome (PAC), yeast artificial chromosome (YAC), mammalian artificial chromosome (MAC), human artificial chromosome (HAC). These chromosomes used for cloning of large segment of DNA and used to launch and control new DNA sequences in host cell, to study chromosomal functions and to map genes.

Keywords: Artificial chromosomes, BAC, PAC, YAC, MAC , HAC.

Introduction

Artificial chromosome is a functional chromosome constructed by genetic engineering, having a centromere and a telomere at each end, if linear rather than circular) and thus transmissible in cell division after introduction into a cell. The main aim of this article is to provide introductory information to stakeholders.

Types Of Artificial Chromosome

Artificial chromosome mainly considered of bacterial artificial chromosome (BAC), P-1 derived artificial chromosome (PAC), yeast artificial chromosome (YAC), mammalian artificial chromosome (MAC), human artificial chromosome (HAC). All these types of vectors will discuss in details as under.

1) YAC (Yeast Artificial Chromosome)

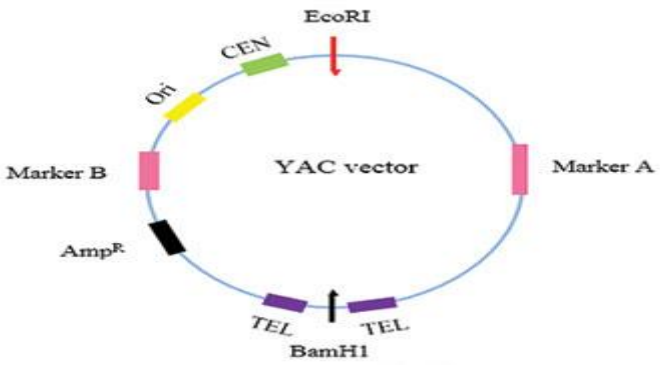
A yeast artificial chromosome (YAC) is a vector and was first described in 1983 by Murray and Szostak. It is used to clone DNA fragments larger than 100 kb and up to 3000 kb. A YAC is built using an initial circular plasmid, which is typically broken into two linear molecules using restriction enzymes; DNA ligase is then used to ligate a sequence or gene of interest between the two linear molecules, forming a single large linear piece of DNA. YAC have following important regions.

i) TEL: The telomere which is located at each chromosome end, protects the linear DNA from degradation by nucleases.

ii) CEN: The centromere which is the attachment site for mitotic spindle fibers, "pulls" one copy of each duplicated chromosome into each new daughter cell.

iii) ORI: Replication origin sequences which are specific DNA sequences that allow the DNA replication machinery to assemble on the DNA it can also called autonomously replicating sequence (ARS).

In addition to above regions, it contains few other specific sequences like yeast selectable marker A and B that allow the easy isolation of yeast cells that have taken up the artificial chromosome, bacterial selectable marker, such as ampicillin resistance marker and recognition site for the two restriction enzymes EcoRI and BamHI.



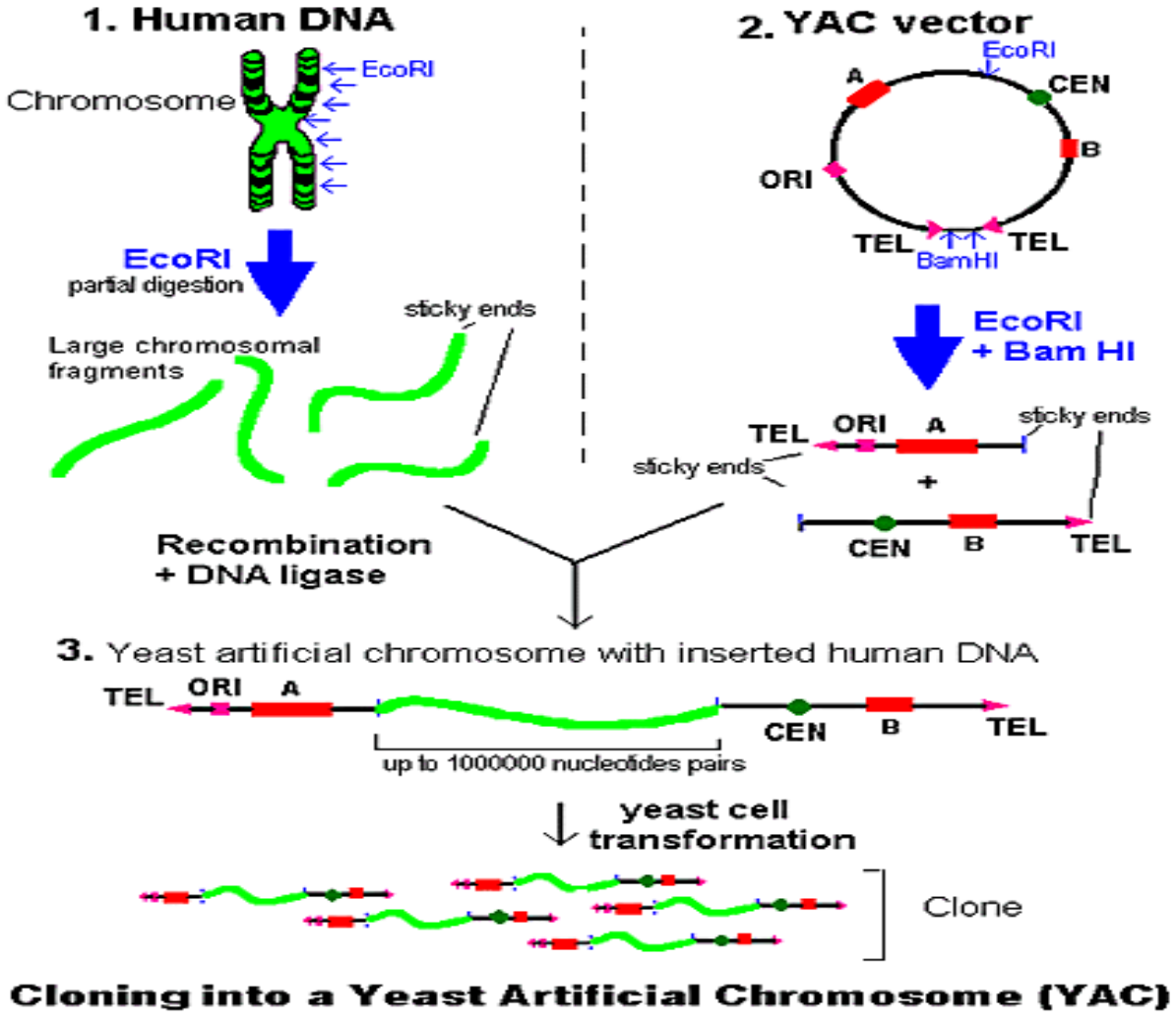
Source: https://microbenotes.com/yeast-artificial-chromosomes-yacs/

Cloning process steps using YAC:

(1) The YAC vectors are linearised by restriction digestion.

(2) The recombinant DNA is then transformed into the protoplast of the yeast cells "a double auxotrophic mutant, *ura3* and *trp1*$^{-}$, yeast strain is used"

(3) Transformants are selected on the minimal medium in which uracil and tryptophan remains absent.



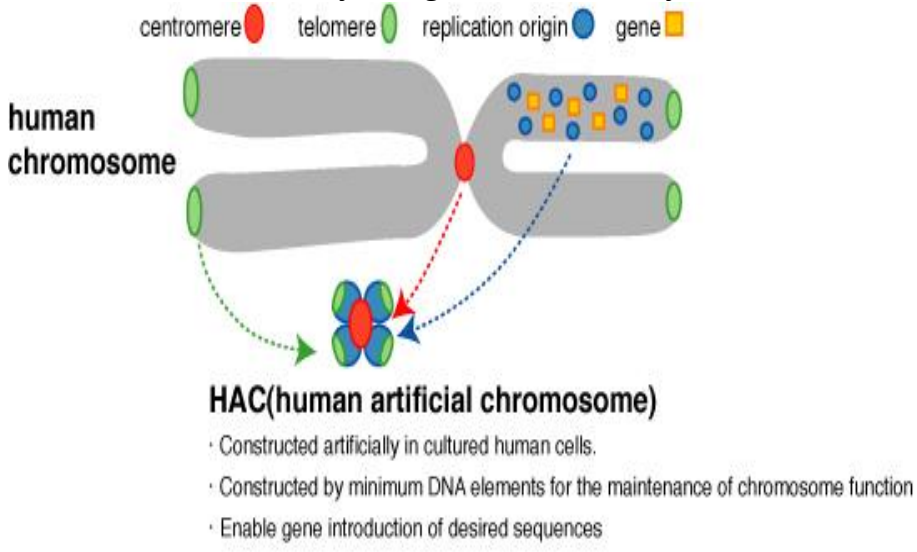
Cloning into a Yeast Artificial Chromosome (YAC)

Source:http://bdatoscucumismelo.blogspot.com/2009/10/

Yeast artificial chromosomes (YAC) are useful for the physical mapping of complex genomes and for the cloning of large genes. These can also be used to express eukaryotic proteins that require posttranslational modification. Besides these YAC also favors cloning of large stretches of DNA. Contrary to these, **YAC**s are unstable and frequently lose parts of the DNA during propagation.

2) HAC (Human Artificial Chromosome)

Human artificial chromosomes (HAC) first described by **Harrington et. al.** (1997) and synthesized by combining portions of alpha satellite DNA with telomeric DNA and genomic DNA into linear micro chromosomes. It was found that Human Artificial Chromosomes (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. HAC is a micro chromosome that can act as a new chromosome in a population of human cells i.e. instead of 46 chromosomes, the cell could have 47 with the 47th being very small, roughly 6-10 megabases in size, and able to carry new genes introduced by human researchers.



Source: https://studiousguy.com/wp-content/uploads/2018/09/Human-Artificial-Chromosome.jpg

They are useful in expression studies as gene transfer vectors and are a tool for elucidating human chromosome function. They are mitotically and cytogenetically stable for up to six months. Human artificial chromosomes (HAC) has three main components viz; replication origin from which the duplication of DNA begins, centromere which functions in proper chromosome segregation during cell division and telomere which protects the ends of linear chromosomes. HAC vector may be useful not only for gene and cell therapy, but also for animal transgenesis.

3. BAC (Bacterial Artificial Chromosome)

It was first developed by *Shizuya in 1992.* It is maintained in *E. coli* as large single copy of plasmids and contain inserts of < 300 kb. It also contains F-plasmid origin of replication and F-plasmid gene controls plasmid replication and plasmid copy number. This Vector commonly used for constructing large-insert genomic libraries for genome sequencing i.e. fragments up to 300 kb can be cloned into BAC. BAC also used to sequence the genome of organisms in genome projects,

e.g. the Human Genome Project and utilized to detect genes or large sequences of interest and then used to map them onto the human chromosome using BAC arrays.

BAC has following Common Gene Components:

i. oriS, F-plasmid origin of replication.

ii. repE, encodes a Rep protein that specifically binds to oriS to initiate replication.

iii. parA and parB loci are involved in regular partitioning of F plasmid during cell division. Loci parA and parB encode proteins that bind parC locus and link to cell membrane; this ensures a regular partitioning of F plasmid/BAC.

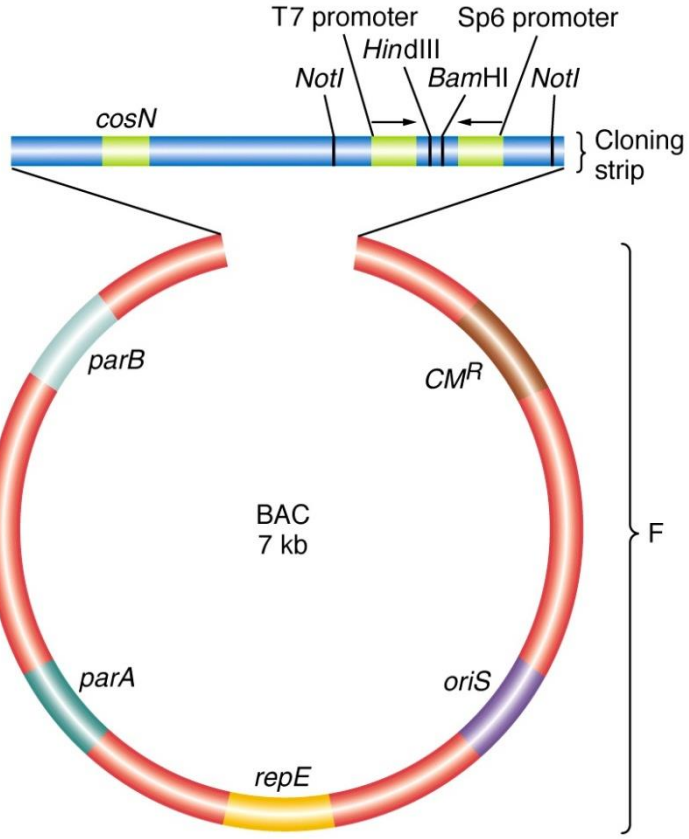
iv. CM^R, chloramphenical resistance.

v. cosN.

vi. T7, bacteriophage T7 RNA polymerase-driven promoter.

vii. SP6, bacteriophage SP6 RNA polymerase-driven promoter.

viii. Restriction enzyme recognition site B and H shown in the example are BamH1 and Hpa1.



4. MAC (Mammalian Artificial Chromosome) Similar to YACs, but instead of yeast sequences they contain mammalian or human ones. they are used to clone DNA fragments larger than **100 kb** and up to **3000 kb.** 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(consists of very large arrays of tandemly repeating, non-coding DNA.). Because the alphoid DNA is needed in units of many kilobases, these MAC DNAs are grown as YACs or, more recently, as BACs. These can carry large fragments of DNA representing an intact eukaryotic split gene with exons and introns permitting its normal expression regulated by the associated promoter sequences. MACs are considered to be suitable for gene therapy, where the inserted DNA will be expressed, yet stably maintained without affecting host genome.

5. PAC (P1-Derived Artificial Chromosome)

PAC is first developed by **Loannou et al.** (1994). In a PAC vector, inserts of size 100-300kb can be cloned and it is devoid of problems such as chimarism and instability of cloned DNA. These vectors incorporated features of both P1 and F-factor systems and can be transformed into *E. coli* host by electroporation. Several micrograms of cloned DNA can be recovered from 5-10ml of the exponential phase of *e.coli.* there are two types of P1 vectors viz: pNS583tet14Ad10 and pAd10sacBII. Both vectors contain genes resistance to the antibiotic kanamycin, Plasmid (origin of replication), loxP site (the cis-acting site specific recognition signal for the P1 recombinase) and a gene that allows resistance to tetracycline, which inserts cloned -tetracycline resistance.

PAC are used in the genome analysis and map based cloning of complex plants and animals, which requires isolation of large pieces of DNA rather than smaller segments. They are also useful in the study of 'phage therapy' and in scientific studies focusing on how antibiotics act on a particular bacterium.

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