



Vectors for Genomic library in genetic engineering: Yeast Artificial Chromosomes

What are Yeast Artificial Chromosomes?



- ❑ 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.
- ❑ By inserting large fragments of DNA, from 100–1000 kb, the inserted sequences can be cloned and physically mapped using a process called chromosome walking.
- ❑ The primary components of a YAC are the ARS, centromere, and telomeres from *S. cerevisiae*. Additionally, selectable marker genes, such as antibiotic resistance and a visible marker, are utilized to select transformed yeast cells.
- ❑ Without these sequences, the chromosome will not be stable during extracellular replication, and would not be distinguishable from colonies without the vector.

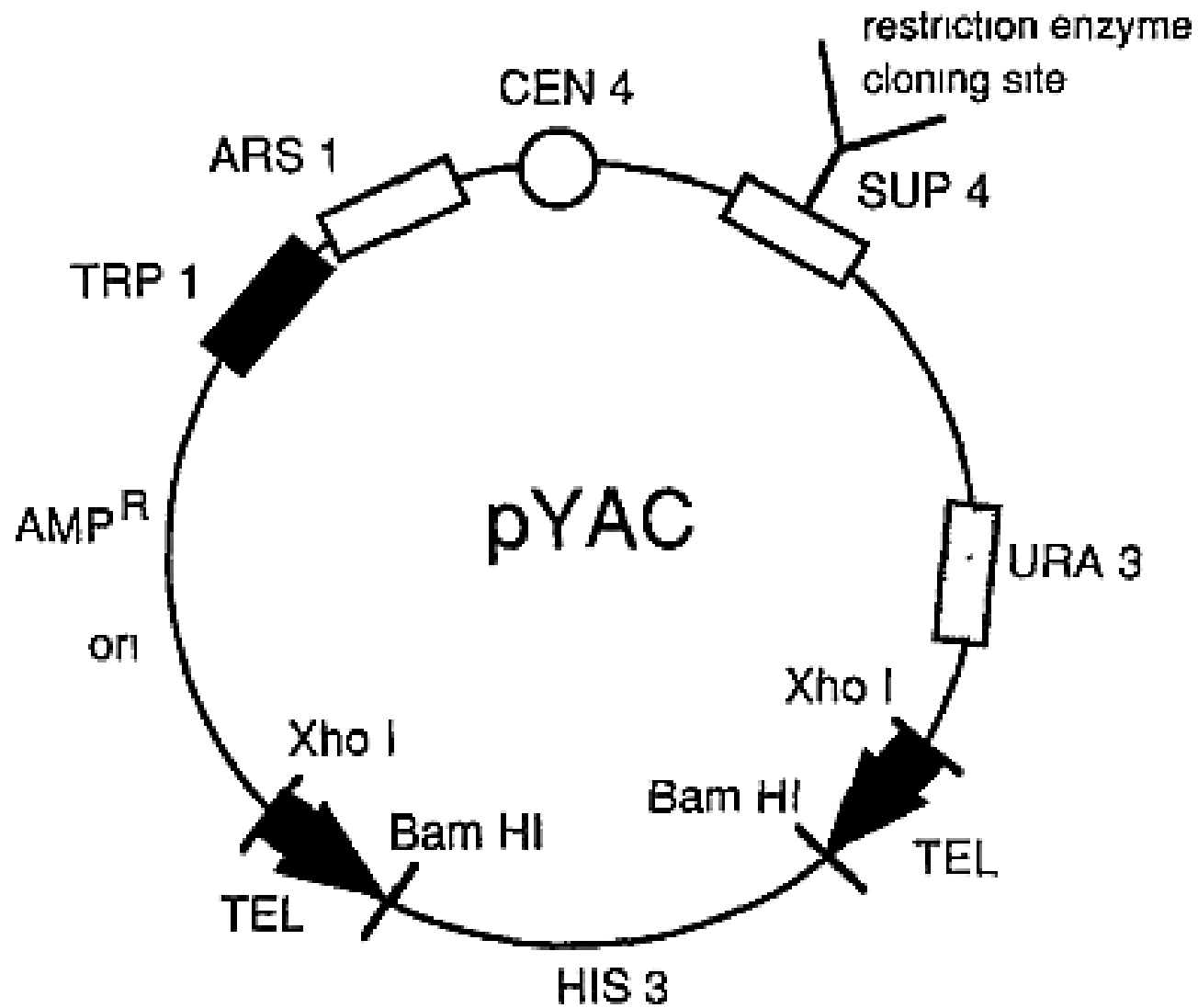
Assembling of YACs



- ❑ A YAC is built using a plasmid and a gene of interest forming a single large recombinant DNA.
- ❑ Then, a selectable marker is ligated into the plasmid vector. This allows for the differential selection of colonies with, or without the marker gene.
- ❑ Ligation of necessary centromeric sequences for mitotic stability.
- ❑ Ligation of Autonomously Replicating Sequences (ARS) providing an origin of replication to undergo mitotic replication. This allows the plasmid to replicate extra-chromosomally, but renders the plasmid highly mitotically unstable, and easily lost without the centromeric sequences.



- ❑ Ligation of artificial telomeric sequences to convert circular plasmid into a linear piece of DNA .
- ❑ Insertion of DNA sequence to be amplified (up to 1000kb).
- ❑ Finally, Transformation yeast colony.
- ❑ YAC was initially used for the Human Genome Project, however due to stability issues, YACs were abandoned for the use of BAC.



Typical structure map of a YAC vector.



Major features of YAC

CEN1

- ✓ It is the centromere sequence that helps in segregation.

TEL

- ✓ It is the telomere sequence that helps in extremity protection.

ARS1

- ✓ It is the autonomous replicating sequence that helps in replication.

Selectable marker

- ✓ Usually the amino acid dependence genes on each arm and helps in selection process.

Ori

- ✓ It is required for the autonomous replication of the YAC.



SUP4

- ✓ Acts as a suppressor tRNA gene which overcomes the effect of the *ade-2* ochre mutation and restores wild-type activity, resulting in colorless colonies.
- ✓ The host cells are also designed to have recessive *trp1* and *ura3* alleles which can be complemented by the corresponding *TRP1* and *URA3* alleles in the vector, providing a selection system for identifying cells containing the YAC vector.

Limitations of YAC



- ❑ Very fragile and prone to breakage.
- ❑ Unstable, with their foreign DNA inserts often being deleted.
- ❑ Loss of the entire YAC during mitotic growth.
- ❑ Difficult to separate the YAC from the other host chromosomes.
- ❑ Chimaerism and poor DNA yield.