Artificial Chromosomes

BZ 302: Biotechnology

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Artificial Chromosome Vectors

• Artificial chromosomes are DNA molecules assembled \textit{in vitro} from defined constituents, which guarantee stable maintenance of large DNA fragments with the properties of natural chromosomes.

• Like other cloning vectors, they contain the DNA sequence elements that are necessary for \textbf{replication} and \textbf{stability} of the molecule in the host cell and for its faithful partitioning to daughter cells upon cell division.

• Yeast artificial chromosome (\textbf{YACs}) vectors, able to carry DNA inserts as large as 2,000 kb (Monaco and Larin 1994), were the \textbf{first} artificial chromosomes (Burke et al. 1987).

• Artificial chromosomes are useful for \textbf{genome sequencing programmes}, for \textbf{functional characterization} of entire genomic regions and for the \textbf{transduction} of large DNA segments into human and nonhuman mammalian cells.

• Types: BAC, YAC, PAC, HAC
### Cloning vectors and their insert capacities

<table>
<thead>
<tr>
<th>Vector System</th>
<th>Host Cell</th>
<th>Insert Capacity (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td><em>E. Coli</em></td>
<td>0.1-10</td>
</tr>
<tr>
<td>Bacteriophage λ</td>
<td><em>E. coli</em></td>
<td>5-25</td>
</tr>
<tr>
<td>Cosmid</td>
<td><em>E. coli</em></td>
<td>35-45</td>
</tr>
<tr>
<td>Bacteriophage P1</td>
<td><em>E. coli</em></td>
<td>70-100</td>
</tr>
<tr>
<td>BAC</td>
<td><em>E. coli</em></td>
<td>50-300</td>
</tr>
<tr>
<td>PAC (P1 phage derived AC)</td>
<td><em>E. coli</em></td>
<td>100-300</td>
</tr>
<tr>
<td>YAC</td>
<td>Yeast (<em>S. cerevisiae</em>)</td>
<td>100-2,000</td>
</tr>
<tr>
<td>HAC</td>
<td>Cultured human cells</td>
<td>&gt;2,000</td>
</tr>
</tbody>
</table>
### Table 10.3: Principal features and applications of different cloning vector systems

<table>
<thead>
<tr>
<th>Vector</th>
<th>Basis</th>
<th>Size limits of insert</th>
<th>Major application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td>Naturally occurring multicopy plasmids</td>
<td>≤ 10 kb</td>
<td>Subcloning and downstream manipulation, cDNA cloning and expression assays</td>
</tr>
<tr>
<td>Phage</td>
<td>Bacteriophage λ</td>
<td>5–20 kb</td>
<td>Genomic DNA cloning, cDNA cloning, and expression libraries</td>
</tr>
<tr>
<td>Cosmid</td>
<td>Plasmid containing a bacteriophage λ cos site</td>
<td>35–45 kb</td>
<td>Genomic library construction</td>
</tr>
<tr>
<td>BAC (bacterial artificial chromosome)</td>
<td><em>Escherichia coli</em> F factor plasmid</td>
<td>75–300 kb</td>
<td>Analysis of large genomes</td>
</tr>
<tr>
<td>YAC (yeast artificial chromosome)</td>
<td><em>Saccharomyces cerevisiae</em> centromere, telomere, and autonomously replicating sequence</td>
<td>100–1000 kb (1 Mb)</td>
<td>Analysis of large genomes, YAC transgenic mice</td>
</tr>
<tr>
<td>MAC (mammalian artificial chromosome)</td>
<td>Mammalian centromere, telomere, and origin of replication</td>
<td>100 kb to &gt; 1 Mb</td>
<td>Under development for use in animal biotechnology and human gene therapy</td>
</tr>
</tbody>
</table>
Bacterial Artificial Chromosomes (BAC)

- 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 up to 300 kb of insert DNA sequence.
- The F (fertility) factor is a plasmid that can be mobilized from F+ male bacteria and F- female bacteria. The gene transfer from one to another bacterial cell is called **conjugation**.
- The F factor controls its own replication.
- It has two origins of replication: oriV is the origin for bidirectional replication; oriS is the origin for unidirectional replication.
- The F factor also has genes that regulate DNA synthesis so that its copy number is kept at a low level; and, genes that regulate the partition into the daughter cells after *E. coli* divides.
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.
CopyControl™ pCC1BAC™ Vector

8.1 kb
Cloning in BAC vector
Applications of BAC

**In disease models: Inherited disease**

- BACs are now being used 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.
In disease models: Infectious disease

- The genomes of several large DNA viruses and RNA viruses have been cloned using 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.

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 (YAC)

- YACs are genetically engineered chromosomes derived from the DNA of the yeast, *Saccharomyces cerevisiae*.
- The first YAC vectors were made in 1987 (Burke et al. 1987)
- YAC vectors allow the cloning, within yeast cells, of fragments of foreign genomic DNA that can approach 2,000 kb in size.
- YAC is essentially pBR322 plasmid into which a number of yeast genes have been inserted.
- YACs are shuttle vectors capable of replicating both in bacteria and yeast.
Components of YAC

• YAC vectors contain all the elements needed to maintain a eukaryotic chromosome in the yeast nucleus.

• The essential functional components of YAC are:
  
  ➢ **Centromeres**: It is required for the disjunction of sister chromatids in mitosis and of homologous chromosomes at the first meiotic division.
  
  ➢ **Telomeres**: It is required for complete replication of linear molecules and for the protection of the ends of the chromosome from nuclease attack.
  
  ➢ **Autonomous replicating sequence (ARS) elements**: It acts as origin of replication.
  
  ➢ **Selectable marker**: It is a gene for YAC selection in yeast. The vector has a functional copy of **URA3**, a gene involved in uracil biosynthesis, and **TRP1**, a gene involved in tryptophan biosynthesis, that allow selection of yeast cells that have taken up the vector.
Components of YAC (contd...)

- **Bacterial replication origin and a bacterial selectable marker:** In order to propagate the YAC vector in bacterial cells, YAC vectors usually contain the ColE1 ori and the ampicillin resistance gene for growth and analysis in *E. coli*.

- **Yeast selectable markers:** All yeast vectors contain marker that allow selection of transformed yeast cells. The most commonly used yeast selectable markers are genes which complement a specific auxotrophy (e.g. Leu-, His-, Trp-, etc.) and thus require the host cell to contain a recessive, non-reverting mutation. The most commonly-used auxotrophic selection markers for the selection of transformants are **LEU2, TRP1, URA3** and **HIS3** used in corresponding mutant strains, which are auxotrophic for leucine, tryptophan, uracil and histidine, respectively.
Figure: Construction of a yeast artificial chromosome (YAC)
Applications of YAC

- Yeast expression vectors, such as YACs, YIps (yeast integrating plasmids), and YEps (yeast episomal plasmids), have an advantage over bacterial artificial chromosomes (BACs) in that they can be used to express eukaryotic proteins that require post translational modification.
- Generation of whole DNA libraries of the genomes of higher organisms.

- **Sequencing**: Since YACs can accommodate large fragments of DNA, it can be utilized to clone and assemble the entire genomes of an organism. The sequence of the whole genome, or region of interest can be obtained by: *Physical Mapping* and *Chromosome Walking*
Limitations of using YAC vectors

- Large DNA molecules are very fragile and prone to breakage, leading to problem of rearrangement.

- High rate of loss of the entire YAC during mitotic growth.

- Difficult to separate YAC from the other host chromosomes because of their similar size.

- Separation requires sophisticated pulse-field gel electrophoresis (PFGE).

- Yield of DNA is not high when the YAC is isolated from yeast cells.

- Clones tend to be unstable, with their foreign DNA inserts often being deleted.

- They typically contain clones that are chimeric, i.e., contain DNA in a single clone from different locations in the genome.

- The efficiency of cloning is low (about 1000 clones are obtained per microgram of vector and insert DNA).
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.
There are currently two accepted models for the creation of human artificial chromosome vectors.

→ Top-down approach (engineered chromosome)

→ Bottom up approach (de novo artificial chromosome)

The generated HACs are 1–10 Mb in size, consisting of multiple copies of rearranged input DNA molecules.
Engineered chromosome by a top-down approach,

- The minichromosomes or chromosomes derived from endogenous chromosomes are generated by
  - natural fragmentation of chromosomes,
  - telomere-directed chromosome breakage, or
  - radiation-induced chromosome breakage
- They contain an endogenous functional centromere.
- The chromosomes can then be transferred into other cell lines by microcell-mediated chromosome transfer (MMCT).
**de novo artificial chromosome by a bottom-up approach**

Exogenous chromosomes can be circular or linear, created *de novo* from cloned chromosomal components, either naturally occurring or synthetic high-order α-satellite DNA arrays introduced on BAC, YAC, or PAC vectors, which have a functional centromere and autonomously replicate and segregate.
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.
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 the delivery of more DNA (including promoters and copy-number variation) than is possible with viral vectors.
Suggested Readings: