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Yeast Artificial Chromosomes

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Yeast artificial chromosomes are shuttle-vectors that can be amplified and modified in bacteria and employed for the cloning of very large DNA inserts (up to 1–2 megabase pairs) in the yeast *Saccharomyces cerevisiae*.

Secondary article

Article Contents

Introduction

Yeast artificial chromosomes (YACs) are plasmid shuttle-vectors capable of replicating and being selected in common bacterial hosts such as *Escherichia coli*, as well as in the budding yeast *Saccharomyces cerevisiae*. They are of relatively small size (approximately 12 kb) and of circular form when they are amplified or manipulated in *E. coli*, but are rendered linear and of very large size, i.e. several hundreds of kilobases (kb), when introduced as cloning vectors in yeast. The latter process involves cleavage at strategically located sites by two restriction enzymes, which break them in two linear DNA arms. These are subsequently ligated with the appropriate DNA insert before transformation into recipient yeast cells converted to spheroplasts, where telomere sequences are added by the cell's telomerase enzymes. In this linear form, these specialized vectors contain all three *cis*-acting structural elements essential for behaving like natural yeast chromosomes: an autonomously replicating sequence (ARS) necessary for replication; a centromere (CEN) for segregation at cell division; and two telomeres (TEL) for maintenance. In addition, their capacity to accept large DNA inserts enables them to reach the minimum size (150 kb) required for chromosome-like stability and for fidelity of transmission in yeast cells. YACs have several advantages over other large capacity vectors: these include accommodation of DNA segments thousands of kilobases in size and stable maintenance of cloned eukaryotic DNA due to the compatibility with the yeast replication machinery. Moreover, they are amenable to large-scale plasmid amplification in *E. coli* and to creation of specific genetic changes within the exogenous DNA sequences by using the faithful and efficient yeast mechanism of homologous recombination.

Overview of Yeast Artificial Chromosomes (pYACs) Plasmids

Many different yeast artificial chromosomes exist as ongoing refinements of the initial pYAC3 and pYAC4 plasmids (**Figure 1**) constructed by Burke *et al*. (1987). The basic structural features of YACs were developed from the yeast centromere shuttle-plasmids YCp series. These are composed of double-stranded circular DNA sequences carrying the β-lactamase gene *bla* and the bacterial pMB1 origin of replication, thus conferring resistance to ampicillin and the ability to replicate in bacteria, respectively. They also include yeast ARS1 with its associated CEN4 DNA sequence, as well as the *URA3* selectable marker. On this basic scaffold plasmid the yeast *HIS3* is cloned, flanked by a telomere-like DNA sequence derived from the termini of the *Tetrahymena pyriformis* macronuclear ribosomal DNA, which allows the formation of functional telomeres in yeast, that are adjacent to two recognition sites for the *Bam*HI restriction enzyme. Most of these YACs also contain the cloning site in the middle of the *SUP4* suppressor of an ochre allele of a tyrosine transfer RNA (tRNA) gene; this enables restoration of the normal white colour phenotype in otherwise red *ade1* and/or *ade2* nonsense mutants (Fischer, 1969). Accordingly, in the

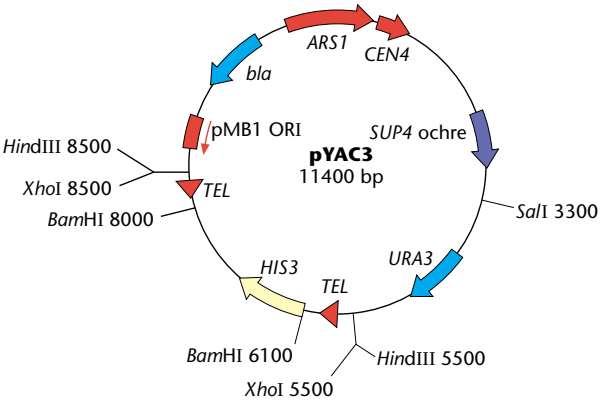

Figure 1 Circular map of plasmid vector pYAC3. All the selectable markers, the origins of replication for bacteria and yeast, and the yeast chromosomal structural elements are indicated, together with the map position of the major restriction sites.

insertional inactivation cloning process, the *SUP4* gene is disrupted by the DNA insert, thus removing the suppression of the *ade* mutations and allowing their phenotypic expression as red colour.

Construction of Yeast Artificial Chromosomes

After plasmid DNA purification, two distinct digestions are performed: the first with *Bam*HI that cuts twice adjacent to the two telomeric DNA sequences flanking the *HIS3* gene, which therefore is excised from the plasmid and lost (**Figure 2a**). This first digestion generates a long linear fragment carrying telomeric sequences at each end. The excision of the *HIS3* gene is used as negative selective marker for uncut pYAC molecules. The second digestion consists of the opening of the cloning site within the *SUP4* gene (**Figure 2a**). As a result of this second digestion, two linear fragments are produced as left and right arms of the future linear YAC (**Figure 2b**). The selective markers are thus separated: *TRP1* adjacent to *ARS1* and *CEN4* on the left arm and *URA3* on the right arm. Large DNA fragments with ends compatible to the cloning site, obtained from the desired genome source by digestion with an appropriate restriction endonuclease, are ligated with phosphatase-treated YAC arms, to create a single yeast-transforming DNA molecule (**Figure 2c**). Primary transformants can be selected for complementation of the *ura3* mutation in the host, and successively for complementation of the host *trp1* mutation, thereby ensuring the presence of both chromosomal arms. Transformant colonies containing the exogenous DNA insert within the *SUP4* gene are detected by their red colour, due to the inactivation of the suppressor activity and the consequent accumulation of a red metabolic precursor in *ade* host cells.

Biological Features of YACs

The stability of YAC vectors in yeast *per se* is similar to that of natural chromosomes ($10^{-5}/10^{-6}$) provided that all three structural elements (ARS, CEN and TEL) are present and functional and, in addition, that the minimal required size is reached by the insertion of enough exogenous DNA.

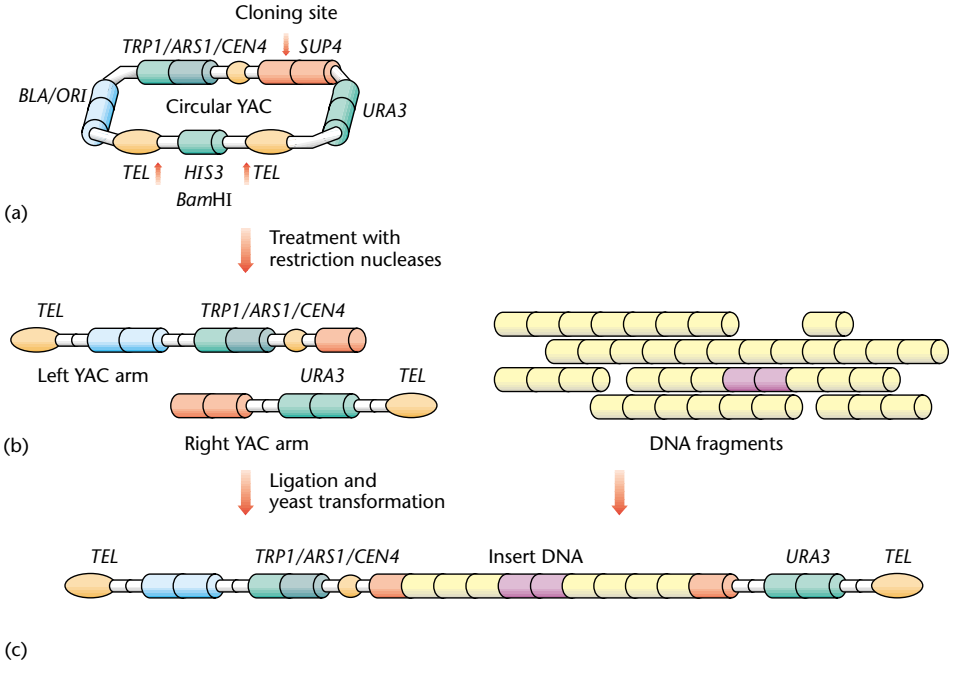

Figure 2 Construction of the yeast artificial chromosome. (a) A circular YAC vector able to replicate in *Escherichia coli* due to the presence of bacterial *ori* and *bla* gene (blue cylinder) and propagated in yeast cells as a linear molecule containing all necessary chromosomal elements: yeast centromere *CEN4* (orange circle), autonomous replication sequence *ARS1* (dark green cylinder) and two *Tetrahymena* telomeric sequences *TEL* (orange ellipse) functional in yeast after linearization with the *Bam*HI restriction endonuclease. The yeast *SUP4* gene (red cylinder) contains a cloning site used as a colour marker for selection of YACs containing exogenous insert DNA. (b) DNA fragments with ends compatible to the YAC cloning site are prepared from source DNA. After double digestion of the YAC vector, the markers used to select for transformants are separated on two chromosomal arms: *TRP1* on the left and *URA3* on the right arm (light green cylinders). (c) Chromosomal arms ligated with exogenous DNA are selected after transformation of appropriate yeast strain (*ura3*, *trp1*, *ade2*). Adapted from Burke *et al.*, (1987).

However, the genetic and biochemical background of the host cell also plays an important role in determining the stability of YACs. Indeed, several mutations are known to affect YAC stability and segregation together with natural chromosomes. For example, alterations in the expressions of genes such as *BUB1*, *BUB2* and *BUB3*, *MAD1*, *MAD2* and *MAD3*, *CDC6*, *CDC20*, *PDS1* and others lead to chromosome losses, including YACs. Another important consideration is that faithful duplication of YACs is guaranteed only if other DNA sequences incompatible with ARS do not exist on the construct. This point is particularly relevant when unknown DNA inserts are cloned in the YAC vector, as is the case for genomic libraries, in which there could be cryptic or otherwise unknown ARS-like sequences able to interfere with the ARS function.

Modifying YACs by Homologous Recombination

Depending on the experimental systems and the yeast strains, different selectable markers and restriction sites are appropriate on the YAC vectors. These can be constructed *in vitro* by standard techniques and then used for subcloning DNA fragments. Additionally, existing YAC clones can be modified by homologous recombination in yeast, a process called 'retrofitting' (**Figure 3**). Accordingly, genetic markers can be modified by simply transforming YAC-containing yeast cells with a disruption cassette carrying the desired genetic marker flanked by short DNA sequences homologous to one of the markers present on the artificial chromosome. The same result can be obtained by using one arm of the artificial chromosome carrying the new genetic marker as the transforming molecule. As shown in **Figure 3a**, the introduction of the yeast *LYS2* gene, together with the mammalian selectable marker *Neo*, into the *URA3* gene on the right arm of a YAC is achieved by using recombination techniques. *Neo* is the usual marker gene for subsequent selection of mammalian cells after YAC transfection (see below). Moreover, disruption of this marker provides a selectable replacement marker for introducing specific genetic changes within the exogenous DNA insert (**Figure 3**).

A yeast integrative plasmid (YIp) carrying the mutated copy of an exogenous DNA segment and the functional copy of a previously disrupted marker can be used for this purpose (Barton *et al*., 1990). After plasmid integration, the target DNA is duplicated in tandem via homologous recombination, generating a YAC with one wild-type and one mutant copy of insert DNA ('pop-in'). At this stage, YACs with only the mutated copy of the marker can be obtained after spontaneous recombination within duplicated DNA regions ('pop-out') by growing the transformed cells in the absence of selection pressure for several generations (**Figure 3b**). However, the frequency is not very high (from 10^{-4} to 10^{-5} per generation) and there is approximately the same probability for both the mutated and wild-type copy of the marker to remain within the cell on the YAC vector. In the latter case it is necessary to perform additional molecular tests of the obtained 'pop-outs'.

Use of Yeast Artificial Chromosomes

YAC vectors were initially created for the cloning of large exogenous DNA segments in *S. cerevisiae* but soon became chromosomal-like platforms for a variety of *in vivo* experiments. Applications of YACs range from 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). The availability of YAC libraries has greatly advanced the analysis of genomes previously cloned in cosmid vectors. For example, YAC clones have been used as hybridization probes for the screening of cDNA libraries, thus simplifying the characterization of unidentified genes. Another major application of YACs is in the study of regulation of gene expression by *cis*-acting, controlling DNA elements, that are present either upstream or downstream of large eukaryotic genes, after the transfer of these YACs from yeast to mammalian cells. Recent technological developments allow the transfer of YACs into mouse embryonal stem (ES) cells and the subsequent generation of transgenic mice. Investigators have begun to employ these artificial chromosomes for the *in vivo* study of multigenic loci in mammalian cells.

YAC Genomic Libraries

Bacterial artificial chromosomes (BACs) and the P1-derived artificial chromosomes (PACs) can accommodate inserts of exogenous DNA up to 300 kb. Although this represents an average insert size close to the one obtained after YAC preparation *in vitro*, it is possible to construct YACs with megabase-long inserts using the precise homologous recombination process of yeast. The prerequisite for this technique is the isolation of YAC clones containing neighbouring genomic DNA inserts. Initial screening for specific sequences usually results in several clones, which most probably contain overlapping inserts. Following insert fingerprinting by conventional Southern hybridization or polymerase chain reaction (PCR)-based methods, using tandemly repeated sequences as markers, it is possible to obtain detailed physical maps allowing an accurate alignment of two inserts by comparing their individual restriction patterns. Further neighbouring

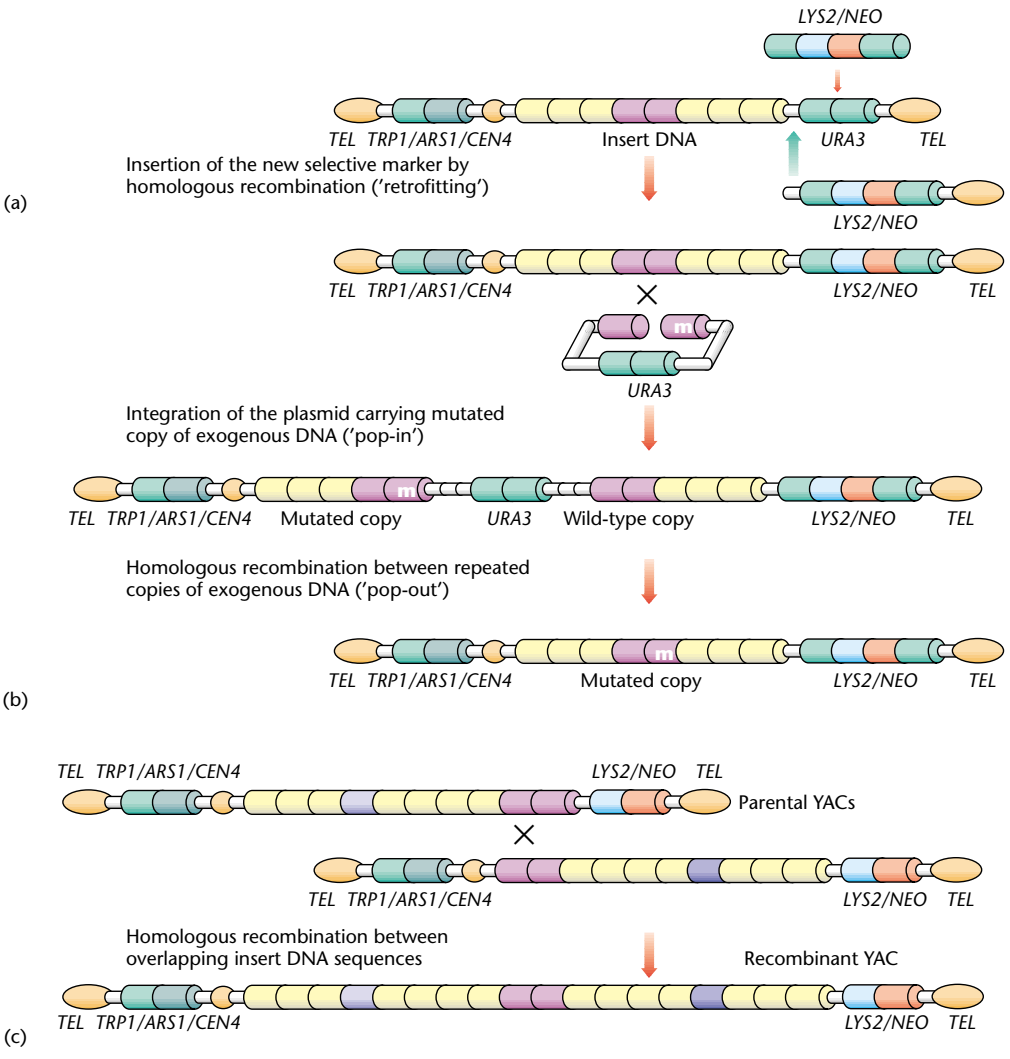

Figure 3 YAC modification by homologous recombination. (a) New selective markers *LYS2* (blue cylinder) and *NEO* (red cylinder) are introduced into the *URA3* gene present on YAC by one-step disruption (transformation with disruption cassette or modified right YAC arm). (b) Inactivation of the *URA3* gene allows subsequent modification of the insert DNA by using linearized yeast integrative plasmid containing a functional copy of the *URA3* gene and a mutagenized copy of the exogenous DNA fragment. After plasmid integration ('pop-in'), two copies of target DNA are present (wild-type and mutated). A YAC containing only the mutated copy of the exogenous DNA is obtained after homologous recombination and loss of the integrative plasmid carrying the wild-type equivalent ('pop-out'). (c) Reconstruction of two smaller overlapping YACs into a larger recombinant YAC by recombination between homologous regions of the insert DNA (violet cylinders).

DNA inserts can be found by chromosome 'walking' techniques that require novel sequence information to design DNA probes at the ends of the existing contigs. One of these techniques is screening by hybridization using contig ends as probes; another is screening by PCR techniques that allow the selective amplification of sequences composed of two neighbouring contigs.

Once clones with overlapping inserts are found, a YAC with a continuous contig can be obtained at high frequency by inducing meiosis in diploid yeast cells formed after mating two haploid strains containing one each of the two YACs (Silverman *et al.*, 1990). This allows meiotic recombination to occur between the two homologous overlapping sequences of the inserts, thus generating a recombinant YAC harbouring both inserts and joined at the point of crossing-over. To prevent the generation of mitotically unstable dicentric or acentric YACs, the insert DNA must be cloned in the same 5′ to 3′ vector orientation. After meiotic division and sporulation, spores containing a single recombinant YAC created by the precise physical exchange of homologous insert DNA between two parental YACs are frequently found among tetrads

(**Figure 2c**). Following several cycles of recombination between overlapping sequences it is possible, therefore, to reconstruct and maintain in a single YAC vector the original DNA sequence of a eukaryotic genome fragment more than 2 Mb in size (Den Dunnen *et al*., 1992).

Production of Transgenic Mice with YACs

YACs carrying an insert of interest, and retrofitted with an appropriate mammalian selective marker, can be transferred to mammalian cells by using several different techniques. Purified vector DNA can be introduced by pronuclear microinjection or by lipofection with YACs previously embedded in a lipid solution. The disadvantage here includes physical damage to the large DNA molecules during the purification process and, consequently, a low yield of intact integrated YAC copies. Another approach, the fusion of yeast spheroplasts containing YACs with mammalian cells, eliminates the problem of *in vitro* YACs disintegration by virtue of the absence of physical DNA isolation. With this method intact YAC molecules can be transferred into the mammalian cell nucleus. However, in addition to the desired YAC vector, a substantial portion of the yeast genome can also be transferred, although this does not seem to affect either the YAC stability or the ability of the cell to differentiate (Pavan *et al*., 1990; Jakobovits *et al*., 1993). Furthermore, it has been found that mouse embryonic stem cells carrying YACs transferred by this technique are able to repopulate mouse tissues and retain the function of human DNA sequences in the developed chimaeric mice.

YACs in the Construction of Mammalian Artificial Chromosomes

YACs were developed following the identification and isolation of the DNA sequence elements essential for chromosome replication and maintenance in yeast cells. Using the same strategy, YACs are being used for isolation of functionally analogous mammalian DNA sequences in order to develop mammalian artificial chromosomes (MACs). Mammalian telomeres can be isolated from DNA fragments by complementation, using modified YAC vectors containing only one *Tetrahymena* telomere, a yeast centromere and a replication origin (Cross *et al*., 1989). After ligation of the modified vector with the insert DNA, only YACs containing the second arm with mammalian telomeric sequences can transform yeast cells, forming linear minichromosomes. Mammalian centromeres and origins of replication are complex genetic elements that cannot be easily defined by sequence, but can only be selected by function. Fortunately, almost all large segments of mammalian DNA are capable of autonomous replication, thus diminishing the need to isolate replication origins. The main difficulty in constructing mammalian artificial chromosomes by using YACs is the isolation and maintenance of the mammalian centromere due to its large size and high instability in the yeast cell (Neil *et al*., 1990). However, construction of α-satellite-based (see Grimes and Cooke, 1998 for a review) and Epstein–Barr virus-based mammalian artificial chromosomes (Tolmachova *et al*., 1999) showed that the use of the YACs is a promising technique for construction of stable MACs.

References

Barton MC, Hoekstra MF and Emerson BM (1990) Site-directed, recombination mediated mutagenesis of a complex gene locus. *Nucleic Acids Research* **18**: 7349–7355.

Burke DT, Georges FC and Olson MV (1987) Cloning of large segments of Exogenous DNA into yeast by means of artificial chromosome vectors. *Science* **236**: 806–811.

Cross SH, Allshire RC, McKay SJ, McGill NI and Cooke HJ (1989) Cloning of human telomeres by complementation in yeast. *Nature* **338**: 771–774.

Den Dunnen JT, Grootscholten PM, Dauwerse JD *et al*. (1992) Reconstruction of the 2.4 Mb human DMD-gene by homologous YAC recombination. *Human Molecular Genetics* **1**: 19–28.

Fischer CR (1969) Enzymology of the pigmented adenine-requiring mutants of *Saccharomyces cerevisiae* and *Schizosaccharomyces*. *Biochemical Biophysical Research Communication* **34**: 306–310.

Grimes B and Cooke H (1998) Engineering mammalian chromosomes. *Human Molecular Genetics* **7**: 1635–1640.

Jakobovits A, Moore AL, Green LL *et al*. (1993) Germ-line transmission and expression of a human-derived yeast artificial chromosome. *Nature* **362**: 255–258.

Neil DL, Villasante A, Fisher RB *et al*. (1990) Structural instability of human tandemly repeated DNA sequences cloned in yeast artificial chromosome vectors. *Nucleic Acids Research* **18**: 1421–1428.

Pavan WJ, Hieter P and Reeves RH (1990) Modification and transfer into an embryonal carcinoma cell line of a 360-kilobase human-derived yeast artificial chromosome. *Molecular and Cell Biology* **10**: 4163–4169.

Silverman GA, Green ED, Young RL *et al*. (1990) Meiotic recombination between yeast artificial chromosomes yields a single clone containing the entire BCL2 protooncogene. *Proceedings of the National Academy of Sciences of the USA* **87**: 9913–9917.

Tolmachova T, Simpson K and Huhley C (1999) Analysis of a YAC with human telomeres and oriP from Epstein-Barr virus in yeast and 293 cells. *Nucleic Acids Research* **27**: 3736–3744.

Further Reading

Broach JR, Pringle IR and Jones EW (1991) *The Molecular and Cellular Biology of the Yeast* Saccharomyces cerevisiae. *Genome Dynamics, Protein Synthesis, and Energetics*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Cai L, Lindpaintner K, Browne J *et al*. (2000) An anchored YAC-STS framework for the rat genome. *Cytogenetics and Cell Genetics* **89**: 168–170.

Call LM, Moore CS, Stetten G and Gearhart JD (2000) A cre-lox recombination system for the targeted integration of circular yeast artificial chromosomes into embryonic stem cells. *Human Molecular Genetics* **9**: 1745–1751.

Cocchia M, Kouprina N, Kim SJ *et al*. (2000) Recovery and potential utility of YACs as circular YACs/BACs. *Nucleic Acid Research* **28**: E81.

Gisselsson D and Hoglund M (2000) Putting chromosome aberrations on the map. *Trends in Genetics* **16**: 420–422.

Ross LO, Zenvirth D, Jardim AR and Dawson D (2000) Double-strand breaks on artificial chromosomes in yeast. *Chromosoma* **109**: 226–234.