**Mammalian artificial chromosomes**

Although plasmid and viral gene delivery systems have been used successfully to introduce genes of interest into mammalian cell lines and transgenic animals, they are limited with regard to the amount of foreign DNA sequence that can be delivered. For applications where cloning and delivery of large genomic loci are desired, such as fragments containing long-range genetic elements required for appropriate regulation of gene expression, developmentally regulated multi-gene loci, or multiple copies of two or more genes in fixed stoichiometry, plasmid or viral vectors may be inadequate

Technologies that can accommodate large DNA payloads and do not require integration into the host genome for long-term stable maintenance would be advantageous, both in terms of having a more predictable gene expression and non-interference in host cell functions. Such systems would also be of great utility in gene therapy applications where safety and stability considerations are paramount. Recently there have been efforts to generate prototype mammalian artificial chromosomes (MACs) that encompass these features.

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.

Because the alphoid DNA is needed in units of many kilobases, these MAC DNAs are grown as YACs or, more recently, as BACs. When added to suitable cell lines, these MAC DNAs form chromosomes that mimic those in the cell, with accurate segregation and the normal complement of proteins at telomeres and centromeres. Their primary use is not in genome mapping but as vectors for delivery of large fragments of DNA to mammalian cells and to whole animals for expression of large genes or sets of genes. They are still in development, and although gene expression has been demonstrated they have not been used in a practical application.

Compared to traditional methodologies, MACs offer significant advantages for cellular protein production, animal transgenesis and gene-based cell therapy applications on account of their high carrying capacity and ability to self-replicate without relying on integration into the host genome.

Despite the numerous advantages of MAC technology, systematic limitations have precluded any widespread implementation. These limitations include the requirement for de novo chromosome synthesis for each individual application, the inability to shuttle MACs easily across various cell types and the inability to precisely engineer gene targets onto the artificial chromosome. For broad applicability of MAC technology, all of these limitations must be addressed.

