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Human artificial chromosomes and TAR cloning technology for genomes studies and biomedicine

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Transformation-associated recombination (TAR) cloning allows selective isolation of full-length genes and genomic loci as large circular Yeast Artificial Chromosomes (YACs) in yeast. The method has a broad application for structural and functional genomics, long-range haplotyping, characterization of chromosomal rearrangements and evolutionary studies. Also, the benefit of combining the TAR gene cloning technology with the HAC gene delivery system for gene expression studies will be discussed. Human artificial chromosome HAC-based vectors offer a promising system for delivery and expression of full-length human genes. HACs avoid the limited cloning capacity, lack of copy number control and insertional mutagenesis due to integration into host chromosomes that plague viral vectors. Recently we engineered the HAC with a single *LoxP gene* adopter site and a defined structure and demonstrated its utility for delivery of several full-length genes and correction of genetic deficiencies in human cells. We also showed that phenotypes arising from stable gene expression can be reversed when cells are "cured" of this HAC by inactivating its kinetochore in proliferating cell populations, a feature that provides a control for phenotypic changes attributed to expression of HAC-encoded genes, thereby aiding in proper interpretation of gene function studies. Also, we demonstrated that HAC-bearing ES cells were indistinguishable from their wild-type counterparts: they retained self-renewal potential and full capacity for multi-lineage differentiation during mouse development, whereas the HAC itself was mitotically and transcriptionally stable during this process. The HAC vectors have a great potential for genes function studies, gene therapy, regenerative medicine, screening of anticancer drugs and biotechnology.

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