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Development of BAC Libraries and Integrated Physical Mapping of Human
Chromosome 22 Using BACs

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BACs and fosmids are stable, nonchimeric, and highly representative cloning systems. BACs maintain large-fragment genomic inserts (100 to 300 kb) that are easily prepared for most types of experiments, including DNA sequencing. We have improved the methods for generating BACs and developed extensive BAC libraries. We have constructed human BAC libraries with more than 175,000 clones from male fibroblast and sperm, and a mouse BAC library with more than 200,000 clones. We are currently expanding human library with the aim of achieving total 50X coverage human genomic library using sperm samples from anonymous donors.

The BAC libraries provide resources to bridge the gap between genetic-cytogenetic information and detailed physical characteristics of genomic regions that include DNA sequence information. They also provide reliable tools for generating a high-resolution, integrated map on which a variety of information and resources are correlated. Using primarily the human BAC library constructed from fibroblasts, we have assembled a physical contig map of chromosome 22 [1]. First, the entire library was screened by most of the known chromosome 22-specific markers that include cDNA, anonymous STS markers, FISH-mapped cosmids and fosmids, YAC-Alu PCR products, FISH-mapped BACs, and flow-sorted chromosome 22 DNA. The positive clones have been assembled into contigs by means of the STS-contents or other markers assigned to BAC clones. Most of the contigs were confirmed by using a restriction fingerprinting scheme originally developed by Sulston and Coulson, and modified in our laboratory. Currently, the contigs cover over 80% of the chromosome arm. Various physical or genetic landmarks on this chromosome can now be precisely localized simply by assigning them to BACs or contigs on the map. Using BAC end sequence information from each of the chromosome 22-specific BACs, it is now possible to close the gaps efficiently by screening deeper BAC libraries with new probes specific to the ends of contigs.

The resulting BAC contig map is now serving as a road map for sequencing the chromosome. Chromosome 22-specific BAC clones have been distributed to our collaborators including The Sanger Center and Dr. Bruce Roe in University of Oklahoma, and many of the clones have already been sequenced. BAC end sequencing scheme[2] will play a crucial role toward the complete sequencing of chromosome 22, and we are currently sequencing the ends of these BACs directly using the miniprep BAC DNA as templates.

[1] Kim et al. (1996) A Bacterial Artificial Chromosome-based framework contig map of human chromosome 22q. *Proc. Natl. Acad. Sci. USA* v93 (13): pp6297-6301.

[2] Venter, C., Smith, H.O., and Hood, L. (1996) *Nature* 381: pp364-366.

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