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 miniprepped BAC DNA as templates.

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Towards a Globally Integrated, Sequence-Ready BAC Map of the Human Genome

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BAC clones are ideal for genome analysis since they are non-chimeric, stably maintain large fragment genomic inserts (100-300 kb)[1], and it is easy to prepare BAC DNA samples for most types of experiments including DNA sequencing[2]. We have improved BAC cloning technique in the past years and constructed >20X human BAC libraries. As BACs are proving to be the most efficient reagents for large scale genomic sequencing, we intend to increase the depth of the library to 50X genomic equivalence. Using the ESTs, especially the Unigenes that have been chromosomally assigned by other means such as Radiation Hybrid mapping and YAC-based STS content mapping, we plan to organize the BAC library into a mapped resource. The resulting BAC-EST framework map will provide a high resolution EST (or gene) map and instant entry points for gene finding and large scale genomic sequencing. We also intend to determine the end sequences of the BAC inserts from a significant number of the clones (at least 350,000 clones or 15X genomic equivalence) within two years [3]. All the BAC-EST mapping data and BAC end sequences will be made available via public databases and WEB servers. The mapping data and end sequence information will dramatically facilitate the process of finding clones that extend the sequenced regions with minimal overlaps. Thus, the tagged BAC libraries will serve as a reliable and facile sequence-ready resource and an organizing tool to support and coordinate simultaneously multiple sequencing projects all over the genome.

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Generation of Normalized and Subtracted cDNA Libraries to Facilitate Gene Discovery

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Large-scale single-pass sequencing of cDNA clones randomly picked from libraries has proven quite powerful to identify genes and the use of normalized libraries in which the frequency of all cDNAs is within a narrow range has been shown to expedite the process by minimizing the redundant identification of the most prevalent mRNAs. In an