Digital imaging microscopy: The Resource supports work in microscopy, image processing and analysis methods needed for CGH and CGHa, 3D FISH, tissue analysis, rare event detection, multi-color image acquisition, aberration scoring for biodosimetry, and analysis of FISH to DNA fibers. Developments this year include an improved package for CGH and prototype systems for analysis of DNA fibers, CGHa arrays and semiautomatic segmentation of nuclei in three dimensions.

Informatics: The Resource maintains a web site at http://rmc-www.lbl.gov that summarizes information about mapped probes. Probes developed by the Resource can be requested directly through this page. In addition, the Resource has developed a Web page for exchange of genomic, genetic and biologic information between geographically disperse collaborators. The page, under password control, carries information about physical maps, genomic sequence, sequence annotation, and gene expression images.

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DNA Sample Manipulation and Automation

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The objective of this project is to develop a high-throughput, fully automated robotic device for the complete automation of the sequencing process. We also aim to further develop DNA sequencing electrophoresis systems and to integrate these devices with our robotics.

We have built the Sequatron, an integrated, robotic device which automates the tasks of DNA purification and setup of thermal cycle sequencing reactions. The major component of our system is an articulated CRS 255A robotic arm which is track mounted. The deck of the robot contains several new or modified XYZ robotic workstations, a novel thermal cycler with automated headed lids, carousels, and custom built plate feeders.

Biochemically, we have employed our Solid-phase reversible immobilization (SPRI) technique to isolate and manipulate the DNA throughout the process.

Specifically we have set up the Sequatron to isolate DNA from M13 phage or crude PCR products using the same protocol and procedures. From M13 phage we obtain approximately 1g of DNA per well, which is sufficient for multiple sequencing reactions.

The current throughput of the system is 80 microtiter plates of samples from M13 phage supernatants or crude PCR products to sequence ready samples every 24 hours. Recently, new enzymes, new energy transfer primers and higher density microtiter plates have opened up possible increases to in excess of 25,000 samples per 24 hour period.

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Construction of a Genome-Wide Characterized Clone Resource for Genome Sequencing

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Bacterial artificial chromosomes (BACs) represent the state of the art cloning system for human DNA because of their stability and ease of manipulation. Venter, Smith and Hood (Nature 381:364-366, 1996) have proposed a strategy based on the use of sequences from the ends of all clones in a deep coverage BAC library to produce a sequence-ready set of clones for the human genome. We propose to demonstrate the effectiveness of this strategy by performing a directed test, initially on chromosomes 16 and 22, and continuing on to chromosome 1. All available markers on chromosome 16 (including the large number of soon-to-be-available radiation hybrid markers) will be used to screen the existing 8x BAC library at CalTech. This will serve to evaluate the quality of the library in terms of representation of broad chromosomal regions. A similar procedure will be used for chromosome 22, except that the existing BAC map will be used to select more evenly spaced markers for screening, including use of end-sequence markers from the current chromosome 22 BAC map constructed in the Simon lab. Each identified clone will be rearrayed from the library and end sequenced. This information will dovetail nicely with ongoing sequencing projects at TIGR and the Sanger Centre, which will in turn provide additional information on the average degree of BAC overlap detectable by this method, the degree of interference with genome-wide repeats, and the appropriate use of fingerprinting as an early or late addition to the end-sequencing information. In addition, we will develop and implement cost-effective, high-throughput methods of preparing and end-sequencing BAC DNA that are suitable for scaling to characterization