tem, we have augmented it with hand-help mobile computing devices (Apple Newtons) that link to the database through RF networking cards.

Base calling software has been developed to support our automated, large scale sequencing effort. 1st stage sequence calling identifies putative bands, however, depending on the number of reader indel errors (2-6%), merging 1st stage sequence without the aide of cutoff information can be difficult. To improve our base calling we have employed Fuzzy Logic to establish confidence metrics. The logic produces a confidence metric for each band using band height, width, uniqueness, shape, and the gaps to adjacent bands. The confidence metric is then used to identify the largest block of highest quality sequence to be merged.

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Reference

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Resource for Molecular Cytogenetics

Donna Albertson, Colin Collins, **Joe Gray**,[1] Steven Lockett, **Daniel Pinkel**,[1] Damir Sudar, Heinz-Ulrich Weier, and Manfred Zorn
Lawrence Berkeley National Laboratory; Berkeley, CA 94720 and [1]University of California; San Francisco, CA 94143
Gray: 415/476-3461, Fax: -8218, *gray@cc.ucsf.edu*
Pinkel: 415/476-3659, Fax: -8218, *pinkel@cc.uscf.edu*
http://rmc-www.lbl.gov

The purpose of the Resource for Molecular Cytogenetics is to develop molecular cytogenetic techniques, instruments and reagents needed to facilitate large scale genomic DNA sequencing and to assist in identification and functional characterization of genes involved in disease susceptibility, genesis and progression. This work is closely coordinated with the LBNL Human Genome Program and directly supports research in the LBNL Life Sciences Division and the UCSF Cancer Center. Work currently is in four areas: a)Genome analysis technology, b)Probe development and physical map assembly, c)Digital imaging microscopy and d)Informatics. The Resource acts as a catalyst for research in several areas so some support comes from Industry, the NIH and NIST.

Probe development and physical map assembly: The Resource maintains a list of over a thousand publicly available probes suitable for molecular cytogenetic studies. These include approximately 600 probes each selected by the Resource to contain a known STS or EST. Probes selected by the Resource can be requested through our web page.

The Resource also participates in the development of low and high resolution physical maps to facilitate analysis and characterization of genetic abnormalities associated with human disease. Low resolution mapping panels with probes distributed at few megabase intervals have been completed this year for chromosomes 1, 2, 3, 7, 8, 10, and 20. The mapped STSs associated with these probes facilitate movement from low to high resolution physical maps. STS content mapping and DNA fingerprinting have been applied to develop a high resolution, sequence-ready map comprised of BAC and P1 clones for the ~1Mb region of chromosome 20 between WI9227 and D20S902. This region is amplified in ~10% of human breast cancers. Approximately 300 kb of this region has been sequenced by the LBNL Human Genome Program.

Quantitative DNA fiber mapping (QDFM) has been developed this year to facilitate high resolution analysis of genomic overlap between cloned probes. In this approach, cloned DNA molecules are uniformly stretched during drying by the hydrodynamic action of a receding meniscus. The position of specific sequences along the stretched DNA molecules is visualized by fluorescence in situ hybridization (FISH) and measured by digital image analysis. QDFM has been used to map gamma alpha transposons, plasmid or cosmid probes along P1 molecules, and P1 or PAC clones along straightened YAC molecules with few kilobase resolution. QDFM is now being studied to determine its utility in the assembly of minimally overlapping, sequence-ready contigs, assessment of the integrity of cloned BACs and mapping of subclones prepared for directed DNA sequencing along the clone from which they were derived.

Genome analysis technology: The Resource has participated in the development of comparative genomic hybridization (CGH) as a tool for detection and mapping of changes in relative DNA sequence copy number in humans and mouse. This year, CGH to arrays of cloned probes (CGHa) has been demonstrated. This is advantageous because it allow aberrations to be mapped with resolution determined by the genomic spacing of probes on the array. CGHa also is attractive since it appears to be linear over a relative copy number range of at least 104 between the two nucleic acid samples being compared.

The Resource has participated in the development of FISH approaches to analysis of relative gene expression in normal and aberrant tissues. FISH with cloned or predicted expressed sequences, previously developed in C. elegans, is now being applied to the assessment of expression of human genes. The C. elegans work suggests a throughput of several dozen sequences per month. Information from this approach will be important in assessment of the function of newly discovered genes, including those predicted from DNA sequencing.

(abstract continued)