includes ensuring that the most stable clones (cosmids, BACs, and PACs) are available for sequencing and that regions with such known physical landmarks as STSs and expressed sequenced tags (ESTs) are annotated to facilitate sequence assembly and analysis. The following targets are emphasized for DNA sequencing:

- Regions of high gene density, including regions containing gene families.
- Chromosome 19, of which at least 42 million bases are sequence ready.
- Selected BAC and PAC clones representing regions of about 0.2 million to 1 million bases throughout the human genome; clones would be selected based on such high-priority biological targets as genes involved in DNA repair, replication, recombination, xenobiotic metabolism, cell-cycle checkpoints, or other specific targets of interest.
- Selected BAC and PAC clones from mouse regions syntenic with the genes indicated above.
- Full-insert cDNAs corresponding to the genomic DNA being sequenced.

The informatics team is continuing to deploy broader-based supporting databases for both mapping and sequencing. Where appropriate, Web- and Java-based tools are being developed to enable biologists to interact with data. Recent reorganization within this group enables better direct support to the sequencing group, including evaluating and interfacing sequence-assembly algorithms and analysis tools, data and process tracking, and other informatics functions that will streamline the sequencing process.

The instrumentation effort has three major thrusts: (1) continued development or implementation of laboratory automation to support high-throughput sequencing; (2) development of the next-generation DNA sequencer; and (3) development of robotics to support high-density BAC clone screening. The last two goals warrant further explanation.

The new DNA sequencer being developed under a grant from the National Institutes of Health, with minor support through the DOE genome center, is designed to run 384 lanes simultaneously with a low-viscosity sieving medium. The entire system would be loaded automatically, run, and set up for the next run at 3-hour intervals. If successful, it should provide a 20- to 40-fold increase in throughput over existing machines.

An LLNL-designed high-precision spotting robot, which should allow a density of 98,304 spots in 96 cm^2, is now operating. The goal of this effort is to create high-density filters representing a 10× BAC coverage of both human and mouse genomes (30,000 clones = 1× coverage). Thus each filter would provide ~3× coverage, and eight such filters would provide the desired coverage for both genomes. The filters would be hybridized with amplicons from individual or region-specific cDNAs and ESTs; given the density of the BAC libraries, clones that hybridize should represent a binned set of BACs for a region of interest. These BACs could be the initial substrate for a BAC sequencing strategy. Performing hybridizations in parallel in mouse and human DNA facilitates the development of the mouse map (with ORNL involvement), and sequencing BACs from both species identifies evolutionarily conserved and, perhaps, regulatory regions.

Information generated by sequencing human and mouse DNA in parallel is expected to expand LLNL efforts in functional genomics. Comparative sequence data will be used to develop a high-resolution synteny map of conserved mouse-human domains and incorporate automated northern expression analysis of newly identified genes. Long range, the center hopes to take advantage of a variety of forms of expression analysis, including site-directed mutation analysis in the mouse.

Summary

The Livermore Human Genome Center has undergone a dramatic shift in emphasis toward commitment to large-scale, high-accuracy sequencing of chromosome 19, other chromosomes, and targeted genomic regions in the human and mouse. The center also is committed to exploiting sequence information for functional genomics studies and for other programs, both in house and collaboratively.