chromosome regions of both human and mouse genomes. Many subtle functional elements can be recognized only by comparing human and mouse sequences. TCRs play a major role in immunity and autoimmune disease, and insights into their mechanisms may one day help treat or even prevent such diseases as arthritis, diabetes, and multiple sclerosis (possibly even AIDS).

Comparative analysis is also used to model human genetic diseases. Given sequence information, researchers can produce targeted mutations in the mouse as a rapid and economical route to elucidating gene function. Such studies continue to be used effectively at Oak Ridge National Laboratory (ORNL).

DNA Sequencing

From the beginning of the genome project, DOE's DNA sequencing-technology program has supported both improvements to established methodologies and innovative higher-risk strategies. The first major sequencing project, a test bed for incremental improvements, culminated with elucidation of the highly complex TCR region (described above) by a team led by Hood.

A novel "directed" sequencing strategy initiated at LBNL in 1993 provides a potential alternative approach that can include automation as a core design feature. In this approach, every sequencing template is first mapped to its original position on a chromosome (resolution, 30 bases). The advantages of this method include a large reduction in the number of sequencing reactions needed and in the sequence-assembly steps that follow. To date, this directed strategy has achieved significant results with simpler, less repetitive nonhuman sequences, particularly in the NIH-funded *Drosophila* genome program. The system also is in use at the Stanford Human Genome Center and Mercator Genetics, Inc.

The preparation of DNA clones for sequencing involves several biochemical processing steps that require different solution environments. At the Whitehead Institute, Trevor Hawkins has improved systems for reversible binding of DNA molecules to magnetic beads that are compatible with complete robotic management. The second-generation Sequatron fits on a tabletop with a single robotic arm moving sample trays between servicing stations. This very compact system, supported by sophisticated software, may be ideal for laboratories with limited or costly floor space.

Fluorescent tags are critical components of conventional automated sequencing approaches. The team of Richard Mathies and Alexander Glazer (University of California, Berkeley) has made a series of improvements in fluorescence systems that have decreased DNA input needs and markedly increased the quality of raw data, thereby supporting longer useful reads of DNA sequence.

Complementary improvements in enzymology have been achieved by the team of Charles Richardson and Stanley Tabor (Harvard Medical School). Current widely used procedures for automated DNA sequencing involve cycling between high and low temperatures. The Harvard researchers used information about the three-dimensional structure of polymerases (enzymes needed for DNA replication) and how they function to engineer an improved Taq polymerase. ThermoSequenase, which is now produced commercially as part of the ThermoSequenase kit, reduces the amount of expensive sequencing reagents required and supports popular cycle-sequencing protocols.

The application of higher electrical fields in gel electrophoresis separation of DNA fragments can increase sequencing speed and efficiency. Conventional thick gels cannot adequately dissipate the additional heat produced, however. Two promising routes to "thinness" are ultrathin slab gels and