capillary systems. An ultrathin gel system was developed by Lloyd Smith (University of Wisconsin, Madison) and licensed for commercial development.

The replacement of gels by pumpable solutions of long polymers is making capillary array electrophoresis (CAE) potentially practical for DNA sequencing. The first CAE system for DNA was demonstrated by the team of Barry Karger (Northeastern University). In 1995, Karger and Norman Dovichi (University of Alberta, Canada) separately identified CAE conditions under which DNA sequencing reads could be extended usefully up to the 1000-base range. Another CAE system, developed by Edward Yeung (Iowa State University), has been licensed for commercial production (see box, p. 23). Mathies has developed a system in which a confocal microscope displays DNA bands. Application of this system to the sizing of larger DNA fragments binding multiple fluors allows single-molecule detection.

Replacing the gel-separation step with mass spectroscopy (MS) is another promising approach for rapid DNA sequencing. MS uses differences in mass-to-charge ratios to separate ionized atoms or molecules. Early efforts at MS sequencing were plagued by chemical reactivity during the "launching" phase of matrix-assisted laser desorption ionization (MALDI). MALDI badly degraded the DNA sample input. However, the degradation chemistry was elucidated in Smith's laboratory, leading to improvements. At ORNL, the team of Chung-Hsuan Chen has performed extensive trials of alternative matrices and has achieved significant improvements that now support sequence reads up to 100 DNA bases. The system is undergoing trials for DNA diagnostic applications.

The most revolutionary sequencing technology is being pursued by the team of Richard Keller and James Jett at LANL. Their goal is to read out sequence from single DNA molecules, work that builds on LANL's expertise in flow cytometry. The strand to be sequenced is labeled first with fluors that distinguish the four DNA subunits and is then suspended in a flow stream. An exonuclease cleaves the subunits, which flow past an interrogating laser system that reports the subunits' identities. All system constituents are operational but limited by the low subunit release rates of commercially available exonucleases. A current developmental focus is on identifying more active exonucleases.

Synthetic DNA strands in the 15- to 30-base range (oligomers) play essential roles in DNA sequencing; in sample-preparation steps for the polymerase chain reaction, which copies DNA strands millions of times; and in DNA-based diagnostics. The cost of custom oligomer synthesis once was a limiting factor in many research projects. A more economical, highly parallel oligomer synthesis technology was developed by Thomas Brennan at Stanford University (see last bullet, p. 22, for further details).

The sequencing by hybridization (SBH) technology provides information only on short stretches of DNA in a single trial (interrogation), but thousands of low-cost interrogations can be performed in parallel. SBH is very useful for rapid classification of short DNAs such as cDNAs, very low cost DNA resequencing, and detection of DNA sequence differences (polymorphisms) over short regions. The team of Radomir Crkvenjakov and Radoje Drmanac invented one format of SBH while in Yugoslavia, made substantial improvements at Argonne National Laboratory (ANL), and later started Hyseq Inc. to commercialize these technologies. At ANL, another implementation, SBH on matrices (SHOM) of gels, holds promise for high-accuracy sequence proofreading and diverse DNA diagnostics. The ANL team, led by Andrei Mirzabekov, collaborates