ENABLING TECHNOLOGY

NEW TRICKS TAME MEGABASE DNA FRAGMENTS

BETHESDA, Md.—Powerful basic laboratory techniques are evolving to match genetic researchers' expanding horizons. Many of these advances in handling megabase pieces of DNA have spun off from efforts to map genes in Drosophila, the mouse, and man, but they also promise to speed less ambitious genetic investigations.

Policy debates over sequencing the human genome have held the spotlight recently. But researchers supplying background for those discussions have gradually unfolded a picture of technology whose value may go far beyond big-science gene mapping projects.

The advisory committee to the director of the National Institutes of Health met here in October to consider what role, if any, NIH should play in funding a formal project to map the human genome (that decision is still pending). In the course of this discussion (and at an earlier *Nature* meeting on "Exploring the Human Genome"), researchers described new methods of DNA sample preparation, separation, and replication that enable them to extract information from single DNA fragments up to 2-million base-pairs long.

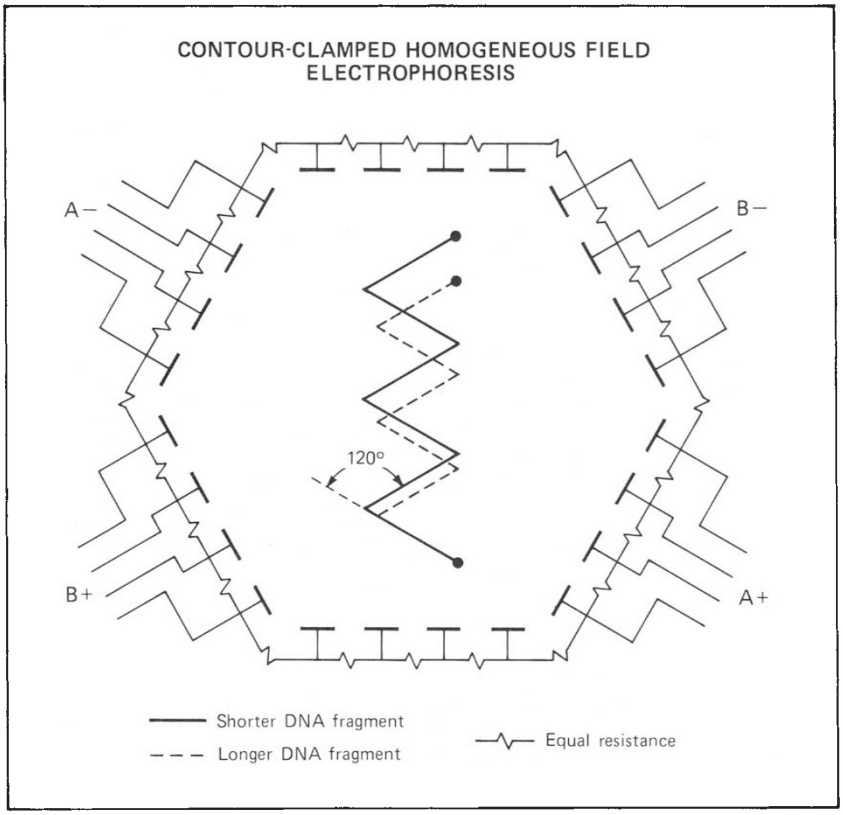
- *Selective restriction.* Researchers are increasingly turning to techniques that produce very long fragments, on the order of 100 Kbp to 1 Mbp. Their tools include restriction endonucleases (like *Not*I) that recognize sequences of nucleic acids 8–10 base pairs long; enzymes like (*Dpn*I) that cut only rare sequences selectively modified by certain methylases; and (in a technique being developed by Peter Dervan at the California Institute of Technology in Pasadena) nucleic-acid probes linked to chelating agents and other moieties that can be activated to snip through the DNA at a precisely determined spot.
- *Sample handling.* Even carefully handled DNA may break when transferred in solution to an electrophoresis gel. Columbia University's Charles Cantor instead immobilizes DNA in agarose before digestion. The agarose block is then inserted in a slot on the electrophoresis gel. Francis Collins of the University of Michigan adds an intermediate step, in which the block is electrophoresed under steady current for about an hour to remove any short strands of broken DNA before the sample is transferred to a second gel for final analysis.
- *Electrophoretic techniques.* When subjected to electric fields that change direction, longer DNA molecules take much more time to "turn the corner" and align with the new potential than shorter bits. This stretches out the gaps between the molecules and increases electrophoretic resolution. Several researchers have developed instruments that exploit this phenomenon, beginning with Cantor's pulsed-field gel electrophoresis device (PFGE, now being produced by LKB Produkter, Bromma, Sweden), to field-inversion techniques which completely reverse the field's polarity. The pulsed-field device produces very sharp resolution in a bowed pattern that some find difficult to read. Field-inversion is said to offer less resolution, and peculiar conditions may occasionally cause overlapping of two bands of very different molecular weights.

Contour-clamped homogeneous electric field (CHEF) electrophoresis, a technique developed by Gilbert Chu, Douglas Vollrath, and Ronald W. Davis of the Stanford University School of Medicine, overcomes some of these difficulties, using a hexagonal grid to mimic the behavior of two infinitely long pairs of electrodes crossing at an angle of 60°. The resulting uniform field eliminates lane bowing, Davis says, and the wide "reorientation angle" of 120° produces sharp separation in the samples.

Using this technique, the Stanford researchers have separated DNA molecules 2-million bases long—including whole chromosomes of yeast and other organisms.

- *Cloning.* Davis's group avoids subdividing megabase fragments of nucleic acid into 50 Kbp bits for cosmid cloning. Instead, they construct an artificial yeast chromosome around the fragment of interest—using circular DNA cassettes that carry a yeast centromere and can integrate into yeast chromosomes carrying selectable markers and yeast telomeres. All that is necessary for competent replication and expression are yeast autonomous replicating sequences (ARS), which are routinely found at intervals of about 50 Kbp throughout the eukaryotic genome, Davis says. For segments longer than about 100 Kbp, the artificial chromosomes are much more stable than plasmids.

—Douglas McCormick



Contour-clamped homogeneous electric field electrophoresis. The lanes begin at the bottom of the illustration, and are subjected to electric fields alternating (at intervals on the order of an hour) between pairs of electrodes. The sample follows a zig-zag path, with light molecules rounding corners far faster than heavy molecules, increasing separation.