REVIEW

Optical Mapping: A Novel, Single-molecule Approach to Genomic Analysis

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Increasingly, it appears likely that the ultimate success of the human genome project, and a majority of advances in molecular diagnosis of human disease, will be driven by advances in genomic analysis that permit ultrarapid physical mapping and DNA sequencing. Molecular biological approaches currently being used were developed primarily for characterization of single genes, not entire genomes and, as such, are not ideally suited to analysis of polygenic diseases, complex trait inheritance, and population-based molecular genetics. Thus, it is imperative to develop new approaches rapidly that deal with entire genomes.

Physical mapping of genomes, using restriction endonucleases, has played a major role in identifying and characterizing various loci, for example, by aiding clone contig formation and by characterizing genetic lesions. Restriction maps provide precise genomic distances, unlike ordered sequence-based landmarks such as sequence tagged sites (STSs), that are essential for optimizing the efficiency of sequencing efforts and for determining the spatial relationships of specific loci (Baxendale et al. 1993). When compared to time-consuming hybridization-based fingerprinting approaches, ordered restriction maps offer relatively unambiguous clone characterization that is useful in contig formation, establishment of minimal tiling paths for sequencing, and preliminary characterization of sequence lesions.

Despite the broad applications of restriction maps, the associated techniques for their generation have changed little over the last 10 years, primarily because they still utilize electrophore-

sis. In contrast, PCR-based techniques have radically changed genomic analysis approaches (Srivastava 1994); for example, they have largely supplanted restriction fragment-length polymorphism (RFLP) analysis for genotyping. This is not surprising because PCR methods are readily automated and make many Southern blot applications unnecessary. In general, fully automated systems for the restriction mapping of a wide size range of cloning vectors have not been widely adopted, because of lack of commercial instrumentation and software. One important exception has been the adaptation of automated electrophoretic methods, originally developed for automated fluorescence sequencing, to genotype small PCR products derived from microsatellite repeats.

However, PCR-based methods alone cannot equal the information content of high-resolution restriction mapping. Consequently, despite the power of restriction mapping to simplify genome analysis, low practical throughput using commonly available techniques has limited its use seriously. To help overcome these shortcomings, our laboratory has developed the first practical nonelectrophoretic approach—optical mapping—to meet this need.

Yanagida and co-workers (1986) first described the molecular motions of fluorescently stained, individual DNA molecules in solution by means of image-enhanced fluorescence microscopy, which uses low-light-sensitive video cameras and image processors to improve visualization. Our laboratory subsequently developed optical mapping, a single-molecule methodology for the rapid production of ordered restriction maps from single DNA molecules (Schwartz et al. 1993; Cai et al. 1995; Meng et al. 1995; Wang et al. 1995). Ordered restriction maps were constructed originally from yeast chromosomes (Schwartz et al. 1995) by imaging restriction en-

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donuclease cutting events on single, stained DNA molecules with fluorescence microscopy. Cut sites appeared as gaps that widened as the DNA fragments relaxed. Maps were then constructed by measuring fragment sizes via relative fluorescence intensity or apparent length measurements.

In the original method, individual fluorescently labeled DNA molecules were elongated in a flow of molten agarose generated between a coverslip and microscope slide (Schwartz et al. 1993). The molten agarose was gelled before the DNA molecules were fully relaxed so that they could be fixed into an elongated state. The endonuclease, added previously to the molten gel, was triggered by addition of magnesium ions, and the resulting cleavage events were recorded by fluorescence microscopy as time-lapse digitized images. Gel fixation was substantial enough to prevent complete molecular relaxation but not enough to prevent DNA at cut sites from relaxing, as evidenced by intensified fluorescence at fragment ends.

Although optical mapping was a valuable approach to genomic mapping, improvements were required if a wide range of cloning vectors (cosmid, bacteriophage, P1, YACs) were to be analyzed. Cosmid and λ clones are valuable genomic reagents, particularly when placed into contigs, because they provide simplified access to a number of high-throughput sequencing routines (Hunkapiller et al. 1991; Baxendale et al. 1993). Contigs constructed from ordered restriction maps of λ bacteriophage or cosmid clones have provided materials for high-resolution restriction maps to characterize large genomic regions. Given the utility of such clones, we developed a second-generation optical mapping approach to produce accurate maps consisting of DNA fragments as small as 500 bp. This approach dispensed with agarose and, instead, fixed elongated DNA molecules onto polylysine-treated glass surfaces. The fixation conditions were carefully controlled to minimize DNA coil relaxation effects, but allowed enough relaxation at cleavage sites for their detection. Time-lapse imaging was not required, because surface-mounted molecules and their associated fragments remained stably in focus.

The exact mechanism of how DNA molecules interact with derivatized surfaces is not known, but we postulated that electrostatic interactions predominate between the anionic DNA and cationic surfaces. Efficient binding and analysis of

molecules reflect a balance between flow and electrostatic forces, whereby sufficient molecular extension occurs to allow access to endonuclease cleavage. Because optical mapping uses fluorescence intensity measurements for mass determination, complete DNA elongation of most molecules is not required for mapping, but it does facilitate the process.

For analysis of λ clones, DNA samples (consisting of DNA, restriction buffer minus magnesium, 2-mercaptoethanol, and a fluorochrome) were fixed onto derivatized glass surfaces by sandwiching between a treated coverslip and glass slide. In these experiments, a cooled charge-coupled device (CCD) camera was used to image molecules from 28 kb down to 800 bp (Meng et al. 1995), representing a major improvement from our prior agarose gel-based optical mapping approach, which had a limit of resolution of 60 kb (Schwartz et al. 1993).

Maps were made after first determining the correct number of fragments and then constructing a histogram for each clone that consisted of the number of imaged restriction fragments per parental molecule and their frequencies. Generally, 100 molecules of each clone were analyzed, and 5–10 molecules were selected for map construction on the basis of fragment number and map content, the latter originating from histogram bins containing the maximum number of restriction fragments. The final map is reported as an average of restriction fragment sizes derived from similar molecules, the latter defined as molecules with appropriate fragment numbers, and with fragment sizes within the stated measurement precision. Histogram analyses are most critical when small numbers of molecules are analyzed and when digestion efficiencies are not entirely quantitative. The overall relative error (which was the same for length and intensity) of 5% for fragments >5 kb is comparable to sizing error by agarose gel electrophoresis. Sizing error is a function of the number of molecules analyzed, and we have achieved rates as low as a few base pairs on restriction digest fragments of bacteriophage λ , representing much higher accuracy than is achievable by agarose gel electrophoresis.

Ordered restriction maps of yeast artificial chromosomes (YACs) have also been generated in this laboratory by optical mapping (Fig. 1) (Cai et al. 1995). Presently, a large fraction of the human genome is covered by YAC contigs (Cohen et al. 1993); however, extensive restriction maps of YACs have not been widely generated. This

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Figure 1 Image of restriction enzyme-digested YAC clone. YAC clone 5L5 derived from human chromosome 11, was digested with *EagI* and *MluI*, stained with the fluorochrome YOYO-1, and visualized by fluorescence microscopy as described in Cai et al. (1995). Five fragments are generated from the ~360-kb parent molecule. Bar, 10 μ m.

fact is attributable primarily to the high frequency of rearrangements/chimerism among YACs, the low complexity of fingerprints generated by hybridization approaches, and the extensive labor required to overcome these problems. The approach taken by this laboratory to optically map YACs involved combining the fluid turbulence damping properties of molten agarose, with the stability and enzymatic accessibility of surface mounting, by fixing YACs (ranging up to 440 kb in size) in molten agarose onto derivatized glass surfaces (Cai et al. 1995). By using a spermine condensation method to avoid DNA shearing, high-resolution maps of YACs have been generated, with overall relative sizing errors based on fluorescence intensity that are comparable to routine pulsed-field gel electrophoretic analysis.

Using the approaches discussed above, this laboratory has generated ordered restriction maps for the Beckwith–Wiedeman locus in humans (in collaboration with Dr. D. Housman's group at MIT, Cambridge, MA), the *Brca2* locus (in collaboration with Dr. S. Fisher's group at Columbia University, NY), and the mouse olfactory locus (in collaboration with Dr. R. Axel's group at

Columbia University). Optical maps are currently being generated from phage, cosmid, YAC, and bacterial artificial chromosome clones. In anticipation of the vastly increased throughput requirements for whole genome analysis, we have devised high-throughput approaches involving obviation of a glass sandwich layer.

Prior to the development of optical mapping, restriction map construction was almost exclusively dependent on gel electrophoretic analysis. With this approach, maps were made for the genomes of *Escherichia coli* (Kohara et al. 1987), *Saccharomyces cerevisiae* (Olson et al. 1986), and *Caenorhabditis elegans* (Coulson et al. 1986). Despite the successes of electrophoretically generated maps, numerous limitations of this approach remain, foremost among them being the need for higher throughput with less labor intensiveness. Automatic image processing of cleaved molecules is a far simpler process than automatic gel band reading because thousands of molecules can be analyzed in parallel per optical mapping workstation. As larger numbers of molecules are analyzed automatically, single-base sizing accuracy can be expected. Fully automated optical mapping approaches would require absolutely no human intervention between the points of sample preparation and map construction. Automation also holds enormous promise for miniaturization, with expected increases in throughput and reductions in cost. Thus, advantages of optical mapping include high throughput and resolution, safety, and low cost. Finally, it is hoped that the throughput and resolution afforded by optical mapping will facilitate completion of the human genome project and when combined with non-restriction-based optical mapping approaches, will enable detection and mapping of most genetic alterations afflicting the human genome.

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