Resolving Proteins Bound to Individual DNA Molecules

David Allison, Bruce Warmack, Mitch Doktycz, Tom Thundat, and Peter Hoyt
Molecular Imaging Group; Health Sciences Research Division; Oak Ridge National Laboratory; Oak Ridge, TN 37831-6123
Allison: 423/574-6199, Fax: -6210, *allisondp@ornl.gov*
Warmack: 423/574-6202, Fax: -6210, *rjw@ornl.gov*

We have precisely located sequence specific proteins bound to individual DNA molecules by direct AFM imaging. Using a mutant *Eco*R I endonuclease that site-specifically binds but doesn't cleave DNA, bound enzyme has been imaged and located, with an accuracy of ±1%, on well characterized plasmids and bacteriophage lambda DNA (48 kb). Cosmids have been mapped and, by incorporating methods for anchoring molecules to surfaces and straightening to prevent molecular entanglement, BAC-sized clones could be analyzed.

This direct imaging approach could be rapidly developed to locate other sequence-specific proteins on genomic clones. Enzymatic proteins, involved in identifying and repairing damaged or mutated regions on DNA molecules, could be imaged bound to lesion sites. Transcription factor proteins that identify gene-start regions and other regulatory proteins that modulate the expression of genes by binding to specific control sequences on DNA molecules could be precisely located on intact cloned DNAs.

Conventional gel-based techniques for identifying site-specific protein binding sites must rely upon fragment analysis for identifying restriction enzyme sites, or, for non-cutting proteins, upon gel-shift methods that can only address small DNA fragments. Conversely, AFM imaging is a general approach that is applicable to the analysis of all site-specific DNA protein interactions on large-insert clones. This technique could be developed for high-throughput analysis, can be accomplished by technicians, uses readily available relatively inexpensive instrumentation, and should be a technology fully transferable to most laboratories.

DOE Contract No. DE-AC05-840R21400.

*Improved Cell Electrotransformation by Macromolecules

Alexandre S. Boitsov, Boris V. Oskin, Anton O. Reshetin, and Stepan A. Boitsov
Department of Biophysics; St.Petersburg State Technical University; 195251 St. Petersburg, Russia
+7-812/277-5959, Fax: /247-2088 or /534-3314, *sasha@bioph.hop.stu.neva.ru*

Our work for 1996 and 1997 will include the following:

1. Comparative study of the kinetics of entry of DNA of different molecular forms into E.coli cells DH10B/r and DH5a during electrotransformation. Study of the optimal regimes of cell-wall permeabilization for the DH10B/r cells.

2. Study of the efficiency of BAC cloning in DH10B/r cells using new electrotransformation method. Optimization of the procedure for DH10B/r cells.

3. Modernization of the electronic equipment in accordance with results of the biological experiments. To expand the studies, we need to extend the capability of the instrumentation to increase its flexibility and to improve the accuracy and reproducibility of the electric fields we generate by incorporating electronic components with higher tolerances.

DOE Grant No. OR00033-93CIS015.

Overcoming Genome Mapping Bottlenecks

Charles R. Cantor
Center for Advanced Biotechnology; Boston University; Boston MA 02215
617/353-8500, Fax: 8501, *crc@enga.bu.edu*
http://eng.bu.edu/CAB

Most traditional DNA analysis is done based on fractionation of DNA by length. We have, instead, begun to explore the use of DNA sequences as capture and detection methods to expedite a number of procedures in genome analysis.

Triplet repeats like $(GGC)_n$ are an important class of human genetic markers, and they are also responsible for a number of inherited diseases involving the central nervous system. For both of these reasons it would be very useful to have a way to monitor the status of large numbers of triplet repeats simultaneously. We are developing methods to isolate and profile classes of such repeats.

In one method, genomic DNA is cut with one or more restriction nucleases, and splints are ligated onto the ends of the fragments. Then fragments containing a specific class of repeats are isolated by capture on magnetic microbeads containing an immobilized simple repeating sequence. The desired material is then released, and, if necessary, a selective PCR is done to reduce the complexity of the sample. Otherwise the entire captured sample is amplified by PCR. The spectrum of repeats is then examined by electrophoresis on an automated fluorescent gel reader. In our case the Pharmacia ALF is used, because of its excellent quantitative signal accuracy. A very complex spectrum of bands is

*Projects designated by an asterisk received small emergency grants following December 1992 site reviews by David Galas (formerly DOE Office of Health and Environmental Research, which was renamed Office of Biological and Environmental Research in 1997), Raymond Gesteland (University of Utah), and Elbert Branscomb (Lawrence Livermore National Laboratory).