Microbial Genome Program Report: Optical approaches for physical mapping and sequence assembly of the *Deinococcus radiodurans* chromosome.

Maps of genomic or cloned DNA are frequently constructed by analyzing the cleavage patterns produced by restriction enzymes. Restriction enzymes are remarkable reagents that faithfully cleave only at specific sequences of between 4 and 8 nucleotides, which vary according to the specific enzymes. Restriction enzymes are reliable, numerous, and easily obtainable and presently, there are approximately 250 different sequences represented among thousands of enzymes. Restriction maps characterize gene structure and even entire genomes. Furthermore, such maps provide a useful scaffold for the alignment and verification of sequence data. Restriction maps generated by computer and predicted from the sequence are aligned with the actual restriction map. Restriction enzyme action has traditionally been assayed by gel electrophoresis. This technique separates cleaved molecules on the basis of their mobilities under the influence of an applied electrical field, within a gel separation matrix (small fragments have a greater mobility than large ones). Although gel electrophoresis distinguishes different sized DNA fragments (known as a "fingerprint"), the original order of these fragments remains unknown. The subsequent task of determining the order of such fragments is a labor intensive task, especially when making restriction maps of whole genomes, and therefore despite its obvious utility to genome analysis, it is not widely used.

Our laboratory has developed Optical Mapping, which is a system for the construction of ordered restriction maps from individual DNA molecules. Optical Mapping was used to construct a whole genome restriction map of *Deinococcus radiodurans* without using any electrophoresis. No cloning of *Deinococcus radiodurans* DNA was required either. The mapping substrate was very large, randomly sheared, genomic DNA fragments which were bound to derivatized glass surfaces and cleaved with the restriction enzyme *Nhe* I. The resulting fragments were imaged by fluorescence microscopy. Cut sites were visualized as gaps between cleaved DNA fragments, which retained their original order. By determining the existence of these sequence-specific cut sites and measuring the DNA mass between them, a landmark map of the DNA sequence was constructed. Maps from a population of about 100 individual molecules were aligned and overlaid to construct an accurate consensus map. We have called this strategy "Shotgun Optical Mapping". The consensus map contained about 90 restriction fragments and would have been extraordinarily difficult to construct by electrophoretic means.

This high resolution restriction map was used by collaborators at The Institute for Genomic Research to verify sequence assembly data from *Deinococcus radiodurans* by aligning the restriction map predicted from their sequence. Optical Mapping of *Deinococcus radiodurans* also rendered insight into the biology of this organism by providing a picture of the basic organization of the entire genome, revealing the genome to be composed of two, rather than one chromosome, as well as demonstrating the presence of other extrachromosomal elements. Whole genome characterization by Optical Mapping may facilitate further understanding of the radiation-resistant nature of *Deinococcus radiodurans*, which is being used as a vehicle for bioremediation of toxic organic pollutants within radioactive waste dumps.

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We have no objection from a patent standpoint to the publication or dissemination of this material.

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We have recently (July, 1999) moved the laboratory to University of Wisconsin, Madison. The move entailed five large moving vans, and our laboratory now appears to be fully enabled for research. The final results of this funded project appeared in a recent issue of Science (Jieyi Lin, Rong Qi, Christopher Aston, Junping Jing, Thomas S. Anantharaman, Bud Mishra, Owen White, J. Craig Venter, David C. Schwartz. "Whole Genome Shotgun Optical Mapping of *Deinococcus radiodurans*", Science, **285**, 1558 (September 3, 1999)). Our work will also be featured in a paper submitted by Owen White to Science.