Development of Microarray-Based Genomic Technology for Environmental Studies

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Development of novel chemistry immobilizing oligonucleotides and proteins on microarrays. Appropriate attachment of nucleic acids and proteins to an array surface is critical to microarray-based biomolecule analysis and detection. A novel three-dimensional surface chemistry was developed and evaluated for immobilizing oligonucleotides and proteins. Unmodified oligonucleotide probes and proteins were immobilized on glass slides coated with multilayered nanofilms through electrostatic adsorption and entrapment within the porous structure of the three-dimensional nanofilm. The binding capacity of oligonucleotides on the nanofilm slide was 2–3 times higher than that on the epoxy-activated and aldehyde-modified slides, and the nanofilm coated slides could differentiate single nucleotide differences. In addition, specific antibody-antigen interactions were obtained with a prototype of the nanofilm protein microarrays. Our results indicated that the nanofilm-based oligonucleotide and protein arrays had increased sensitivity and decreased background when compared to commercially available slides.

Microbial community diversity at FRC. Clonal libraries of the SSU rRNA gene were constructed from different groundwater samples at the FRC, and these sites differed with respect to geochemical characteristics. Inserts from the clones were amplified, partial nucleotide sequences were determined for 1,600 clones, and the sequences compared with cluster analysis. The results indicated that the microbial community composition at contaminated sites was altered compared to background. All unique clones from the six samples were selected (approximately n=325), and full-length sequences determined for the selection of distinctive oligonucleotides.

SSU rRNA Gene Oligonucleotide Microarray. Oligonucleotide microarrays were developed for the assessment of microbial communities based on conserved SSU rRNA gene sequences, and to determine whether single mismatch discrimination could be achieved. Our results indicate that the position of the mismatch, the type of mismatch, and the concentration of hybridization additives, such as formamide and tetramethylammonium chloride (TAMCl), significantly affected the specificity and signal intensity. The hybridization signal intensity of the probes with a single-base mismatch was decreased approximately 3- to 10-fold, depending on the oligonucleotide sequence. These results indicate that single-base discrimination for SSU rRNA genes can be achieved with array-based hybridization. The effects of probe length and GC content on microarray hybridization were also determined.

Bioremediation Gene Array (BGA). A bioremediation gene array (BGA) was also constructed based on more than 2,000 available sequences involved in biodegradation of pollutants and metal resistance. We have optimized the conditions for the discrimination of probes (50-mer) with up to 85% similarity without a significant reduction in sensitivity. Probes of less than 85% similarity showed less than 10% hybridization signal intensity with perfect match probes. For assessment of BGA, we used several reference strains that could degrade different pollutants. The results indicated that BGAs could be successfully used for the determination of metabolic pathways and biochemical activity.

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