

Research Objective:

The objective of this program is to develop innovative DNA detection technologies to achieve fast mutation screening and to reveal the linkage between gene mutation and contaminants. The specific approach are (1) to develop innovative multiplexing hybridization detection for DNA mutation detection, (2) to develop sequence-proof microarray hybridization technology (3) to develop hybridization on disk and (4) to validate the pollution-mediated mutation can be used for sound risk analysis for setting up the priorities for waste cleanup.

Research Progress and Implication:

AS of 2 year of a 3 year project, we have significant achievement in mass spectrometry and computer disk DNA analysis for hybridization. Since this program is to address the DNA mutation due to the exposure to contaminated media and to promote a better understanding of the relationship between exposure and health impact, high throughput DNA analysis technology is critically needed. During the past few years, microarray hybridization has been considered as a candidate for high throughput DNA analysis. However, there are two disadvantages with present hybridization detection technology. At present, all hybridization-on-chip is detected by laser induced fluorescence. DNA probes need to be tagged with dye molecules. At each hybridization site, only single hybridization reaction can be pursued. However, with mass spectrometry for microarray hybridization detection, no tagging is needed. In addition, multiplexing hybridization can be achieved. Thus, the speed can be one order of magnitude faster and the cost is also significantly less. During the past year, we succeeded in using mass spectrometry for multiplexing hybridization detection. As microarray hybridization becomes a very important tool for genetic analysis, it is critically needed to have a new hybridization technology which can be significantly cheaper than present hybridization technology. In addition, it can also be used for in-situ real time analysis. During the past year, we have tried to pursue the idea of using a magnetic disk for hybridization. The feasibility of this approach has been proved.

The major achievements during the past year are listed and discussed in the follows.

(1) Rapid multiplexing hybridization detection by mass spectrometry.

Since there are between 50,000 to 100,000 genes in human genome and each gene can have more than hundreds of different mutations, unusual high throughput DNA analysis will be needed to evaluate mutation due to the environmental impact. At present, all the available hybridization detection can only have one hybridization reaction at one site. Recently, we demonstrated the successful multiplexing hybridization detection with laser desorption mass spectrometry. Six different hybridization reaction with probes tagged with different molecular weights have been detected at a single hybridization site. A long single-stranded DNA hybridized with DNA fragments with different lengths has also been demonstrated. Furthermore, hybridization without the need of PCR was also successfully demonstrated. The implication of this work is high throughput microarray DNA analysis will become feasible for risk analysis in the future.

(2) Laser synthesis of magnetic nanoparticles:

In this program, we detect bio-molecules based on the magnetic particle attached. Thus, a simple and reliable method to produce magnetic particles is very valuable. Synthesis of fine magnetic particles in aqueous solution have been performed through co-precipitation of various salts of Fe(III) and Fe(II) in alkaline media. The target used for ablation was a foil of metal immersed in water in an optical cell. Typically, 3000 to 10000 laser shots were used to generate 1 cm³ of colloid solution. Spectral absorption of synthesized colloid solution was monitored in the spectral range 350-800 nm. Precipitation can be observed when an external magnetic field was applied. It clearly indicates that nano/micro-magnetic particles can be produced by the laser ablation process. We also observed that the size of the particle produced is a strong function of laser fluence. Our results indicate laser ablation in liquids can be used as new technique for producing colloids for biomedical applications. We believe the technique can provide a unique opportunity for the development of new methods for detection of DNA hybridization based on DNA tagging by magnetic particles.

(3) Hybridization on magnetic disk substrate:

Synthetic DNA tagging with magnetic nanoparticles has been used to give demonstration on hybridization on computer disk substrate. It implies that DNA hybridization on computer disk can be feasible. Future in-situ real time DNA analysis can become practical.

Planned activities:

The following tasks will be pursued during the coming year:

- (1) To demonstrate hybridization on disk with PCR products
- (2) To apply hybridization on disk technology to gene mutation.
- (3) To evaluate the mutation due to environmental impact
- (4) To pursue high throughput DNA analysis with microarray arrangement with magnetic disks.

Information Access:

- (1) C. H. Chen , "Laser Desorption Mass Spectrometry of DNA: Mechanism and Applications", Research Trend in Chemical Physics, 8, 1-17 (2001)
- (2) Z. Liu, N. R. Isola, S. L. Allman and C. H. Chen, "Biotin Enhanced Fragmentation for Direct DNA Sequencing Using MALDI", Anal. Chem. (Submitted)
- (3) N. I. Taranenko, R. Hurt, J. Z. Zhou, N. R. Isola, H. Huang, S. H. Lee, and C. H. Chen, " Laser desorption mass spectrometry for microbial DNA analysis" J. Micro.Meth. 48, 101-106 (2002)