

Research Objective:

The objective of this program is to develop innovative DNA detection technologies to achieve fast microbial community assessment. The specific approaches are (1) to develop inexpensive and reliable sequence-proof hybridization DNA detection technology (2) to develop quantitative DNA hybridization technology for microbial community assessment and (3) to study the microbes which have demonstrated the potential to have nuclear waste bioremediation

Research Progress and Implication:

As the first year of a three year project, we put most of our effort in developing microarray hybridization technology. During the past few years, microarray hybridization has been considered as a necessary tool for high throughput DNA analysis especially for environmental applications. However, there are several disadvantages for present laser induced fluorescence detection technology for microarray hybridization. In general, each target DNA needs to be labeled with a dye molecule. The minimum detection level of the number of fluorescence photon from laser induced fluorescence process is $\sim 10^8$ due to the background of scattering of the laser beam. Thus, the minimum quantity of target DNA required is ~ 1 femtomole. Due to this relatively low detection sensitivity, polymerase chain reaction (PCR) is always needed to replicate DNA samples. Since quantitative information is easy to get lost in general PCR process, quantitative determination of microbes in the soil samples becomes extremely difficult. For present microarray DNA hybridization detector, a confocal geometry for laser induced fluorescence measurement is required. The fluorescence measurements need to be obtained spot by spot. It takes longer analysis time. Due to the limitation of detection sensitivity, an expensive photomultiplier with low dark current and a high power laser are required. Thus, the cost of the present microarray hybridization detector is in general higher than \$100,000. Only major research institutes can afford such an expensive instrument. Since it is expensive and non-portable, *in-situ* analysis for environmental application becomes impossible. We tried to take the advantage of well developed personal computer (PC) hardware as a platform for hybridization detection so that the cost can be less than \$1000. Instead of dye molecule labeling, we label DNA with a micro or nano particle. Since a particle contains millions molecule or more, the detection sensitivity can be greatly improved. During the past year, we tried to develop the technology to label DNA fragments with micro/nano magnetic particles and use a floppy drive for hybridization detection. With this approach, there is a potential to detect a single hybridized DNA duplex. PCR may not be necessary. The cost of the detection device can be less than \$1000. It can be a portable DNA detector.

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

(1) Magnetic particle production and characterization:

Since our approach is to detect hybridization based on the detection of magnetic particle, it is necessary to produce magnetic particles for DNA labeling. We have produced iron oxide and cobalt magnetic particles with laser ablation of metal and metal oxide compound in solution. In general, it takes 1 hour to produce 1 cm³ of magnetic particle colloid solution. Absorption spectroscopy has been used to estimate the size distribution of these nano magnetic particles. It was found that the size distribution is narrower with laser ablation in solution than other methods to produce nanoparticles. Precipitation can be observed when an external magnetic field is applied. It indicates that magnetic particles were successfully produced.

(2) Design construct and test a floppy drive DNA detector

We examined floppy disk drive mechanism and electronics, then designed and constructed an external amplifier circuit to process the analog signal from the disk drive circuit board. This allows us to monitor more exactly what magnetic head is sensing in real time on the disk, since the original disk drive electronics was optimized for reading digital information in a particular format and of a particular signal strength. We also designed and constructed control circuits to spin the disk and move the heads independently of a host computer. This was necessary to provide simplified operation while testing modified disks. A digital oscilloscope then can be used to examine and store the waveform produced as the disk rotates and the head steps through eighty circular tracks on the disk in concentric circles. This stored information then provides a “map” of the magnetic states on the surface of the disk. For a floppy disk with magnetic coating removed at certain selective spots, there are no magnetic materials to be detected by the magnetic sensor. When DNA attached magnetic particles are introduced through hybridization process, the same spot can be detected by the magnetic sensor.

(3) Measurements based on “repair” of magnetic film:

We have use laser ablation to clean the magnetic coating of a floppy disk. Then we subsequently applied the magnetic particles produce from laser ablation in liquid to “repair” the damage spot. We found the floppy diskette can come back to normal operation. It indicates the good feasibility that DNA can be detected by a floppy drive with magnetic particle labeling.

(4) Successful immobilization of probe DNA onto floppy surface:

The immobilization of DNA onto a Mylar surface was achieved by an alkaline hydrolysis procedure for the liberation of free carboxylic acid groups. Activation of the free carboxylic acid groups then allows the reaction of the amino group of an amino modified oligonucleotide thereby covalently immobilizing the oligonucleotide onto the surface of the Mylar.

(5) Hybridization on a floppy disk:

We have a preliminary demonstration of DNA hybridization on a floppy disk with synthetic oligonucleotide. However, the improvement of reproducibility is still needed.

Planned Activities in the coming year:

The following tasks will be pursued

- (1) Improvement of reliability of hybridization on diskette
- (2) Determination of detection sensitivity
- (3) Test hybridization on diskette with microbial DNA
- (4) Test hybridization with DNAs from soil samples
- (5) Test on quantitative microarray with floppy diskette approach.