VIII-U Fundamental Study on Electrostatic Manipulation of Biomolecules and its Application to Gene Analysis

Since conventional DNA sequencing method can determine up to 1000 base pairs at one time, longer DNA must be cut into small fragments. However, order information among these fragments is inevitably lost resulting in tremendous post sequencing process to do a puzzle. To cope with the problem, we have studied DNA sequencing method based on one-by-one DNA handling. The method includes (1) electrostatic manipulation of genomic DNA, (2) fixation in a stretched from, (3) cut from the terminus, (4) recovery and amplification of the fragments.

VIII-U-1 Manipulation of Single Coiled DNA Molecules by Laser Clustering of Microparticles

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A method of manipulating single DNA molecules for application in single-molecule analysis was developed. Manipulation of laser clustered beads allowed manipulation of a single DNA molecule without modification. Figure 1 shows sequential photographs of a DNA molecule anchored at both end to a glass surface. The DNA molecule was tweezed at one point and successfully stretched (figure1(b)–(d)). The stretched DNA molecule returned to its original form after releasing by turning off the laser beam (figure1(e)).

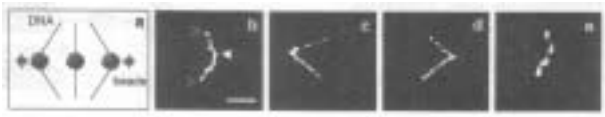

Figure 1. Sequential images of the manipulation of a single DNA molecule using laser clustering of 0.2 μm latex beads.

VIII-U-2 One-End Immobilization of Individual DNA Molecules on a Functional Hydrophobic Glass Surface

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An extremely simple technique of DNA immobilization on a hydrophobic glass surface was developed. The technique includes hydrophobic processing of a cover slip with dichlorodimethylsilane and modification of the terminus of DNA with sulfhydryl group. Dichlorodimethylsilane reacts with silanol groups on a cover slip surface (figure1(a)) and forms hydrophobic monolayer of dichlorodimethylsilane (figure1(b)). Sulfhydryl group of DNA molecule modified at one terminus reacts on the dichlorodimethylsilane layer resulting in anchoring of a single DNA molecule to the cover slip (figure1(c))

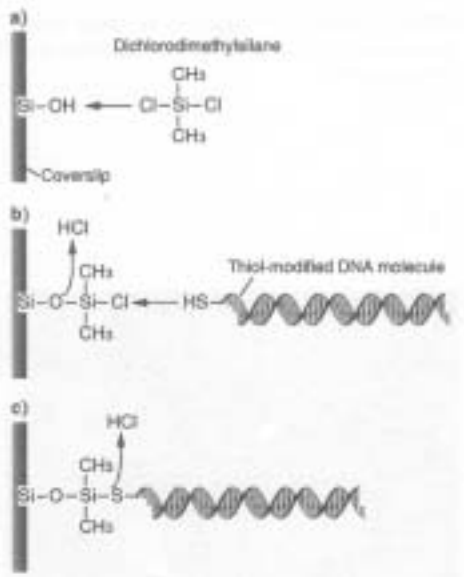

Figure 1. Schematic diagram of reaction mechanism of DNA immobilization on a dichlorodimethylsilane-coated surface.

VIII-U-3 Single-Molecule PCR using Water-in-Oil Emulsion

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[*J. Biotechnology* **102**, 117–124 (2003)]

A simple PCR method utilizing a water-in-oil (W/O) emulsion was developed. Numerous numbers of droplets serving as reaction mixture are included in bulk oil phase in this system. The method allows amplification of very low concentration DNA samples because such droplets increase effective concentration of template DNA. In this method, PCR was started with emulsified samples, in which template DNA was amplified to sufficient amount applicable to conventional PCR next step. After the first PCR in emulsified state was completed, W/O emulsion was broken by centrifuging to start the conventional PCR. By using this method consisting of 13 and 25 cycles in the first and the second step, target DNA of which concentration is 1molecule/tube was successfully amplified.

VIII-U-4 FIM Observation of DNA Molecules

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An application of field ion microscope (FIM) to DNA sequencing has been experimentally studied. By applying a DC voltage to a FIM tip on which a DNA molecule is attached, the bases may be released and carried along the electric field. Ultra high-speed genome analysis will be feasible by detecting the digested bases. We constructed a DNA sequencing system based on a field ion microscope. An electro-polished gold was used as a substrate. DNA molecules modified with sulfhydryl (-SH) group prepared by PCR (polymerase chain reaction) were attached on the needle tip. High voltage up to 7 kV was applied to DNA-bound tip to observe the FIM images. It was found that binding of DNA on samples decreased on-set voltage of FIM images suggesting the existence of an atomically rough surface.

VIII-U-5 Micro Reactor System Based on Water-in-Oil Emulsion

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A method called as “combinatory chemistry” has been proposed to create useful enzymes higher active and stable. Since the combinatory chemistry is based on multiple mutations of enzymes and evaluation of those mutants, high through-put production and analysis of enzymes is required to promote this method. For this purpose, miniaturization of reactors has been developed by using micro fabrication techniques. However, the miniaturization accompanies with difficulty of liquid handling, therefore size of reactors was limited by liquid handling technique.

To overcome this problem, we have been engaged in development of reaction system based on water-in-oil emulsion. W/O emulsion contains large amount of water droplets in continuous oil phase. Because many biological molecules are hydrophilic, those can be enclosed in the droplets. This indicates that the droplets play a role of small reactors, however reaction control of the droplets has not been established. We are developing unit operations such as transport, fusion to achieve reaction control of the droplets. In this term, we have developed transport methods based on electro-osmosis and charge injection. We have succeeded to drive the droplets by both methods. Now, we are engaged in improving controllability of droplet transport.