IX-T 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.

IX-T-1 On-Demand Mixing Droplet Spotter for Preparing Picoliter Droplets on Surfaces

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An on-demand mixing droplet spotter for generating and mixing picoliter droplet has been developed for ultrasmall reaction vessels. The droplets were generated by appling a ~500 V, ~2 ms pulsed voltage to the tips of capillary tubes (o.d. ~20 μm; i.d. ~12 μm) filled with solution. The mixing process was achieved using electrostatic force. The initial droplet was formed by applying the pulsed voltage between one capillary and the substrate, and the second jet of the other solution was generated from the other capillary and collided with the initial droplet automatically because the electric field lines concentrated on the initial droplet. Using this mixing process, a microarray having a concentration gradient was obtained by spotting ~6 pL droplets on a surface with a density of one spot per 75 × 75 μm^2.

IX-T-2 Ice-Water Interface Migration by Temperature Controlling for Stretching of DNA Molecules

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This report shows a new DNA stretching method using migration of an ice-water interface. DNA molecules were stretched accompanying the migration of the solid-liquid interface and immobilized in frozen area. This simple method needs no chemical modification to keep DNA in the stretched form. For full stretching of DNA molecules, one terminus of the DNA molecules were anchored on silanized substrate. The anchored DNA molecules were stretched by freezing the DNA solution. The stretched DNA molecules were observed after sublimation of the frozen solution keeping its stretched form on silanized surface which had no attractive interaction with DNA molecules except for the SH-modified terminus in solution. An infrared (IR) laser beam was introduced to a frozen DNA solution through an objective lens for local area melting of the solution. Scanning of the laser irradiation caused stretching and enclosing of DNA molecules in the frozen area followed by migration of the solid-liquid interface.

IX-T-3 Activation of Restriction Enzyme by Electrochemically Released Magnesium Ion

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Observation and cutting of DNA molecules at intended positions permit several new experimental methods that are completely different from conventional molecular biology methods; therefore several cutting methods have been proposed and studied. In this paper, a new cutting method for a DNA molecule by localizing the activity of a restriction enzyme is presented. Since most restriction enzymes require magnesium ions for their activation, local restriction enzyme activity can be controlled by the local concentration of magnesium ions. Applying a dc voltage to a needle electrode of metallic magnesium made it possible to control the local magnesium ion concentration at the tip of the needle. The restriction enzyme was activated only when magnesium ions were electrochemically supplied.