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Micro- and Nanofabridcated Device Technology for Single Biomolecule Analysis

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1. Introduction

Human genome sequencing has been almost completed [1], but determination of the genome sequence of many other organisms is still required, since comparative genomics allows biologists to identify conserved functional features and recognize new innovations in specific lineages. To realize the full promise of comparative genomics, however, it needs to become simple and inexpensive to sequence the genome of any organism. Microfabricated chip technology is one of the promising technology for future sequencing and more revolutionary method. Single-molecule sequencing from a specific single cell, has not yet been fully developed, but is one of our goals to develop biochemical analysis based on chip-technology.

Here we propose a novel and fundamental method toward realizing a single chromosomal DNA analysis for future chromosomal sequencing. Long dsDNA, especially chromosomal DNA, exhibits random-coiled conformation in a solution. However, to read a single DNA sequence, stretched conformation is more suitable than random-coiled one. In this paper, firstly we propose a novel method for stretching of chromosomal DNA in a miniaturized agarose gel system applying an AC electric field [2]. Secondly we suggest that a chip-based stretching method of DNA without gel matrix is available. Lastly we mention a possible application of chip-technology to be combined with other methods like optical tweezers [3]. Based on chip-technology, we aim to integrate several kinds of tools for a single molecule and a single cell manipulation on one chip.

2. Method

DNA stretching in gel

The direct observation of stretching process of a phage DNA (T4 DNA; 165.6 kbp) and a chromosomal DNA (*Saccharomyces cerevisiae*; 0.225 – 2.2 Mbp) was carried out using fluorescence microscope equipped with miniaturized gel electrophoresis set (Fig. 1). After Sample DNA were injected into working gel applying low steady electric field, we investigated effective condition for stretching DNA.

DNA stretching by chip-technology

Microfabricated devices with narrow and shallow channels were made of quartz. For stretching of T4 DNA (166 kbp, Rg = ca. 3 μm), the channel was fabricated into 5 μm in width and 0.5 μm in depth. Experimental set up was similar to one expressed in Fig. 1.

DNA manipulation by optical assembling

Optical tweezers can concentrate several microparticles into one laser spot reversibly. We call this phenomena optical assembling. The trapping capacity depends on particle size, laser power, and solvent composition etc. By optical assembling of 0.2 μm latex beads, a single DNA molecule can be manipulated in a solution. Nd:YAG laser was used for the manipulating at 400 mW, 1064 nm in wavelength.

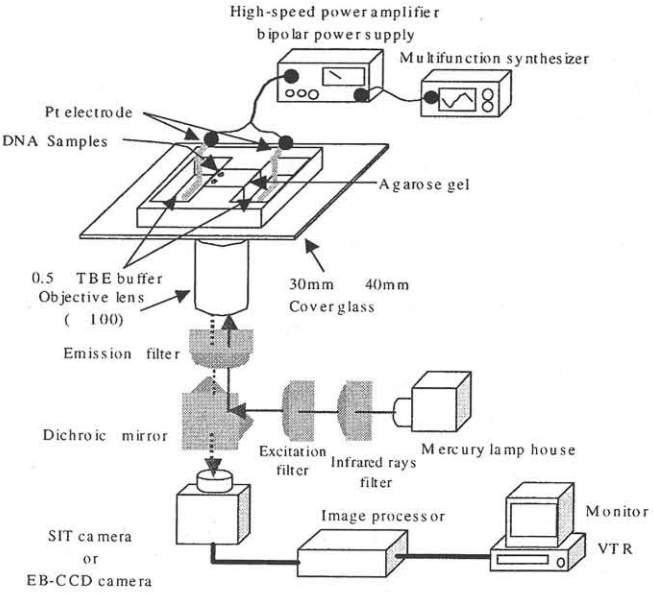

Fig.1 Schematic diagram of experimental setup.

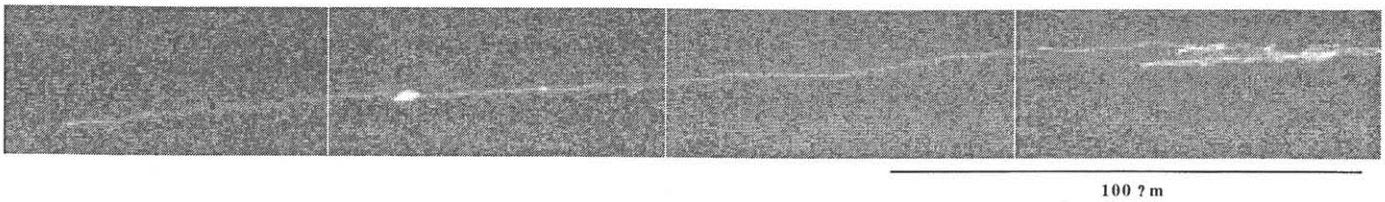

Fig. 2 Fluorescence image of *Saccharomyces cerevisiae* chromosomal DNA, 2.2 Mbp fragments, under AC field (200Vpp/cm, 10Hz) in 1% agarose gel. Coil and bent parts never solute in spite of applying AC field more than few minutes. This image was recovered from four images to obtain well-focused one.

3. Results and Discussion

DNA stretching in gel

We examined several conditions, including field frequency, field strength, concentration of gel, and different DNA fragments. The frequency is the most efficient parameter for the stretching of DNA molecule. We found applying 10 Hz sinusoidal AC electric field is extremely effective to stretch single DNA molecule in a miniaturized agarose gel.

Optimized condition was successfully applied to stretching of *Saccharomyces cerevisiae* chromosomal DNA as shown in Fig. 2. The 10 Hz sinusoidal AC electric field is remarkably suitable to stretch even over 300 μm long DNA molecule corresponding to 2.2 Mega base pairs. The method reported here is promising to contribute to more rapid and inexpensive single genome analysis for future chromosomal sequencing.

DNA stretching by chip-technology

Similar DNA stretching can be actualized on microfabricated devices under almost same electric condition to these in gel. Figure 3A and 3B show fluorescence images of T4DNA (166 kbp, Rg = ca. 3 μm) confined in the shallow straight channel (0.5 μm in depth, 5 μm in width). DNA takes a two-dimensional random conformation without an electric field (Fig. 3A). On the other hand, under a higher AC electric field (200 V/cm, 10 Hz), DNA takes a one-dimensional conformation (Fig. 3B) because of the physical confinement from the shallow channel. DNA stretching by chip-technology should be expected to overcome same demerits like Joule's heat in DNA stretching in gel.

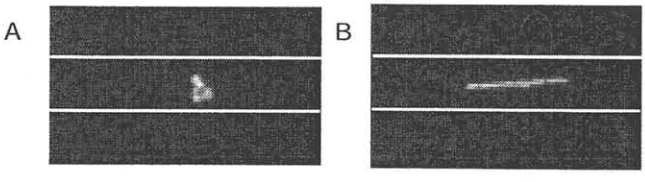

Fig. 3 Fluorescence images of T4DNA in channel (5μm in width, 0.5μm in depth). A) without an electric field. B) in an AC electric field (200V/cm, 10Hz).

DNA manipulation by laser assembling

Figure 4 shows snapshots of manipulation of a single DNA molecule by laser assembling of 0.2 μm latex beads. The laser assembling was carried out at 400mW by Nd:YAG laser, 1064nm in wavelength. The laser beam turned on over DNA molecule at which time, a single T4 phage DNA (166kbp) was grasped (Fig. 4a). When a microscope stage was moved, the held DNA molecule was manipulated following stage moved direction to upward (Fig. 4b) and leftward (Fig. 4c-d). At turned off the laser beam during manipulation, the DNA molecule could be released from trapped point immediately. This method provides simple way for manipulation of a coiled state DNA molecule.

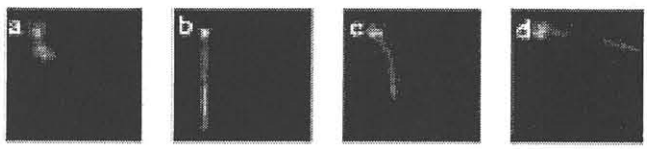

Fig. 4 Manipulation of a single T4 DNA by laser-assembling method. Without shear force (a). Microscope stage was moved to downward (b) and rightward (c-d).

4. Conclusions

We proposed a novel and fundamental method toward realizing a single chromosomal DNA analysis for future chromosomal sequencing based on chip-technology.

References

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