SIZE DEPENDENT MOBILITY OF DNA IN ELECTRIC AND HYDRO DRAG FORCE FIELD
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ABSTRACT
The migration velocities of DNA were measured in microfabricated taper-shape channels where both the hydro pressure and the electric force were applied at the same time in mutually reverse directions. If the both force were larger than a threshold, DNA was trapped near the narrowest position of the channel. In this case, the trap probability appeared to depend on DNA size; larger DNA has larger probability. On the other hand, when the both force were lower than the threshold, DNA migrated in the channel. In some conditions of this case, the migration velocity showed strong dependence on DNA size.

Keywords: DNA electrophoresis, size selection, selective trap, hydro drag

INTRODUCTION
The size separation is a basic technique for DNA analysis. Gel electrophoresis is a most popular one for this purpose and has been used for many applications successfully. However, the gel has a few weak points that it should be kept wet and is not suitable for high molecular weight DNA separation. The needs of large DNA separation are considered to be increasing in cellome field, chromosome analysis, and on-chip single cell analysis.

Recently, as a substitution of the gel, many groups reported attempts of DNA electrophoresis employing microfabricated sieving structures such as nano-pillar [1-3], entropic trap [4], curved nano-channel [5], nano-pores [6], and so on. These methods fit well to semiconductor fabrication techniques. However, most of them require very fine nano-lithography and their size separation resolutions are still not satisfactory.

At the last microTAS, we reported that DNA was trapped in a resultant force field of mutually reverse electric force and hydro drag force in micro fabricated taper-shape channels [7]. The trap is selective and so strong that it can extract and concentrate DNA from mixtures containing other substances. Therefore, the method is expected to be used for DNA pretreatment on a chip. One advance of this trap to other sieving nano-structure is that it does not always demand a fine nano-lithography. It can trap DNA by relatively wide channel, for example, 10 μm, which reduces clogging problem. Moreover, a remarkable feature of the trap is that the trap efficiency has size dependence. The larger
DNA is trapped strongly.

In this paper, we investigate the trap probability (in trap conditions) and the mobility (in non-trap condition) of DNA in the resultant force field as a function of DNA size and strengths of both forces. We found that the mobility depends strongly on DNA size even in the non-trap condition. A size separation employing the trapping field is suggested.

**EXPERIMENTAL**

Figure 1 shows the micro channel pattern fabricated on the quartz chip employing electron beam lithography, dry etching, and HF-bonding techniques. The channel is totally 5 mm long, 100 μm wide, 0.5 μm deep, and there are 8 taper-shaped channels in-line at the center. Each taper is 50 μm long, and 0.6 μm and 5 μm wide at the both ends. Figure 2 shows the experimental setup. A solution of T4 (165kbp) DNA and its fragments stained by YOYO1 in 0.5 Tris borate EDTA buffer was introduced into the chip, and the motion of each DNA was recorded by a fluorescence microscope with highly sensitive CCD video camera. The hydro drag force was generated by pressure driven flow of the buffer solution in the channel when a constant air pressure was applied to one of the inlets using a syringe and pressure gauge. The electric force was applied by Pt electrode. By the resultant force field of two different forces, DNA either migrated through or got trapped near the narrowest position in the channel. The size of DNA was estimated by microscope image, according to its length and brightness, on the assumption that T4 DNA fragments consists of ~80kbp, ~40kbp, and under 20kbp. The migrating velocity of each DNA was measured from video by image processing.
RESULTS AND DISCUSSION
When the applied forces were stronger than a threshold (3kPa, 4V), almost all DNA of T4 and its fragments were trapped. Decreasing a force field close to the threshold value resulted in releasing of some DNA from the trap. Figure 3 shows the size distribution of DNA at upstream and downstream of the trap when the strengths of electric force and hydro drag force were just above the threshold. The inlet solution contained T4 (165kbp), half size (~80kbp), and smaller ones (<40kbp). However, the outlet solution contained only smaller one (<40kbp). It clearly indicates that the size dependency of the trapping force, i.e., larger DNA has larger trapping probability.

By decreasing the two forces to below the threshold, DNA flowed by the resultant field through the taper-shape channel without stopping. Figure 4 shows the motion of a large DNA (~80kbp) and a small DNA (~20kbp) in the force condition about 20% below the threshold. Both DNA migrate with changing their velocities repeatedly; fast at the narrow position and slow at the wide position in the channel. Noteworthy, the overall migrating time has significantly large dependence on DNA size. This fact may be related to the size dependency of the trapping force on DNA size, mentioned above. Figures 5 and 6 show DNA motion in the same channel under only electric force and under only hydro drag force, through one taper in weakest force condition. Both of them do not show any size dependence. This means that the size dependence is not due to entropic trap effect [4] in this case, and that the resultant force field of two different forces is essential.
CONCLUSION
The DNA motion was investigated in resultant force field of mutually reverse electric and hydro drag force in microfabricated taper-shape channel. The trapping probability in the trap condition and migrating velocity in the non-trap conditions appears to have significantly large dependencies on DNA size. This was applicable to novel size separation methods for DNA analysis.

REFERENCES