HIGH-THROUGHPUT GENE ANALYSIS USING SUSPENDING DNA FIBERS (SDFs) ON A MICRO GLASS-PHONORECORD

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ABSTRACT

Manipulation of DNA fibers is very useful technique for gene analysis such as Fluorescent In Situ Hybridization (FISH). In conventional methods for manipulation of DNA fibers, FISH efficiency often decreases due to the tight adhesion between DNA fibers and substrates. In this paper, we propose a micro glass-phonorecord with concentric grooves for suspending DNA fibers with centrifugal force. As a result of FISH analysis with the proposed technique, FISH efficiency of suspending DNA fibers was increased five times than one of the conventional stretching DNA fibers.

KEYWORDS: micro glass-phonorecord, In Situ Hybridization, suspending DNA fibers

INTRODUCTION

DNA fibers have been known very useful for DNA analysis, such as genome mapping by FISH [1]. In conventional manipulation methods of DNA fibers such as stretching DNA with cover slip sliding [2] and electro-osmosis flow (EOF) [3], the breakage and the transform of DNA fibers are often occurred due to the tight adhesion of DNA fibers to a glass substrate [4]. Regarding the demerit, we propose a micro glass-phonorecord consisted of several groove on a grass slide to avoid the breakage and the transformation of DNA fibers due to the adhesion. To confirm the validity of the propose substrate and suspending DNA fibers (SDFs), FISH analyses were carried out on the proposed micro glass-phonorecord.

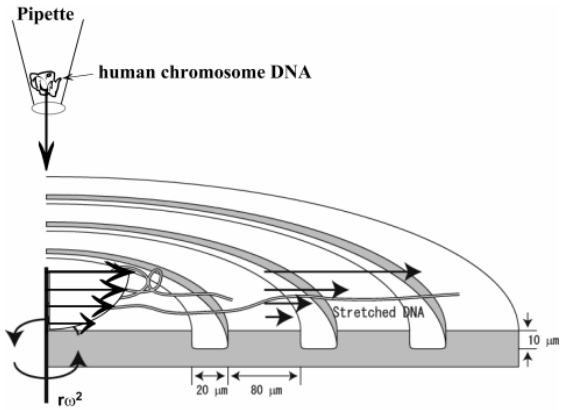

Figure 1: Schematic of stretching DNA

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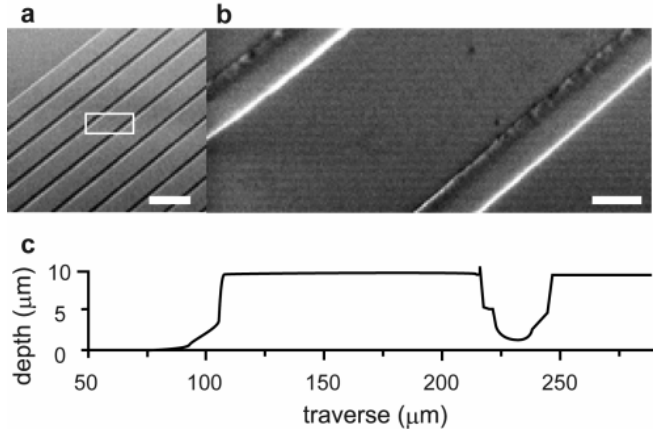


Figure2: Structure of microgrooves

EXPERIMENTAL

A proposed micro glass-phonorecord is composed of concentric micro-grooves and a circular flat surface at the center of rotation as a dropping spots of chromosomes suspension, and suspends a stretching DNA over micro-grooves by the centrifugal force of the rotation as shown in Fig.1. The micro-grooves were fabricated on a glass slide by anisotropic etching after scratching with a micro-needle as shown in Fig.2. By stretching DNA over the grooves, DNA fibers are suspended over the concave as shown in Fig.3. Then, FISH analysis of SDFs can be demonstrated by Myc/CEN-8 probe mix as shown in Fig.4.

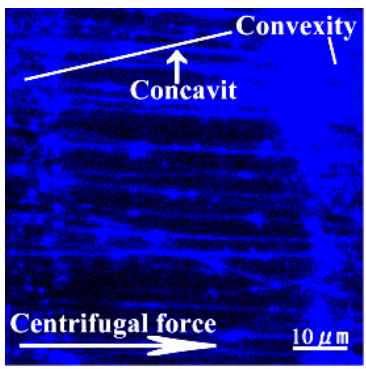


Figure 3: SDFs on the microgrooves

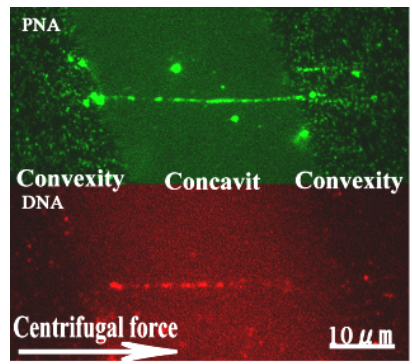
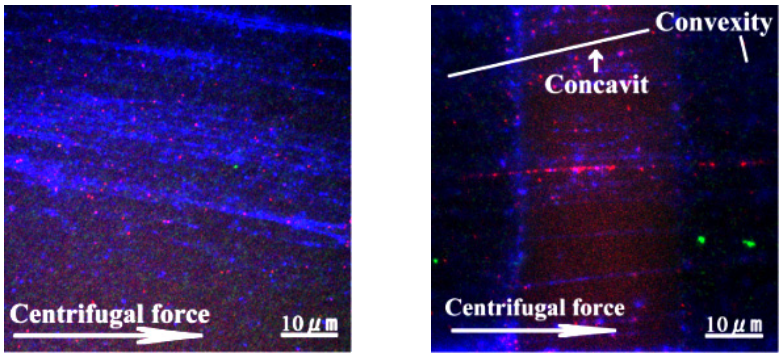


Figure 4: FISH image of SDFs

RESULTS AND DISCUSSION

FISH analysis was performed to confirm the validity of SDFs using the micro glass-phonorecord for DNA analysis as shown in Fig.5. Stretching DNA fibers on flat surface and SDFs of HeLa cells were treated by Myc/CEN-8 probe mix at a short time (one hour, normally treat over five hours). Observable labeling cannot be seen over stretching DNA fibers on the flat surface as shown in Fig.5 (a). On the other

hand, SDFs were clearly labeled with DNA probe (red signals) as shown in Fig.5 (b). Furthermore, in the case of SDFs-FISH, non-specific absorption on the glass surface is excluded from the field of vision using the vertical interval of the micro-grooves. In conclusion, it is confirmed that the proposed micro glass-phonorecord can achieve SDFs appropriate to genome mapping by FISH.



(a) Flat surface *(b) Over the microgrooves*

Figure5: FISH images of DNA fibers on flat surface and microgrooves

ACKNOWLEDGMENTS

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