SPOT-SELECTIVE DNA RECOVERY FROM DNA MICROARRAY CHIPS FOR ON-CHIP DIRECTED EVOLUTION

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ABSTRACT

A novel class of DNA chips, on which immobilized DNA molecules are selectively recoverable, was developed for the directed evolution of biomolecules. DNA molecules immobilized on a gold surface via a DNA linker, which contains a nitrobenzyl group as a photocleavable moiety, were recovered from each chip by spot-selective photoirradiation, and the recovered DNA molecules were successfully amplified by PCR.

KEYWORDS

Protein chip, DNA chip, High through put screening, Photo cleavage, On-chip screening

INTRODUCTION

Directed evolution is a powerful tool to create functional biomolecules and is expected to be performed more rapidly and effectively using a microchip device. Figure 1 shows the concept of directed evolution using a microchip device. Firstly, a mutant library of DNA is prepared and distributed on a chip. Secondly, the functions or activities of the DNAs or DNA-protein conjugates, which are synthesized on the chip [1], are evaluated. Thirdly, DNAs or DNA-protein conjugates with high functions or activities are collected from the chip. Finally, the collected DNA molecules are amplified with mutation to prepare the mutant DNA library for the next round of on-chip selection. Functional molecules could be created by these subsequent on-chip selection rounds. In these procedures, the recovery of DNA from a chip is essential, but the method has not yet been developed.

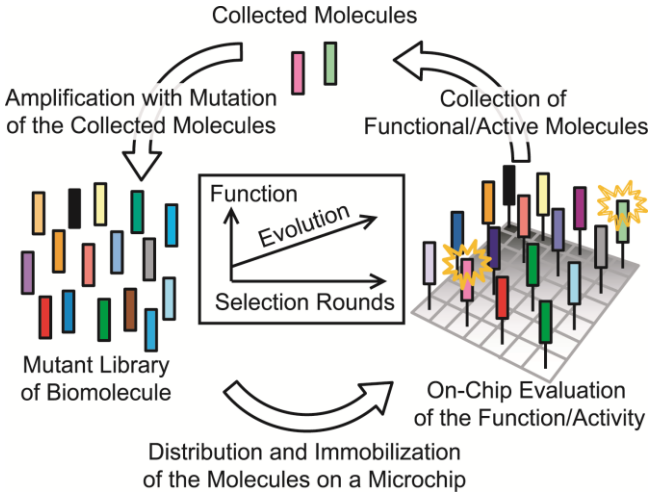

Figure 1. Concept of on-chip directed evolution. Functional molecules are evolved by performing subsequent selection steps.

EXPERIMENTAL

Figure 2 shows the experimental procedures used in this study. A photocleavable DNA linker (PC-linker, details are shown in Figure 3) and 6-mercaptohexanol (MCH) were immobilized on a gold surface. Cy5-modified mRNA was hybridized on the PC-linker in a micrometer-scale spot pattern, as previously reported [1], and the mRNA was reverse-transcribed on the chip to make a spot pattern of Cy3-modified cDNA. The cDNA patterned substrate was covered with a 3-aminopropyltriethoxysilane (APTES)-modified glass and 377-nm laser irradiation was performed on selected spots. cDNA molecules detached via photocleavage were adsorbed onto the APTES substrate and the adsorbed cDNA molecules were recovered and amplified by PCR.

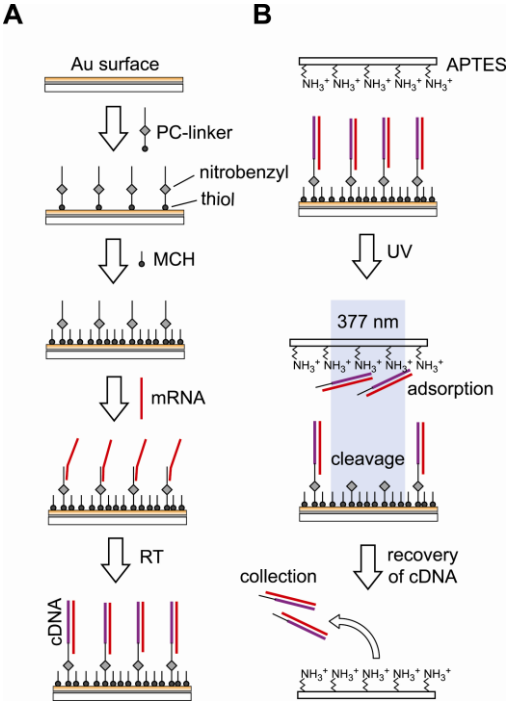

Figure 2. Experimental procedure.
A. preparation of SAM of cDNA on Au surface.
B. photocleavage and recovery of cDNA.

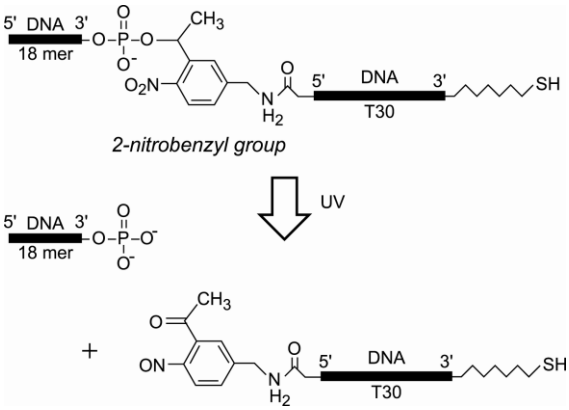

Figure 3. Structure of photocleavable DNA linker (PC-linker). A 2-nitrobenzyl group is easily cleaved by UV irradiation.

RESULTS AND DISCUSSION

Prior to the photorecovery experiment, the adsorption of DNA on the APTES substrate was investigated in a series of buffers with different pHs. Figure 4 shows the result of the adsorption of fluorescence-modified DNA in different pH buffer solutions dropped onto the APTES surface. DNA was efficiently adsorbed at pHs below 10 and hardly adsorbed at pHs 10 and 11. Therefore, we used the buffer of pH 7.4 as the adsorption solution and the buffer of pH 10 as the desorption solution.

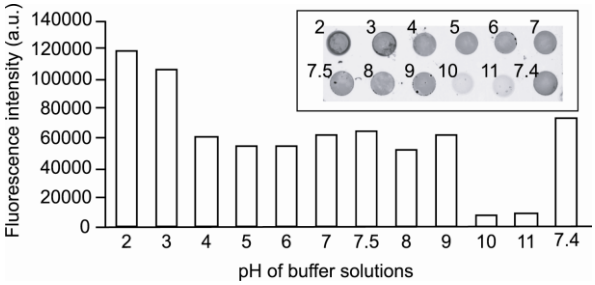

Figure 4. Adsorption of DNA onto APTES substrate in a series of buffers with different pHs. Inset; fluorescence image of adsorbed Cy5-modified DNA.

A micropattern of a 50-µm-diameter spot of Cy3-modified cDNA was generated, and the spot-selective photocleavage and adsorption of the cDNA on the APTES substrate were successfully achieved in the adsorption solution (Figure 5). Finally, the cDNA molecules recovered from the APTES surface using the desorption solution were amplified by PCR. As a result, the sample recovered from the photoirradiated area of the APTES substrate was highly amplified compared with the sample recovered from the nonirradiated area (Figure 6). This result indicates that cDNA molecules were successfully recovered spot-selectively without significant damage.

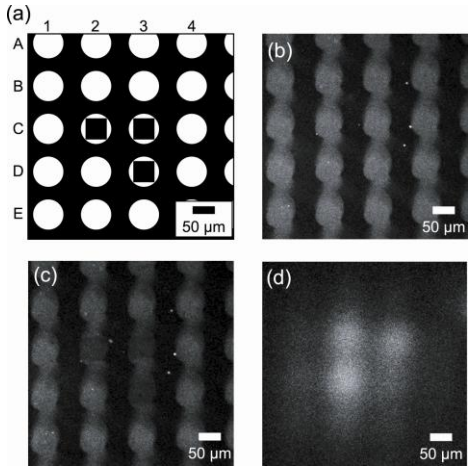

Figure 5. Result of spot-selective photocleavage and recovery of micropatterned cDNA. Three spots (C2, C3, and D3) were irradiated by 377 nm laser (a). (b) preirradiaton, (c) postirradiation, and (d) cDNA adsorbed onto APTES substrate.

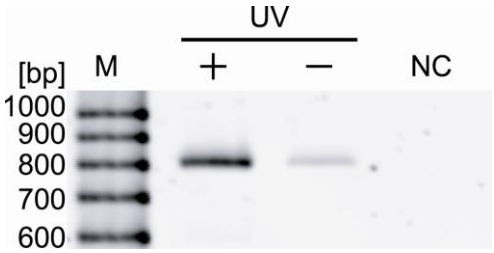

Figure 6. Result of PCR amplification of recovered cDNA. The cDNA recovered from the irradiated area of the APTES substrate (UV+) was more highly amplified than that from the nonirradiated area (UV-). NC: negative control without template DNA.

CONCLUSION

A novel DNA chip with a function of photoassisted spot-selective DNA recovery has been developed. The micro spot array of DNA was fabricated on a gold surface via a photocleavable linker, which contains a nitrobenzyl group as a photocleavable moiety. These DNAs were detached and recovered from the gold surface by spot-selective photoirradiation and amplified by PCR successfully. This novel DNA chip is expected to be used for on-chip directed evolution.

ACKNOWLEDGEMENTS

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