Development of DNA-arrayed column for sensitive and selective analysis of DNA

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ABSTRACT
Quantitative separation of target DNA molecules was performed by DNA-arrayed silica capillary column on the basis of base pairing interaction of nucleic acids. We prepared DNA-arrayed silica capillary column by conjugating 5'-aminoethoxy oligonucleotide (probe) on inner surface of the capillary column pre-treated with 3-aminopropyltriethoxysilane (APS) and disuccinimidyl glutarate (DSG) cross-linker. Sufficient resolution was observed by controlling of target-DNAs concentration, salt gradients, and temperature gradients. Finally, we succeeded to separate two different DNA targets even with same melting temperature according to their own concentrations. These results would be useful for developing quantitative analysis of cDNA, which is related to mRNA levels in biological sample.

INTRODUCTION
Nowadays, the requirements of a technique used for nucleic acids detection generally include high resolution, fast analysis, high sensitivity, small sample consumption, and ease of coupling with substrate12. For analyzing mRNA levels in biological sample, cDNA pool is usually employed by converting mRNA to cDNA by RT-PCR. Therefore, quantitative analysis method of cDNA is strongly required to be established for accurate investigation of mRNA levels. Up to now, DNA microarray has been the mostly-used method for analyzing cDNA pool. The principle of this detection method is based on hybridizing interaction between probe DNA attached on the solid support and target cDNA, which is complementary to probe DNA. However, DNA chip technology possesses some limitations in target cDNA molecule detection such as non-specific adsorption of target cDNA, variances in quantity and area of the spotting probe DNA, non-linear relationship between fluorescence intensity and sample amount. These problems make it difficult to analyze cDNA level quantitatively and accurately.

Affinity-chromatographic methods have often been exploited for selective separation of single strand DNA or mRNA. The discovery, that most of the mRNA from various animal tissues contains poly-adenine sequences (ploy(A)), has prompted a valuable procedure for the isolation of poly(A)-containing mRNA by poly(T)-containing affinity chromatography9,10. Recently, rapid progress in micro- and nano-scale technologies could enable us to fabricate affinity-chromatographic miniatures for separation of biomolecules in minimum volume of sample. Moreover, silica capillary column with 20-100 μm of inner diameter and long length can be employed as good substrate material for developing efficient micro- and nano-scale affinity-chromatographic system.

In this study, we considered affinity-chromatographic analysis as one of promising methods alternative to DNA chip technology for quantitative analysis of DNA samples. Especially, DNA-arrayed capillary column was prepared and employed for sensitive and selective analysis of target DNA molecules. We also optimized temperature, salt concentration and flow rates for quantitative detection and separation of target DNA molecules.

RESULTS AND DISCUSSION
Fabrication of DNA-arrayed column
DNA-arrayed capillary columns were prepared by using the following procedure; bare silica columns were treated with H2O2/NH4OH/H2O (1:1:5 v/v/v) and H2O2/HCl/H2O (1:1:5 v/v/v), washed and dried7. Then, the capillary was treated with 3-aminopropyltriethoxysilane (APS) in toluene, washed and dried. Capping-treatment was additionally performed by following the same silanization procedure as mentioned above except that butyltrimethoxysilane (BMS) was used in place of APS5. The APS-modified capillary was further treated with disuccinimidyl glutarate (DSG) cross-linker in DMSO. Finally, the APS/DSG-treated capillary column was filled with oligodeoxynucleotide Probe 1 and Probe 2 solution (Table 1), sealed at both ends and allowed to stand at 37°C for 24 hrs. As shown in Figure 1, DNA-arrayed capillary column was prepared.
Figure 1. Schematic diagram of DNA-arrayed capillary column.

Table 1. Oligonucleotide sequences (probes and targets) used in this work.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Probe 1</td>
<td>5’-Amine-TGTTGCTGAAAAATGTCAAGC-3’</td>
</tr>
<tr>
<td>Probe 2</td>
<td>5’-Amine-ATGGCGTGATCGTTGTCCA-3’</td>
</tr>
<tr>
<td>Target-1</td>
<td>5’-CGTTGACATTTTCGACAA-3’ (for the probe1)</td>
</tr>
<tr>
<td>Target-2</td>
<td>5’-TTGAACAGATCGACGACAT-3’ (for the probe2)</td>
</tr>
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Separation of two different DNA targets

Chromatogram in Figure 2A showed that the targets (Target 1 and Target 2 (Table 1)) eluted at nearly 1 min without affinity-interaction with arrayed-probe DNA: flow rate; 10 µL/min, eluent; 10mM sodium phosphate buffer containing 100 mM NaCl; temp.; 55°C. By adding the 5 mM MgCl2 to the buffer, the targets eluted at nearly 8 min as presented in Figure 2B, but only one peak was observed. However, when the different concentration sample of Target 1 (1 µM) and Target 2 (10 µM) was employed in the above condition, we observed two different peaks at 7 min and 8 min, respectively, as shown in Figure 2C. These results showed that the lower concentration target (Target 1) elutes early than the high concentration target (Target 2) because the lower concentration duplexes have the lower melting temperature in microscopic equilibrium.

CONCLUSION

We prepared DNA-arrayed capillary column for sensitive and selective analysis of target DNA. Using this developed column, we successfully demonstrated that target DNAs can be separated on DNA-arrayed column according to the target DNA’s concentration, which are thermodynamically correlated with melting temperature. This approach will be useful for quantitative separation of cDNA for analyzing the mRNA levels in biological samples.

Figure 2. Separation of Target 1 and Target 2. Analytical conditions: column, DNA-arrayed capillary column (100 µm i.d. and 20cm length) with Probe 1 and Probe 2 (each 18 µM); running buffer, 10mM sodium phosphate and 100mM NaCl2 (A), 10mM sodium phosphate and 5mM MgCl2 (B and C); sample concentration, Target 1 (5 µM) and Target 2 (5 µM) (A and B), Target 1 (1 µM) and Target 2 (10 µM) (C); sample volume, 20 nL; temperature, 55°C.

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