Oligonucleotide chip for the diagnosis of HNF-1α mutations

Hyun Gyu Park a, *, Hyun Ok Ham a, Kyung Hee Kim a, Nam Huh b

a Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-ku, Daejeon 305-701, Republic of Korea

b Biochip Project Team, Samsung Advanced Institute of Technology, Suwon 440-600, Republic of Korea

Received 21 December 2004; received in revised form 23 December 2004; accepted 23 December 2004

Abstract

Mutations in HNF-1α cause maturity-onset diabetes of the young (MODY) type 3, which is the most prevalent MODY subtype in most countries. In the present study, we investigated an oligonucleotide microchip for the detection of the known HNF-1α mutations. We first optimized the coupling chemistries for covalent immobilization of allele-specific oligonucleotides on aldehyde (CHO)- and thiocyanate (NCS)-activated glass slides and compared their hybridization efficiencies. CHO-glass was found to provide a more favorable environment for hybridization than NCS-glass, whereas the binding capacity of NCS-glass for amine-activated oligonucleotide was much greater than with CHO-glass. We also investigated the effects of the length of the capture probes on the hybridized signals. To determine the presence of HNF-1α mutations in a human sample, we prepared an oligonucleotide chip from selected mutation sites of exon2 from HNF-1α. Cy3-labeled RNA target probes were obtained by in vitro transcription of promoter-tagged PCR products from a wild-type blood sample and subsequent fragmentation. Hybridization of the chip with the RNA target probes successfully identified all of the genotypes for the tested sites. This work demonstrates that oligonucleotide chip-based analysis is a good candidate for routine clinical testing for HNF-1α mutations.

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Keywords: Oligonucleotide chip; HNF; MODY; Mutation detection

1. Introduction

Maturity-onset diabetes of the young (MODY) is a monogenic form of type 2 diabetes characterized by early onset, usually before 25 years of age. This contrasts with most cases of type 2 diabetes which often appear in overweight and middle-aged individuals. At least six MODY genes, termed MODY1 to MODY6, have been genetically mapped to human chromosomes (Fajans et al., 2001).

Of these, MODY3 is the most prevalent in most countries, including Western and Asian countries (Fajans et al., 2001; Iwatsuki et al., 1997), although the distributions vary in different populations (Velho et al., 1997). MODY3 is characterized by a severe insulin secretory defect. MODY3 results from mutations in the gene encoding hepatocyte nuclear factor (HNF)-1α, which is located on chromosome 12 and is composed of 3 functional domains and 10 exons. To date, more than 100 different mutations have been identified in the promoter or coding regions of HNF-1α (Fajans et al., 2001; Ellard, 2000). Conventional methods to diagnose such human genetic mutations include single strand conformation polymorphism assay, heteroduplex analysis, and direct nucleotide sequencing alone or in combination with denaturing high performance liquid chromatography (Castilla et al., 1994; Friedman et al., 1994; Simard et al., 1994; Gayther et al., 1995, 1996; Hogervorst et al., 1995). These methods, however, may not be suitable for the routine detection of DNA mutations because they are relatively time-consuming, expensive, and technically demanding.

An alternative method that can resolve these problems is DNA microchip technology (Pirrung, 2002; Wang, 2000). A DNA chip, which is suitable for targeted analysis of defined genetic sites, can be prepared by robotic deposition of
allelic-specific oligonucleotides on an appropriately derivatized support (Lockhart et al., 1996; Schena et al., 1996). Use of a suitably labeled nucleotide in PCR amplification of target samples yields a labeled target strand, which can then be used directly in hybridization to the support-bound oligonucleotides. With an appropriate selection of oligonucleotide capture probes and hybridization conditions, it is possible to discriminate between a wild-type and a mutant sample differing by only a single base change (Waldmuller et al., 2002; Hacia et al., 1996; Zhou et al., 2002; Cowie et al., 2004).

For the preparation of DNA microchip, the surface chemistry plays an important role in the overall performance of the chip and various immobilization schemes have been developed (Lucarelli et al., 2004). The coupling schemes can be generally categorized into noncovalent methods (Ivanova et al., 2004; Chiu et al., 2003; Taylor et al., 2003; Belosludtsev et al., 2001; Diehl et al., 2001) and covalent methods (Taylor et al., 2003; Chiu et al., 2003; Zammartite et al., 2000; Ivanova et al., 2004; Berre et al., 2003; Fixe et al., 2004; Beier and Hohesei, 1999). Noncovalent strategies have a drawback that DNA probes bound to a solid substrate are susceptible to detachment from the surface under harsh hybridization conditions and, therefore, covalent binding methods are preferred due to their chemical stability. UV mediated covalent cross-linking (Kimura et al., 2004; Duggan et al., 1999; Taylor et al., 2003; Belosludtsev et al., 2001; Diehl et al., 2001) and silanated (amine) glass slides were from CEL-associates (Houston, TX). All other chemicals used in this work were of analytical grade.

2.2. Oligonucleotides

Oligonucleotides were synthesized and purified on SDS-PAGE by Bioneer (Daejeon, Korea). Allele-specific oligonucleotide probes used in the fabrication of the HNF-1α microarray were 5′ aminoethyl-terminated. Synthetic target probes were 25 bases long and were conjugated with Cy3 dye at their 5′ ends. For all probes, the mutation points were located in their central positions. For the optimization of immobilization buffer, we used oligonucleotides terminated with both a 5′ aminoethyl group and a 3′ Cy3 group. All oligonucleotides were quantified by measuring the absorbance at 260 nm based on the calculated molar extinction coefficient, and their identities were confirmed by MALDI-TOF.

2.3. Preparation of NCS-activated glass slides

Silanated glass slides were treated for 2 h with a solution of 0.2% 1,4-phenylene diisothiocyanate solution in 10% pyridine/dimethyl formamide, washed with methanol and acetone, and dried under vacuum. The activated glass slides were stored at 4 °C in a vacuum dessicator containing anhydrous calcium chloride until use.

2.4. Immobilization of amminated oligonucleotides on CHO- or NCS-activated glass

Aminated capture probes were diluted to a final concentration of typically 25 μM in an appropriate buffer, printed on activated glass using a Pixys5500 microspotter (Cartesian Technologies, Irvine, CA) according to the manufacturer’s instructions, and allowed to covalently bond to the glass surface overnight in a humid chamber. To verify the reproducibility, four or nine spots were printed for each capture probe on the same glass surface. The final buffer concentrations were 100 mM and the pH of buffer solutions was adjusted by adding diluted HCl or NaOH solution. For CHO-activated glass, the printed glass was washed twice with 0.2% SDS and twice with distilled water, submerged in water at 95–100 °C for 2 min, and transferred into a sodium borohydride solution (1.0 g NaBH₄ dissolved in 300 ml of PBS and 100 ml of 100% ethanol) for 5 min at room temperature to reduce Shiff bases and free aldehydes. The slides were then rinsed three times.
with 0.2% SDS and twice with distilled water. In the case of NCS-activated glass, the printed glass was washed twice with 0.2% SDS and once with distilled water. Both types of slide were air dried at room temperature and stored at 4 °C in a vacuum dessicator until use. The spots were 100–150 μm in diameter, and the volume dispensed was about 1 nl.

2.5. Genomic DNA isolation and nucleotide sequencing

Genomic DNA was isolated from blood of an apparently healthy subject using a genomic DNA extraction kit (Qiagen, Hilden, Germany). After PCR amplification using an appropriate primer set, direct sequencing was performed using an ABI Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions, followed by analysis on an ABI3700 DNA sequencer (Applied Biosystems).

2.6. PCR amplification of the exon2 region of HNF-1a gene

In vitro transcription templates were generated by PCR amplification of exon2 region of HNF-1a from genomic DNA using a forward primer containing the T7 promoter sequence (E2F-T7) and a reverse primer (E2R). The sequence for E2F-T7 was 5′-taatacgactcactatagggCGAAGATGGTCAAGTCCTACCT-3′ (lower case letters indicate the bases for the T7 promoter sequence) and a reverse primer (E2R) was 5′-GCCACCTCTCGCTGCTTGC-3′. PCR was performed in a 50 μl volume containing 50 ng of the purified genomic DNA, 50 mM Tris–HCl, pH 8.3, 40 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTP (Boehringer Mannheim, Mannheim, Germany), and 2.5 U Taq DNA polymerase (Boehringer Mannheim). The reaction tubes were heated to 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and then a final 5 min extension at 72 °C. After the amplification reaction, the PCR products were purified with a Qiaquick purification kit (Qiagen) and fragmented RNA transcript was adjusted to a final concentration of 95 μM MgCl2 and incubating for 5 min at 94 °C.

2.8. Hybridization and scanning

With the synthesized target probe or the RNA target probe from a real sample, hybridization on the MODY3 chip was performed for 12 h at 37 °C in 40 μl of solution placed between the slide and a coverslip within a closed humid chamber. The hybridization solution with the synthetic probe or the fragmented RNA transcript was adjusted to a final concentration of 6× SSPE (0.9 M NaCl, 60 mM Na2HPO4, 6 mM EDTA [pH 8.0]) containing 0.1% (v/v) Triton X-100. Hybridization was followed by washing once for 5 min on a rotating mixer with 6× SSPE containing 0.005% (v/v) Triton X-100 and then once with 3× SSPE containing 0.005% (v/v) Triton X-100. After drying the slide for 15 min at room temperature, image data was processed using a confocal chip scanner (Scan Array 5000 from GSI Lumonics, Billerica, MA) with a typical laser power of 90 and a PMT gain of 95.

3. Results and discussion

3.1. Support chemistry and optimization of the immobilization conditions

Many solid supports can be used for DNA chips, and several technical issues must be considered in choosing an appropriate support. The issues include the level of background fluorescence and light scattering, the degree of nonspecific binding, and the amenability to chemical modification. In the present study, glass was chosen as a solid support because it has good properties for fluorescence detection and because silane surface modification chemistry is well developed (Rogers et al., 1999; Halliwell and Cass, 2001; Chiu et al., 2003). With the glass support, the next aspect is to choose or develop the coupling chemistry for linking the capture oligonucleotides to the support, which is one of the key factors determining the efficiency of the DNA chip. In our preliminary experiments, the other immobilization schemes, including ionic bonding of negatively charged oligonucleotides to positively charged aminated glass and UV-crosslinking to polysine-coated glass, were also evaluated. For both schemes, comparable immobilization efficiencies were obtained in comparison with the covalent bonding scheme using CHO-glass, but their experimental variations of the resulting hybridized signals were much greater (30%) than the two terminal-linking schemes using CHO- or NCS-glass (<10%), presumably due to the unpredictable detachment of the DNA probes or/and different binding modes of the probes at multiple sites. Therefore, the terminal-linking strategies are considered to be suitable for the preparation of oligonucleotide microarray and
Fig. 1. Diagram of covalent linkage chemistries employed for the attachment of the aminated capture probe DNA to the CHO-activated glass (A) and NCS-activated glass (B).

Further studies were performed for the two strategies. The two schemes are depicted in Fig. 1. CHO-activated glass was purchased from commercial sources, and NCS-activated glass was generated by chemical activation of silanated glass slides with 1,4-phenylene diisothiocyanate (Guo et al., 1994).

We next optimized the conditions for covalently linking aminated oligonucleotides to CHO- and NCS-activated glass, and we compared their binding capacities. Throughout this study, antisense oligonucleotides corresponding to the sequences in Table 1 were immobilized as capture probes. Wild-type probes of 15 mer length (2-1 and 2-4 in Table 1) were used as model capture probes for this experiment. Because the capture probes were 5′ aminohexyl-terminated and labeled at their 3′ ends with Cy3, their binding capacities could be directly evaluated just after immobilization without any further hybridization reaction. To determine the best buffer conditions for the two coupling chemistries, we immobilized the capture probes in various buffer solutions and detected the Cy3 fluorescence. The results in Table 2 show that pH 9.0 bicarbonate buffer is the best for NCS-activated glass, whereas the binding capacity on CHO-activated glass was the greatest in pH 9.5 bicarbonate buffer.

Furthermore, the background signal of NCS-glass was much lower than that of CHO-glass, presumably due to its lower hydrophilicity. When optimized with respect to a perfectly matched signal, the resulting signal to noise ratio of NCS-glass was 162.5, and that of CHO-glass was 15.6. Due to the hydrophobic nature of the NCS-glass, the spots on the glass formed well-defined circles that were smaller in diameter than those on CHO-glass. These results indicate that immobilization of aminated DNA on an NCS-modified glass surface could be a good choice when considering only the binding capacity.

Table 1 HNF-1α mutations represented on the MODY3 chip

<table>
<thead>
<tr>
<th>Probe number</th>
<th>Region</th>
<th>Sense sequence (5′ ↔ 3′)</th>
<th>Mutation effect</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Exon2</td>
<td>AAGTCCTAGCTGAGCAG</td>
<td>Y122C</td>
<td>1794</td>
</tr>
<tr>
<td>2-2</td>
<td>Exon2</td>
<td>CCACACGCGGAAGCTGGT</td>
<td>R133Q</td>
<td>1821</td>
</tr>
<tr>
<td>2-3</td>
<td>Exon2</td>
<td>CCCACAGCGGAAGCTGGT</td>
<td>R131W</td>
<td>1820</td>
</tr>
<tr>
<td>2-4</td>
<td>Exon2</td>
<td>AAACATGCTCCAGCGTT</td>
<td>S142F</td>
<td>1854</td>
</tr>
<tr>
<td>2-5</td>
<td>Exon2</td>
<td>CACACGCGGGCCGCC</td>
<td>R159Q</td>
<td>1905</td>
</tr>
<tr>
<td>2-6</td>
<td>Exon2</td>
<td>CAAGCGGCGGAGCTGG</td>
<td>R171X</td>
<td>1940</td>
</tr>
<tr>
<td>3</td>
<td>Exon3</td>
<td>GAGGAGGCGAGAGAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Exon4</td>
<td>CTGGCTCGGTTGGGGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Optimization of immobilization buffer conditions for different coupling chemistries

<table>
<thead>
<tr>
<th>Buffer</th>
<th>CHO-glass</th>
<th>NCS-glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background signal</td>
<td>500</td>
<td>120</td>
</tr>
<tr>
<td>(1) MES, pH 6.5</td>
<td>4200</td>
<td>4800</td>
</tr>
<tr>
<td>(2) Distilled water</td>
<td>5200</td>
<td>7300</td>
</tr>
<tr>
<td>(3) 3× SSC, pH 7.0</td>
<td>6000</td>
<td>12000</td>
</tr>
<tr>
<td>(4) NaHCO₃, pH 9.0</td>
<td>7300</td>
<td>19500</td>
</tr>
<tr>
<td>(5) NaHCO₃, pH 9.5</td>
<td>7800</td>
<td>16400</td>
</tr>
<tr>
<td>(6) NaHCO₃, pH 10.0</td>
<td>7600</td>
<td>13900</td>
</tr>
</tbody>
</table>

* The two wild-type capture probes 2-1 and 2-4 in Table 1 were immobilized to the glass surfaces in a final concentration of 5×10² M. Both probes have a 5′ aminohexyl group and a 3′ Cy3 group. The immobilized slides were scanned and the signal intensities for the two probes were averaged. The experiments were repeated three times, and the results were averaged. The coefficients of variation were less than 10%.

* MES = 2-(N-morpholino) ethanesulfonic acid.

* 3× SSC: 0.45 M NaCl, 45 mM C₆H₅Na₃O₇·2H₂O (pH 8.0).
3.2. Hybridization efficiency

The binding capacity, however, is not the only factor important for the final hybridization signal; the accessibility of the immobilized capture probes to a target sample DNA should also be considered. Therefore, we also prepared DNA microchips with the same two wild-type capture probes (2-1 and 2-4 in Table 1), although these capture probes were only 5'-aminohexyl-terminated and were not labeled with Cy3. Using the two coupling chemistries, the probes were immobilized under the respective optimized buffer conditions and subsequently hybridized with Cy3-labeled target probe. Interestingly, the hybridized signal with CHO-glass was comparable to that with NCS-glass, even though our initial experiments showed that the binding capacity of CHO-glass was much lower than that of NCS-glass. This result was reproducible, and it is probably due to the CHO-glass providing a more favorable environment for hybridization than the NCS-glass. CHO-glass is more hydrophilic than that of NCS-glass, and it likely provides the target probes with better accessibility. Moreover, the high surface density of the NCS-glass might cause steric interference between the covalently immobilized probes, impeding interaction with the target DNA and resulting in diminished signal intensity. Because the final hybridized signals of the two kinds of glass are similar, we employed both of the coupling chemistries for further experiments.

3.3. Selection of probes

The design of capture probe oligonucleotides is critical for the ability of the DNA chip to reliably detect human genetic mutations such as MODY3. The ability to discriminate single base differences between a wild-type and mutant sample is largely determined by the nature of the capture probes. We limited the scope of our study to single base mutations, and all the capture probes were designed to have their mutation points in their central positions. Using a Cy3-labeled synthetic target probe complementary to the wild-type capture probe, we investigated the effects of capture probe length on the hybridized signal intensity and specificity for each mutation site. We used capture probe pairs (wild-type and mutant probes) of different lengths for 2-1 and 2-4 in Table 1. For the evaluation of the specificity, signal intensities obtained with wild-type probes (perfectly matched probe) were compared to the respective intensities obtained with corresponding mutant probes (single-base mismatched probe). The discrimination ratio ($Q_{pm}$) for a mutation site was defined as the ratio of the hybridization signal intensity for the wild-type capture probe to that for the mutant capture probe, and it was used to indicate the ability to determine the correct genotype at the site. To verify the reproducibility, the experiments were performed with both CHO- and NCS-activated glass, and their results were averaged. Of three pairs of capture probes (15, 21, and 25 mer), the hybridized signal intensity was stronger for the longer probes (Table 3 and Fig. 3). However, the discrimination ratio was greatest for the shortest probe (15 mer). Because the specificity was considered to be more important than the detection sensitivity, we adopted the 15 mer probes for the generation of a MODY3 chip for diagnosing real human samples.

Table 3

<table>
<thead>
<tr>
<th>Length of capture probe (mer)</th>
<th>Relative signal intensity</th>
<th>$Q_{pm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>3.45</td>
</tr>
<tr>
<td>21</td>
<td>1.26</td>
<td>2.23</td>
</tr>
<tr>
<td>25</td>
<td>1.60</td>
<td>1.62</td>
</tr>
</tbody>
</table>

*a Wild-type and mutant capture probes 2-1 and 2-4 in Table 1 were immobilized on CHO- and NCS-glass at the corresponding optimum buffer conditions and hybridized with 10 nM of complementary synthetic target probes labeled with Cy3 at their 5' ends. Signals from the two types of glass slides were used to give the relative signal intensities and $Q_{pm}$ values. The experiments were repeated three times and the results were averaged. Coefficients of variation (CV) were less than 10%.*
3.4. MODY3 chip-based diagnosis

We next examined the ability of the oligonucleotide chip-based assay to simultaneously detect six mutations in exon2 of HNF-1α. For the chip, wild-type, mutant, and two negative control sequences for each mutation site in Table 1 were immobilized on NCS-activated glass, and each capture probe was located in a defined position on the glass surface. The two control probe sequences are the same as the wild-type or mutant capture probe sequences except for a substitution at the mutation site. Excluding the two bases corresponding to the wild-type and mutant sequence, they have one of the other two possible bases at that position. Capture probes for two unrelated sites, containing sequences from exon3 and exon4 (probes 3 and 4 in Table 1), were also included as negative controls.

Genomic DNA was isolated from the blood of an apparently healthy female, and direct nucleotide sequencing confirmed that it has wild genotypes for all of the six different HNF-1α mutation sites. Using the promoter-tagged PCR products of the genomic region, Cy3-labeled RNA target probes were prepared by in vitro transcription, followed by fragmentation to give probes with an average length of 50 bases. Alternately, a DNA-based strategy can be employed to generate the labeled target products. The RNA probe, however, has some great advantages over DNA capture probe: (1) because RNA is single-stranded, the target strands cannot rehybridize with the original complementary strands in the solution instead of the capture strand on the glass surface; and (2) because the size of fragmented RNA target probes can be controlled much better than DNA probes, RNA probes with uniform length can be consistently obtained. Therefore, although an extra step of transcription is required, we employed an RNA target probe in this study because it enhances the sensitivity and consistency of the DNA chip.

When the MODY3 chip analysis was performed with the RNA target probes, all the $Q_{pm}$ values were greater than 1, indicating correct identification of the genotypes for all six mutation sites (Table 4). The discrimination ratios for each genotype were different, ranging from 1.7 to 7.4. The signal intensities for the two control probes for each site, which also differed from the wild-type sequence by only a single base at the mutation site, were similar with those of the corresponding mutant probe (Table 4). As expected the intensities of the hybridization signals for negative controls (exon3 and exon4 probes) were much lower than those of the capture probes for exon2 (Fig. 4), indicating that the signals were caused by the specific hybridization between complementary sequences. Three independent experiments all conducted in a blind manner reproducibly showed the same results.

Although the correct determinations for all of the mutation sites were achieved with the DNA chip analysis, discriminations between perfectly matched and single-base mismatched duplexes were not as desirable, especially for probes 2-2 and 2-5. For the two probe pairs, $Q_{pm}$ values lower than 2.0 were observed, whereas much higher values (>7.0) were reproducibly observed with the probe pairs for 2-1 and 2-4. It is not currently clear why the $Q_{pm}$ values for the same mutation type are so different from each other.

<table>
<thead>
<tr>
<th>Probe number</th>
<th>$Q_{pm}$</th>
<th>Ratio of perfectly matched signal to negative control signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>7.4</td>
<td>7.5</td>
</tr>
<tr>
<td>2-2</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>2-3</td>
<td>4.5</td>
<td>3.2</td>
</tr>
<tr>
<td>2-4</td>
<td>7.6</td>
<td>7.4</td>
</tr>
<tr>
<td>2-5</td>
<td>1.8</td>
<td>3.8</td>
</tr>
<tr>
<td>2-6</td>
<td>2.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* Wild-type, mutant, and two negative control probes for each mutation in Table 1 were immobilized on NCS-glass, and the glass was hybridized with the labeled RNA target probe prepared by in vitro transcription and fragmentation. The experiments were repeated three times and the results were averaged. Coefficients of variation were less than 10%.

The hybridization signals of two negative control probes for each mutation were averaged and used to calculate the ratio to that of wild-type probe.
4. Conclusions

Enormous progresses have been made toward the development of oligonucleotide chip for the detection of human genetic mutations over the past decade, but there are still some limitations remaining in the technology. They include the problems for reliability, sensitivity, and reproducibility. Of these, the most significant one is the limitation for the reliability of the chip, which can be usually represented by the discriminating power of target DNA between perfectly matched probe and mismatched probe. The discriminating power is not great enough to achieve reliable mutation detections with the oligonucleotide chip, as also illustrated with some mutation sites in Table 4. Better discrimination could be achieved by using novel DNA mimic molecules such as peptide nucleic acids (PNAs). PNAs are novel oligonucleotide mimics in which the sugar phosphate backbone is replaced by a pseudo-peptide skeleton (Nielsen et al., 1991; Nielsen, 1998).

Due to their neutral backbone, PNA can bind more strongly to complementary DNA than DNA probe and PNA–DNA hybridization is known to be more specific than the corresponding DNA–DNA hybridization (Weiler et al., 1997; Brandt et al., 1999). The discrimination of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarray immobilization and hybridization. Biochim. Biophys. Acta 1732, 1970–1977.

In the present study, we demonstrated that DNA chip analysis of the mutations in exon2 of HNF-1α. The mutations on our MODY3 DNA chip were selected only from single base substituted mutations in exon2 of HNF-1α. However, there are several deletion and insertion hot spots in these mutations and these mutations should be also included to increase the clinical utility of the chip by using some novel methods, such as single base extension and ligation (Favis et al., 2000). We are currently developing by using some novel methods, such as single base extension be also included to increase the clinical utility of the chip and ligation (Favis et al., 2000). We are currently developing by using some novel methods, such as single base extension and ligation (Favis et al., 2000).

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Fig. 4. DNA chip analysis of the mutations in exon2 of HNF-1α. Circles denote the positions of the wild-type probe and squares denote the positions of the mutant probe.

4. Conclusions

study takes approximately 24 h and does not require laborious experimental steps involving gel electrophoresis. These advantages could make our DNA chip analysis a good candidate for routine clinical testing of HNF-1α mutations. The approaches developed in this study will greatly benefit the DNA microarray-based technology for the detection of human genetic mutations.

Acknowledgements

This work was supported by the Brain Korea 21 (BK21) Program and Center for Ultramicrochemical Process Systems sponsored by KOSEF.


