Effective two-color SNP typing based on photoligation

Takehiro Ami and Kenzo Fujimoto
School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan

ABSTRACT

We report a method for the rapid differentiation of nucleic acid sequences with 5-carboxyvinyl-2'-deoxyuridine that allows the simultaneous observation of two colors and facilitates the SNP typing of a heterozygous sample.

INTRODUCTION

Single nucleotide polymorphisms (SNPs) are point mutations that represent the most widespread type of sequence variation in genomes and provide the most commonly used genetic markers for the mapping of human disease genes. An accurate and sensitive analysis of SNPs will play a vital role in future genetic diagnostics. Several different techniques for SNP genotyping have been developed in attempts to establish an ideal typing system that would be highly sensitive, robust, and multiplexed, without involving costly, time-consuming steps. Most current SNP typing techniques utilize the difference in hybridization efficiency between the target DNA and the probe oligodeoxynucleotides (ODNs), or the difference in enzymatic recognition between full-matched and mismatched duplexes. However, these methods have some disadvantage, such as hybridization errors, the high cost of enzymes, and the time-consuming steps required. For the purpose of high sequence selectivity, it is necessary to carefully select the hybridization and washing conditions to minimize any undesirable responses from mismatched hybridization probes. There are limitations in their selectivity to DNA probes that rely on only hybridization events. In our previous study, we reported an SNP-typing method with high selectivity and sensitivity by introducing a form of photochemical ligation with 5-carboxyvinyl-2'-deoxyuridine into an existing allele-specific hybridization. In this report, we show this SNP-typing method with 5'-UT and two fluorophors, and we demonstrate that this method facilitates the SNP typing of a heterozygous sample. In the previous method, fluorescence had to be labeled at every operation. But in this method, fluorescence is labeled before operation, so this method is easier and quicker than the previous method. We tested the SNP detection of the SNP sequence of the human aldehyde dehydrogenase 2 (ALDH2) gene by means of photoligation.

Scheme 1 Strategy for the detection of DNA point mutation on a DNA chip. Conceptual scheme showing how the target is detected by photochemical ligation and hybridization specificity.

Table 1 Oligodeoxynucleotides (ODNs) used in this study

<table>
<thead>
<tr>
<th>Sequences</th>
<th>a,b</th>
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<tbody>
<tr>
<td>Capture strand</td>
<td>5'-NH2(S)-ACTCACATTTTCAC-3'</td>
</tr>
<tr>
<td>WT-ODN</td>
<td>5'-GGAGTGGCCGGGAATGACGTCG</td>
</tr>
<tr>
<td>MUT-ODN</td>
<td>5'-GGAGTGGCCGGGAATGTCAATC</td>
</tr>
<tr>
<td>Cy3-ODN</td>
<td>5'-UTAGGTGCTGCAAGGCAATAGGA</td>
</tr>
<tr>
<td>Cy5-ODN</td>
<td>5'-UTAGGTGCTGCAAGGCAATAGGA</td>
</tr>
</tbody>
</table>

a,b The underlined letters indicate a mutation point. b,c S corresponds to a hex(ethylene glycol) linker fragment. WT and MUT represent wild type and mutant respectively.

RESULTS AND DISCUSSION

To investigate the difference in the ligation rate between the matched and mismatched sequences on the DNA chip, we prepared capture strands, two target strands (mutation and wild type), and Cy3-ODN and Cy5-ODN (Table 1). The capture strands were immobilized onto an activated glass surface by spotting with solutions of the appropriate 5'-aminomethylated capture strands in sodium cacodylate.
buffer. Liguations were carried out on the DNA chip by 366-nm irradiation at room temperature with Cy3-ODN and Cy5-ODN and the target, in sodium cacodylate buffer. After the chip was washed with ultrapure water at 40°C for 3 minutes, surface fluorescence measurements were performed on a CRBIO Ile microscanner array (Hitachi Software Engineering Co.). When WT-ODN or MUT-ODN were added, the fluorescence emission at 585 nm or 695 nm, respectively, increased substantially over 1 h (see the time course in Fig. 1). Likely sources of the small background (that is, “wrong”-color) signal are 1) overlap of Cy3 emission into the Cy5 channel of the camera, and 2) ligation of the mismatch probe on the target.

We also tested the heterozygous state detection of ALDH2 by means of photoligation. Cy3-ODN and Cy5-ODN were mixed with a sample solution of the target sequence, WT-ODN, MT-ODN, or a 1:1 mixture of WT-ODN and MT-ODN, to mimic the heterozygous state, and the fluorescence of the mixture was immediately read at room temperature with CR-BIO Ile (Figure 2). As a result of the photoligation with WT-ODN, a strong emission of Cy3 was obtained, whereas the emission of Cy5 was negligible. In contrast, for a sample solution containing MUT-ODN, Cy5 showed strong fluorescence, whereas very weak fluorescence was observed for Cy3. When Cy3-ODN and Cy5-ODN were added to a 1:1 mixture of WT-ODN and MUT-ODN, fluorescence emission was observed for both Cy3 and Cy5, and was clearly distinguishable from those of the homozygous samples. Therefore, the present method using a combination of Cy3-ODN and Cy5-ODN constitutes a very powerful tool for SNP typing.

CONCLUSION

In summary, we have described an approach for rapid differentiation of nucleic acid sequences with Cy5 that allows the simultaneous observation of two colors. The results are reproducible, and the color-based screening allows a qualitative determination to be made easily. This SNP typing method is a very powerful homogeneous assay that does not require enzymes or time-consuming steps. In addition, this method facilitates the SNP typing of a heterozygous sample. Future work will be directed at applying this strategy to simultaneous multiple-sequence sensing, and to imaging of DNA and RNA from living cells by using fluorescent probes in a wide variety of colors.

REFERENCES


*Corresponding author. E-mail: kenzo@jaist.ac.jp