DNA Photoligation in Two-color Detection of DNA Point Mutation

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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.

Single nucleotide polymorphisms (SNPs) constitute the most widespread type of sequence variation in genomes[1] and serve as the most commonly used genetic markers for the mapping of human disease genes.[2] 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.[3] Most current SNP typing techniques utilize the difference in hybridization efficiency between target DNA and probe oligodeoxynucleotides (ODNs),[4] or the difference in enzymatic recognition between full-matched and mismatched duplexes.[5] However, these methods have some disadvantages, such as hybridization errors, the high cost of enzymes, and the time-consuming steps required. Differences in hybridization efficiency are affected by the sequence context, and these are often very subtle for the detection of a single-base mismatch in long target strands of DNA. 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 the selectivity of 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 (^{CV}U) into an existing allele-specific hybridization.[6] In this report, we show a new SNP-typing method with ^{CV}U and two fluorophores, and we demonstrate that this new 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 the operation, so this method is easier and quicker than the previous method (Figure 1). We tested the SNP detection of the SNP sequence of the human aldehyde dehydrogenase 2 (ALDH2) gene[7] by means of photoligation. The synthesized ODNs are summarized in Table 1.

Table 1. Oligodeoxynucleotides (ODNs) used in this study

	Sequences[a,b]
Capture strand	5′-NH_2-$(S)_4$-ACTCACAGTTTTCAC-3′
ODN(C)	5′-ACTCACAGTTTTCAC-3′
ODN(WT)[c]	5′-GGAGTGGCCGGGAGTTGGGCGAGT ACGGGCTGCAGGCATACACTGAAGTG AAAACTGTGAGTGTGGGACCTGCTGG GGGCTCAGGGCCTGTTGGGGCTTG-3′
ODN(MUT)[c]	5′-GGAGTGGCCGGGAGTTGGGCGAGT ACGGGCTGCAGGCATACACTAAAGTG AAAACTGTGAGTGTGGGACCTGCTGG GGGCTCAGGGCCTGTTGGGGCTTG-3′
ODN(Cy3)	5′-^{CV}UTCAGTGTA-Cy3-3′
ODN(Cy5)	5′-^{CV}UTTAGTGTA-Cy5-3′

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

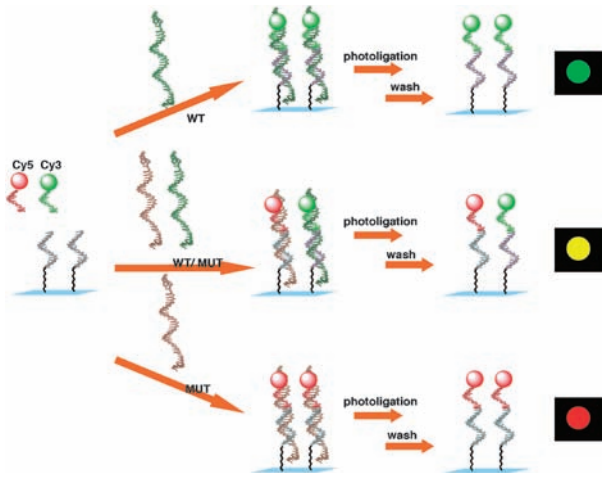

Figure 1. Strategy for the detection of DNA point mutation on a DNA chip.

We determined the feasibility of the DNA photoligation via ODN(Cy3) or ODN(Cy5). When ODN(Cy3) and ODN(C) were irradiated at 366 nm for 30 min in the presence of ODN(WT), capillary gel electrophoresis (CGE) showed the appearance of a peak relating to photoligated product in 98% yield (see Supporting Information).[8] On the other hand, when ODN(Cy5) and ODN(C) were irradiated at 366 nm for 30 min in the presence of ODN(MUT), CGE showed the appearance of a peak relating to photoligated product in 97% yield. As show in Figure 2, results show that a single mismatch yielded very little photoli-

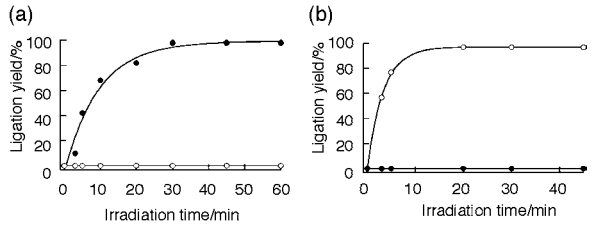

Figure 2. Comparison of photoligation rates with ODN(WT) (filled symbols) versus ODN(MUT) (open symbols). (a) Template-directed DNA photoligation by using ODN(Cy3). (b) Template-directed DNA photoligation by using ODN(Cy5).

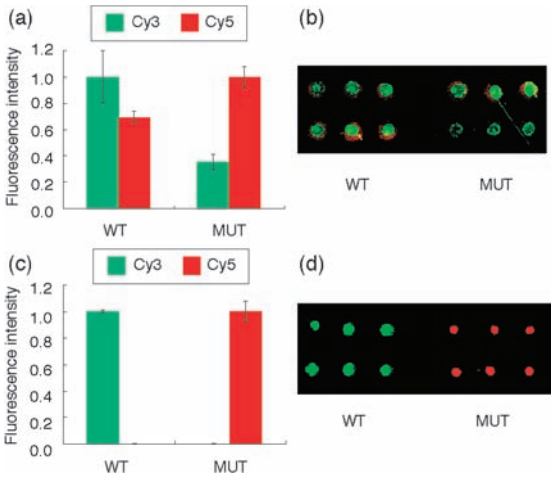

Figure 3. Influence of DNA photoligation on specificity. The capture strands were attached to the surface, and targets (left: ODN(WT), 2 × 3 spots; right: ODN(MUT), 2 × 3 spots) were applied. (a) Use of only hybridization specificity and washing with buffer under stringent conditions. (b) Fluorescence images. (c) Use of both hybridization specificity and photoligation specificity and washing with the deionized water at 98 °C for 30 s. (d) Fluorescence images.

gated product and that the photoligation has high sequence selectivity.

To compare the specificity between the conventional method and the photoligation method, we constructed a DNA chip by attaching a capture strand. Ligations were carried out on the DNA chip by 366-nm irradiation at room temperature with ODN(Cy3) and ODN(Cy5), and the target, in sodium cacodylate buffer. After the chip was washed with deionized water at 98 °C for 30 s, surface fluorescence measurements were performed on a CRBIO IIe microarray scanner. In the conventional method, which shows only hybridization specificity, the observed selectivity for ODN(WT) and ODN(MUT) were 1.4 and 2.9, respectively, even after stringent washing with a solution at the appropriate temperature (Figures 3a and 3b). On the contrary, in the photoligation method, mismatches are discriminated by a factor greater than 10^3-fold, as compared to the corresponding matched sequence (Figures 3c and 3d).

We also tested heterozygous state detection of ALDH2 by means of photoligation. ODN(Cy3) and ODN(Cy5) were mixed with a sample solution of the target sequence, ODN(WT), ODN(MUT), or a 1:1 mixture of ODN(WT) and ODN(MUT), to mimic the heterozygous state (Figure 4). As a result of the photoligation with ODN(WT), a strong emission of Cy3 was obtained, whereas the emission of Cy5 was negligible. On the contrary, for a sample solution containing ODN(MUT), Cy5 showed strong fluorescence, whereas very weak fluorescence was observed for Cy3. When ODN(Cy3) and ODN(Cy5) were added to a 1:1 mixture of ODN(WT) and ODN(MUT), 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 ODN(Cy3) and ODN(Cy5) constitutes a very powerful tool for SNP typing. Photoligation-coupled SNP typing has been capable of detecting attomolar levels of single-nucleotide mismatches, as reported previously.[6b]

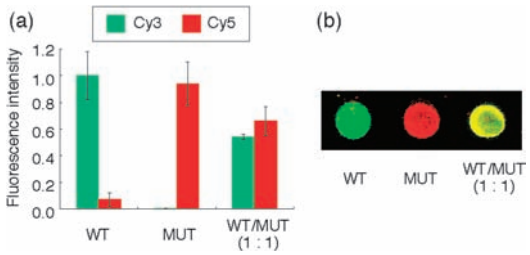

Figure 4. (a) Fluorecence intensity acquired on microarray scanner for the products of photochemical ligation on wild-type, mutant, and hetero target DNA. (b) Fluorescence images.

In summary, we have described an approach for rapid differentiation of nucleic acid sequences with ^{CV}U 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. By using the photoreversibility of the ODN containing ^{CV}U,[6a] this method has the advantage of offering recycle of the DNA chip. 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.

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8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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