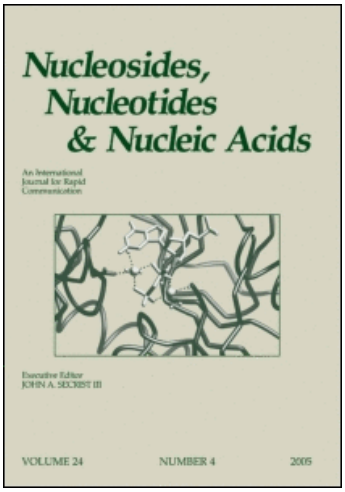


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# Mismatch Base Pair Detection by Fluorescence Spectral Change Upon Addition of Metal Cation—Toward Efficient Analysis of Single Nucleotide Polymorphism

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# MISMATCH BASE PAIR DETECTION BY FLUORESCENCE SPECTRAL CHANGE UPON ADDITION OF METAL CATION—TOWARD EFFICIENT ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM

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□ *Addition of mercury (II) cation to fluorescent-labeled duplex involving a T:T mismatch base pair and silver (I) cation to fluorescent-labeled duplex involving a C:C mismatch base pair significantly changed the fluorescence intensity, but no significant change in the fluorescence intensity was observed for duplexes involving the other base pairs. The fluorescence spectral change upon addition of the metal cation can discriminate T:T and C:C mismatch base pairs from the other base pairs. Our results certainly support the idea that the fluorescence spectral change upon addition of the metal cation could be a convenient strategy for the mismatch base pair detection by the heteroduplex analysis, and may eventually lead to progress in single nucleotide polymorphism genotyping.*

**Keywords** Single nucleotide polymorphism; mismatch base pairs; fluorescence

## INTRODUCTION

Single nucleotide polymorphism (SNP) is the most abundant form of genetic variation in the human genomic DNA, accounting for most of all differences between individuals.[1−3] Analyses of SNP can help to identify genes affecting many human phenotype variations, including complex diseases and drug responses.[1−3] When two kinds of duplex DNA with a different

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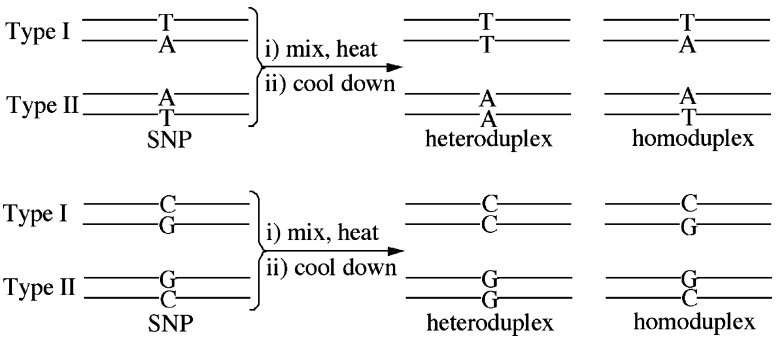


**FIGURE 1** Strategy for heteroduplex analysis.

type of SNP are mixed, heated, and annealed, novel two kinds of mismatch base paired heteroduplexes are formed in addition to the initial two kinds of perfectly matched homoduplexes (Figure 1). The heteroduplex analysis to detect the newly formed mismatch base pair is one of useful approaches for SNP genotyping. We have already found that mercury (II) and silver (I) cations specifically bind to T:T and C:C mismatch base pairs in heteroduplexes, respectively, which significantly increased the melting temperature of a heteroduplex involving the corresponding mismatch base pair.[4,5] The result shows that addition of the metal cation is a promising strategy for mismatch base pair detection in a heteroduplex, but determination of the melting temperature by UV melting is time-consuming. Thus, in the present study, we examined fluorescence spectral change of fluorescent-labeled duplexes upon addition of the metal cation to develop a more convenient way for the mismatch base pair detection.



## RESULTS AND DISCUSSION

Dye-labeled complementary oligonucleotides INS-F11**X**-FAM: 5′-Fam-CTGTC**X**CCCAG-3′ (**X**=A or T) and INS-R11**Y**-DAB: 5′-CTGGG**Y**GACAG-Dab-3′ (**Y**=A or T), where 6-carboxyfluorescein (Fam) is a fluorophore and dabcyl (Dab) is a quencher, were annealed to form a duplex. The base pair **X:Y** corresponds to a type 2 diabetes-related SNP found at the upstream of human insulin gene.[6] Figure 2 shows a fluorescence spectral change of INS-F11**X**-FAM:INS-R11**Y**-DAB (**X:Y**=**T:T**, **T:A**, **A:T**, and **A:A**) upon addition of mercury (II) cation. When the molar ratio of mercury (II) cation to the duplex with **X:Y**=**T:T** was increased, the intensity of Fam emission at 520 nm was decreased. Each strand of the duplex may be separated due to the T:T mismatch base pair without mercury (II) cation, but addition of mercury (II) cation may induce the assembly of each strand by the T-Hg-T formation,[4,5] which decreases the distance between the fluorophore (Fam) and the quencher (Dab). The Dab-mediated quenching may decrease the

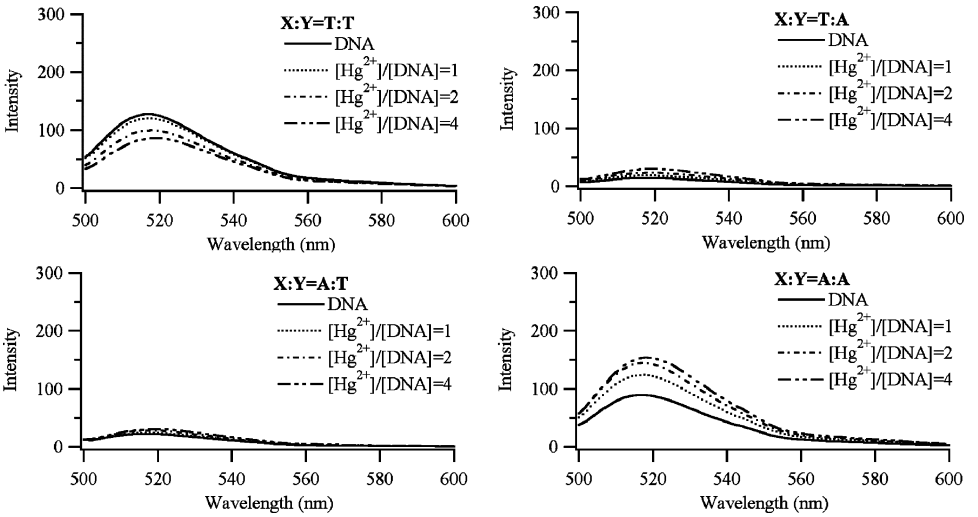


**FIGURE 2** Fluorescence spectral change of INS-F11**X**-FAM:INS-R11**Y**-DAB (**X**:**Y**=**T:T**, **T:A**. **A:T**, and **A:A**) in 10 mM sodium cacodylate-cacodylic acid and 100 mM sodium perchlorate at pH 6.8 upon addition of mercury (II) perchlorate.



intensity at 520 nm. In contrast, a little increase in the intensity at 520 nm was observed by addition of mercury (II) cation to the duplex with **X:Y=A:A**. Each strand of the duplex may be separated due to the A:A mismatch base pair without mercury (II) cation, and no significant structural change is expected upon addition of mercury (II) cation. Unknown interaction with mercury (II) cation may induce the observed small increase in the intensity at 520 nm. On the other hand, addition of mercury (II) cation to each of the duplexes with **X:Y=T:A** and **X:Y=A:T** did not induce any significant change in the intensity at 520 nm, which is quite reasonable because no significant structural change of the perfectly matched duplexes is expected upon addition of mercury (II) cation.

Simiarly, dye-labeled complementary oligonucleotides APM-F11**X**-FAM: 5′-Fam-CCTGC**X**CTTCA-3′ (**X=C** or **G**) and APM-R11**Y**-DAB: 5′-TGAAG**Y**GCAGG-Dab-3′ (**Y=C** or **G**), where 6-carboxyfluorescein (Fam) is a fluorophore and dabcyl (Dab) is a quencher, were annealed to form duplex. The base pair **X:Y** corresponds to a type 2 diabetes-related SNP found at the upstream of human adiponectin gene.[7] Figure 3 shows a fluorescence spectral change of APM-F11**X**-FAM:APM-R11**Y**-DAB (**X:Y=C:C**, **C:G**, **G:C**, and **G:G**) upon addition of silver (I) cation. When the molar ratio of silver (I) cation to the duplex with **X:Y=C:C** was increased, the intensity of Fam emission at 520 nm was decreased. Each strand of the duplex may be separated due to the C:C mismatch base pair without silver (I) cation, but addition of silver (I) cation may induce the assembly of each strand by the C-Ag-C formation,[4] which decreases the distance between the

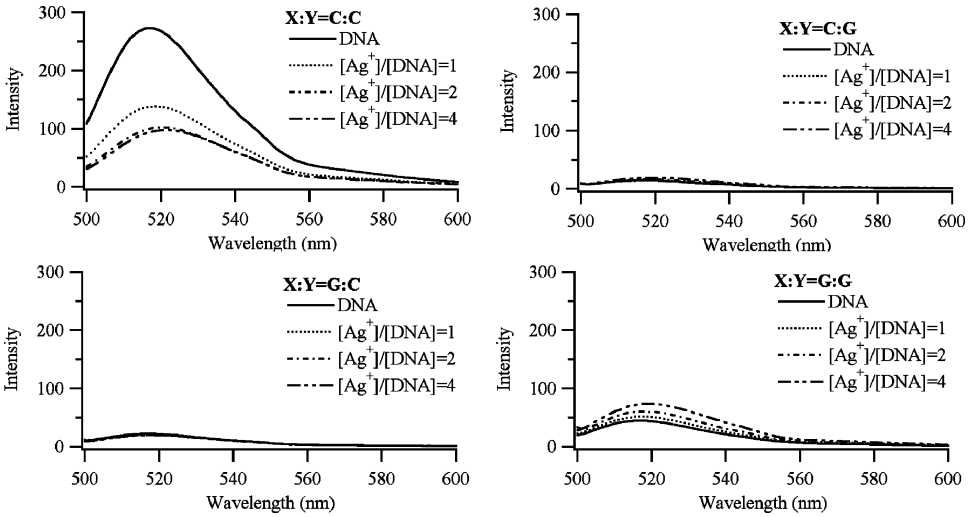


**FIGURE 3** Fluorescence spectral change of APM-F11**X**-FAM:APM-R11**Y**-DAB (**X:Y=C:C**, **C:G**. **G:C**, and **G:G**) in 10 mM sodium cacodylate-cacodylic acid and 100 mM sodium nitrate at pH 6.8 upon addition of silver (I) nitrate.



fluorophore (Fam) and the quencher (Dab). The Dab-mediated quenching may decrease the intensity at 520 nm. In contrast, a little increase in the intensity at 520 nm was observed by addition of silver (I) cation to the duplex with **X:Y=G:G**. Each strand of the duplex may be separated due to the G:G mismatch base pair without silver (I) cation, and no significant structural change is expected upon addition of silver (I) cation. Unknown interaction with silver (I) cation may induce the observed small increase in the intensity at 520 nm. On the other hand, addition of silver (I) cation to each of the duplexes with **X:Y=C:G** and **X:Y=G:C** did not induce any significant change in the intensity at 520 nm, which is quite reasonable because no significant structural change of the perfectly matched duplexes is expected upon addition of silver (I) cation.

On the basis of these results, we conclude that the fluorescence spectral change upon addition of the metal cation can discriminate T:T and C:C mismatch base pairs from the other base pairs. Our results certainly support the idea that the fluorescence spectral change upon addition of the metal cation could be a convenient strategy for the mismatch base pair detection in heteroduplex analysis and may eventually lead to progress in SNP genotyping.

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