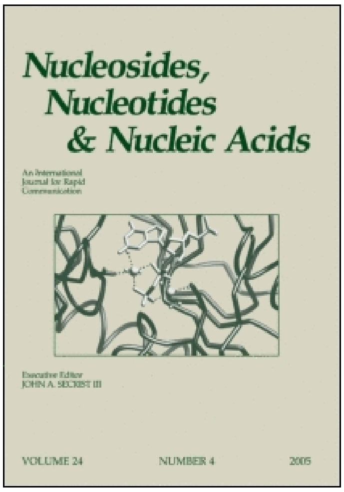

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Novel Strategy for Single Nucleotide Polymorphism (SNP) Genotyping by Heteroduplex Analysis: Specific Stabilization of TT Mismatch Base Pair by Mercury (II) Cation and CC Mismatch Base Pair by Silver (I) Cation

Hidetaka Torigoe[a]; Kouji Kawahashi[a]; Ayako Takamori[a]; Akira Ono[b]
[a] Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan [b] Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan

To cite this Article Torigoe, Hidetaka , Kawahashi, Kouji , Takamori, Ayako and Ono, Akira(2005) 'Novel Strategy for Single Nucleotide Polymorphism (SNP) Genotyping by Heteroduplex Analysis: Specific Stabilization of TT Mismatch Base Pair by Mercury (II) Cation and CC Mismatch Base Pair by Silver (I) Cation', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 915 — 917

To link to this Article: DOI: 10.1081/NCN-200059274
URL: http://dx.doi.org/10.1081/NCN-200059274

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):915–917, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059274

NOVEL STRATEGY FOR SINGLE NUCLEOTIDE POLYMORPHISM (SNP) GENOTYPING BY HETERODUPLEX ANALYSIS: SPECIFIC STABILIZATION OF TT MISMATCH BASE PAIR BY MERCURY (II) CATION AND CC MISMATCH BASE PAIR BY SILVER (I) CATION

Hidetaka Torigoe, Kouji Kawahashi, and Ayako Takamori ▫ *Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan*

Akira Ono ▫ *Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan*

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. 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 containing different SNPs are mixed, heated, and annealed, novel two kinds of mismatch base paired heteroduplex are formed in addition to the initial two kinds of perfectly matched homoduplex (Figure 1). The heteroduplex analysis is one of useful approaches for SNP genotyping.[1] To develop a novel strategy for SNP genotyping by heteroduplex analysis, in this study, we have examined the effect of metal cation on the thermal stability of heteroduplex and homoduplex.

RESULTS AND DISCUSSION

Table 1 summarizes melting temperatures of a series of duplexes, INS-F25X•INS-R25Y: 5′-GCCCTGCCTGTC**X**CCCAGATCACTG-3′/3′-CGGGACG-GACAG**Y**GGGTCTAGTG AC-5′ (**X:Y** = T:T, T:A, A:T, and A:A), with or without mercury (II) cation.[4,5] Without mercury (II) cation the melting temperatures of the homoduplex containing the perfectly matched base pairs (**X:Y** = T:A and A:T) were significantly higher than those of the heteroduplex containing the

Address correspondence to Hidetaka Torigoe, Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan; E-mail: htorigoe@ch.kagu.tus.ac.jp

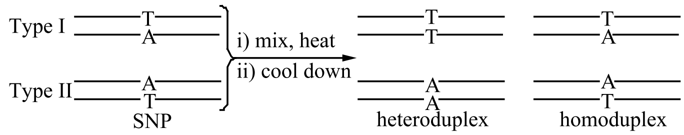

FIGURE 1 Strategy for heteroduplex analysis.

mismatch base pairs (**X:Y** = T:T and A:A). Addition of mercury (II) cation increased the melting temperature of the heteroduplex containing the T:T mismatch base pair by about 4°C. On the other hand, the melting temperatures of the homoduplex containing the T:A and A:T perfectly matched base pairs and that of the heteroduplex containing the A:A mismatch base pair were not significantly changed by the addition of mercury (II) cation. These results indicate that mercury (II) cation specifically increases the thermal stability of the heteroduplex containing the T:T mismatch base pair.

Table 2 summarizes melting temperatures of a series of duplexes, APM-F25X•APM-R25Y: 5′-CTCAGATCCTGC**X**CTTCAAAAACAA-3′/3′-GAGTC-TAGGACG**Y**GAAGT TTTTGTT-5′ (**X:Y** = C:C, C:G, G:C and G:G), in the absence or presence of silver (I) cation.[4,6] In the absence of silver (I) cation, the melting temperatures of the homoduplex containing the perfectly matched base pairs (**X:Y** = C:G and G:C) were significantly higher than those of the heteroduplex containing the mismatch base pairs (**X:Y** = C:C and G:G). Addition of silver (I) cation increased the melting temperature of the heteroduplex containing the C:C mismatch base pair by more than 4°C. In contrast, the melting temperatures of the homoduplex containing the C:G and G:C perfectly matched base pairs in the presence of silver (I) cation were almost the same as those in the absence of silver (I) cation. In addition, the heteroduplex containing the G:G mismatch base pair was not stabilized by the addition of silver (I) cation. These results indicate that silver (I) cation specifically stabilizes the heteroduplex containing the C:C mismatch base pair.

We conclude that mercury (II) and silver (I) cation specifically stabilizes TT and CC mismatch base pair in heteroduplex, respectively. The addition of the metal cation may be a convenient strategy for SNP genotyping.

TABLE 1 Melting Temperatures of the Duplex, INS-F25X•INS-R25Y (**X:Y** = T:T, T:A, A:T, and A:A), at pH 6.8[a] with or without Mercury (II) Perchlorate, Obtained from UV Melting

X:Y	$T_m(-Hg)$ (°C)	$T_m(+Hg)$ (°C)	ΔT_m (°C)[b]
T:T	65.7	69.4	3.7
T:A	70.7	70.5	−0.2
A:T	70.4	69.8	−0.6
A:A	66.6	65.8	−0.8

[a]10 mM sodium cacodylate-cacodylic acid and 100 mM sodium perchlorate (pH 6.8).
[b]$\Delta T_m = T_m(+Hg) - T_m(-Hg)$.

TABLE 2 Melting Temperatures of the Duplex, APM-F25X•APM-R25Y (**X:Y** = C:C, C:G, G:C, and G:G), at pH 6.8[a] with or without Silver (I) Nitrate, Obtained from UV Melting

X:Y	$T_m(-Ag)$ (°C)	$T_m(+Ag)$ (°C)	ΔT_m (°C)[b]
C:C	55.4	59.9	4.5
C:G	64.4	64.2	−0.2
G:C	66.2	66.4	0.2
G:G	61.1	60.9	−0.2

[a]10 mM sodium cacodylate-cacodylic acid and 100 mM sodium perchlorate (pH 6.8).
[b]$\Delta T_m = T_m(+Ag) - T_m(-Ag)$.

REFERENCES

1. Kwok, P.-Y.; Chen, X. Detection of single nucleotide polymorphisms. Curr. Issues Mol. Biol. **2003**, *5*, 43–60.
2. Chen, X.; Sullivan, P.F. Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. Pharmacogenomics J. **2003**, *3*, 77–96.
3. Twyman, R.M.; Primrose, S.B. Techniques patents for SNP genotyping. Pharmacogenomics **2003**, *4*, 67–79.
4. Ono, A.; Miyake, Y. Highly selective binding of metal ions to thymine-thymine and cytosine-cytosine base pairs in DNA duplexes. Nucleic Acids Res. Suppl. **2003**, *3*, 227–228.
5. Le Stunff, C.; Fallin, D.; Schork, N.J.; Bougneres, P. The insulin gene VNTR is associated with fasting insulin levels and development of juvenile obesity. Nat. Genet. **2000**, *26*, 444–446.
6. Vasseur, F.; Helbecque, N.; Dina, C.; Lobbens, S.; Delannoy, V.; Gaget, S.; Boutin, P.; Vaxillaire, M.; Lepretre, F.; Dupont, S.; Hara, K.; Clement, K.; Bihain, B.; Kadowaki, T.; Froguel, P. Single-nucleotide polymorphism halotypes in the both proximal promoter and Exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French caucasians. Hum. Mol. Gen. **2002**, *11*, 2607–2614.