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Genome Res. 2000 10: 1403-1413
Access the most recent version at doi:10.1101/gr.132700

Rapid Detection of Deletion, Insertion, and Substitution Mutations via Heteroduplex Analysis Using Capillary- and Microchip-Based Electrophoresis

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In this report, we explore the potential of capillary and microchip electrophoresis for heteroduplex analysis–(HDA) based mutation detection. Fluorescent dye-labeled primers (6-FAM-tagged) were used to amplify the DNA fragments ranging from 130 to 400 bp. The effects of DNA fragment length, matrix additives, pH, and salt were evaluated for capillary electrophoresis– (CE) and/or microchip electrophoresis–based HDA, using six heterozygous mutations, *185delAG*, *E1250X* (*3867GT*), *R1443G* (*4446CG*), *5382insC*, *5677insA* in *BRCA1*, and *6174delT* in *BRCA2*. For this system, the effective fragment size for CE-based HDA was found in the range of 200–300 bp, however, the effective range was 150–260 bp for microchip-based HDA. Sensitivity studies show CE-based HDA could detect a mutated DNA present at only 1%–10% of the total DNA. Discrimination between wild-type and deletion or insertion mutations in BRCA1 and BRCA2 with CE-based HDA could be achieved in <8 min, while the substitution mutations required 14 min of analysis time. For each mutation region, 15 samples were run to confirm the accuracy and reproducibility of the method. Using the method described, two previously reported mutations, *E1038G* (*3232AG*, missense) and *4427 C/T* (*4427CT*, polymorphism), were detected in the tested samples and confirmed by DNA sequencing. Translation of the CE-based methodology to the microchip format allowed the analysis time for each mutation to be decreased to 130 sec. Based on the results obtained with this model system, it is possible that CE-based HDA methodologies can be developed and used effectively in genetic testing. The fast separation time and automated operation afforded with CE instrumentation provide a powerful system for screening mutations that include small deletions, insertions, and point mutations. Translation to the microchip platform, especially to a multichannel microchip system, would allow for screening mutations with high throughput.

With the efforts of the Human Genome Project, our ability to identify genes that are responsible for human diseases will increase immensely. The identification of new genes, the detection of variations in these genes, and the relationship between the disease states and these variants will not only improve our understanding of human disease but also affect the clinical practice (Cantor and Smith 1999; Felsenfeld et al. 1999). As a result of both the DNA sequence information collected by the Human Genome Project and the increasing number of genes linked to specific diseases, it is increasingly more important to develop simple, low-cost, reliable, high-speed, high-throughput methods to detect sequence variations in specific genes.

Although DNA sequencing is the "gold standard" for identifying specific nucleotide variations, the high cost of screening samples for mutations by DNA sequencing and the difficulty of detecting heterozygotes remain issues. Accordingly, efforts have been made to develop alternative mutation detection methods. These methods can be operationally divided into allele-specific and sequence-scanning methods. Primer extension (Piggee et al. 1997), allele-specific amplification (Struewing et al. 1997), allele-specific oligonucleotide hybridization (Hacia et al. 1996) and oligonucleotide ligation (Iannone et al. 2000) are specific mutation detection methods that are currently used. Other methods such as heteroduplex analysis (HDA; Gerrard and Dean 1998), single-strand conformation polymorphism (SSCP; Nataraj et al. 1999), denaturing gradient gel electrophoresis (DGGE; De Santis and Azzi 2000), temperature gradient gel electrophoresis (TGGE; Toliat et al. 2000), denaturing high-performance liquid chromatography (DHPLC; Liu et al. 1998; Arnold et al.

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Article and publication are at www.genome.org/cgi/doi/10.1101/gr.132700.

1999; Gross et al. 1999), RNase cleavage (Faudoa et al. 2000), and methods using either DNA repair enzymes or resolvases for the detection of mismatches (Hsu et al. 1994) represent sequence-scanning (or nonspecific) approaches to mutation detection. Among these methods, HDA and SSCP are the most widely used mutation scanning approaches (Gerrard and Dean 1998; Nataraj et al. 1999). Heteroduplex analysis has a distinct advantage over SSCP in that there is either no need or minimal need for manipulating the PCR product and, with the use of a duplex generator, it can also be used to detect allele-specific mutations (Bowen et al. 1997, Jackson et al. 1997, Nataraj et al. 1999).

The principles underlying mutation detection using heteroduplex analysis are based on conformational differences of duplex DNA, which are produced in the amplification process by the polymerase chain reaction (PCR). Wild-type duplex DNA consists of two complementary strands (homoduplex) while the duplex DNA from the heterozygous individual contains two complementary strands (wild-type homoduplex and mutant homoduplex) and two mismatched strands (two heteroduplexes). The goal of heteroduplex analysis is to be able to discriminate the homoduplex DNA from the heteroduplex DNA fragments based on their conformations under native conditions (Gerrard and Dean 1998). Traditionally, [^{32}P]-labeled deoxynucleoside triphosphates (dNTPs) are incorporated into the PCR products for the detection and slab-gel electrophoresis (with long-track-length polyacrylamide gels or mutation detection enhancement gel [MDE]) is used in HDA (Gerrard and Dean 1998; Nataraj et al. 1999). However, in the interest of higher efficiency detection, greater convenience and safety, a few studies have evaluated the use of fluorescent dye-labeled primers or dNTPs instead of radioactive chemicals for HDA via slab-gel electrophoresis and capillary electrophoresis (CE; Cheng et al. 1994; Jackson et al. 1997; Nataraj et al. 1999). Although Jackson et al. (1997) and Bowen et al. (1997) showed that CE-based HDA could be used to detect point mutations in the HFE (*HLA-H*) gene responsible for haemochromatosis via duplex generation, a proprietary polymer was used as the sieving matrix. Capillary electrophoresis offers several unique advantages over the traditional gel electrophoresis, the most important of which are high-speed, high-resolution, automation, small-reagent consumption and miniscule sample requirements. The microvolume characteristics of CE provide obvious advantages over slab-gel electrophoresis for biomedical and clinical applications (Landers 1997).

In this report, we describe HDA by CE using a fluorocarbon- (FC) coated capillary and hydroxyethylcellulose (HEC) as the sieving polymer for detecting mutations in two breast cancer susceptibility genes, *BRCA1* and *BRCA2*. Six mutations, *185delAG*, *E1250X* (*3867GT*), *R1443G* (*4446CG*), *5382insC*, *5677insA* in *BRCA1*, and *6174delT* in *BRCA2*, were used to demonstrate the fast, simple, and semiautomated HDA. Using DNA purified directly from blood or from cell lines via either a silica-based micro-solid phase extraction method (Tian et al. 2000a) or conventional extraction protocols, screening for each mutation could be completed in less than 2.5 hr. This included DNA purification (~10 min), DNA amplification (1–2 hr), and HDA by CE (<15 min). Ultrasensitive laser-induced fluorescence detection was possible as a result of the use of fluorescent-labeled primers for amplification. Further reduction in analysis time was afforded by carrying out the electrophoresis portion of the assay on an electrophoretic microchip. HD analysis time in this format was sixfold faster than in the capillary.

RESULTS

Optimizing the Separation Conditions for CE-Based Heteroduplex Analysis

The goal of heteroduplex analysis is to separate the homoduplex DNA (wild type, mutant) and heteroduplex DNA fragments based on their conformations under native conditions (Gerrard and Dean 1998). Conventional HDA typically involves the use of polyacrylamide slab-gel electrophoresis in the presence of neutral additives, such as glycerol, urea, ethylene glycol, formamide, and sucrose to improve the discrimination between homo- and heteroduplexes (Keen et al. 1991; White et al. 1992; Gerrard and Dean 1998; Nataraj et al. 1999). It is important to test the effect of neutral additives on the resolution by CE-based HDA, where an entangled polymer solution (in the capillary) is used instead of polyacrylamide gel for the size-based DNA separation. In this study, we evaluated the effect that varying the glycerol and urea concentrations in the polymer solution had on the ability to detect deletion, insertion, and substitution mutations by CE-based HDA.

Hydroxyethylcellulose (HEC) was utilized as the sieving matrix for analysis of PCR-amplified DNA containing the heterozygous mutation *5677insA* (Fig. 1) and *4446CG* (Fig. 2). Although the wild-type and mutant homoduplexes were still not resolved, addition of glycerol to the HEC solution in the 5%–15% range did improve the resolution in the duplex region, allowing the homoduplexes to be resolved from the heteroduplexes with *5677insA* mutant (Fig. 1A–D). While increasing the concentration of glycerol to 15% was beneficial for improving the duplex region resolution with the *6174delT* mutant (data not shown), 10% glycerol was found to be optimal for both mutations with the separation time in <8 min, as shown in Figure 1C.

While addition of glycerol (10% glycerol) to the HEC solution (2.5% HEC) was adequate for discrimi-

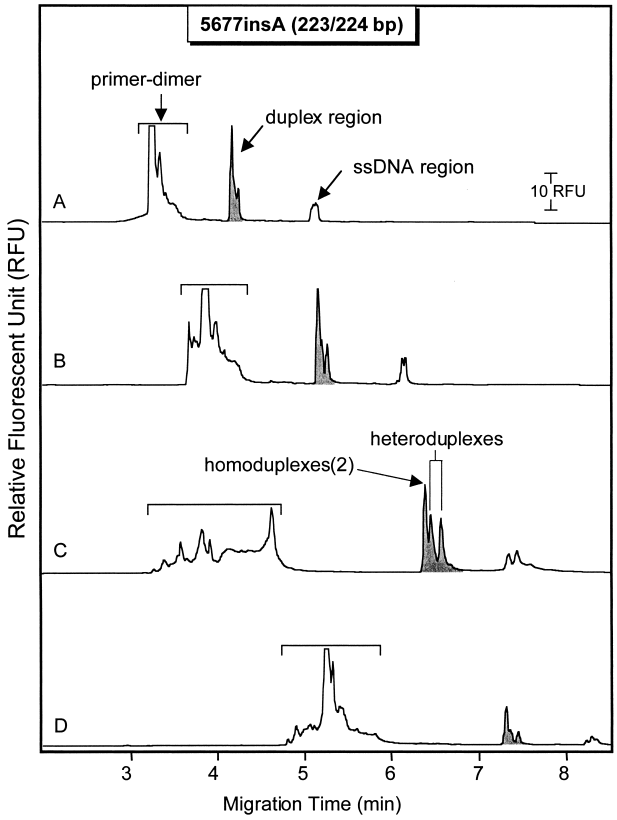

Figure 1 Effect of glycerol on capillary electrophoresis-based heteroduplex analysis of *5677insA* mutant. PCR conditions were described within the text. CE conditions were as following: the FC-coated capillary was 50 μm (I.D.) by 27 cm (effective length was 20 cm); PCR products were directly injected into the capillary for 20 sec at 370 V/cm. The separation was carried out at 370 V/cm using the reversed polarity (inlet as cathode and outlet as anode), and the capillary was maintained at 30°C. LIF detection (em/ex: 520 nm/ 488 nm) was used. The separation buffers were 2.5% HEC (Mr 360,000) in 1 × TBE buffer (pH = 8.6) containing the additive, glycerol as following: (*A*) no glycerol, (*B*) 5% glycerol, (*C*) 10% glycerol, (*D*) 15% glycerol. Shaded areas represent the duplex regions.

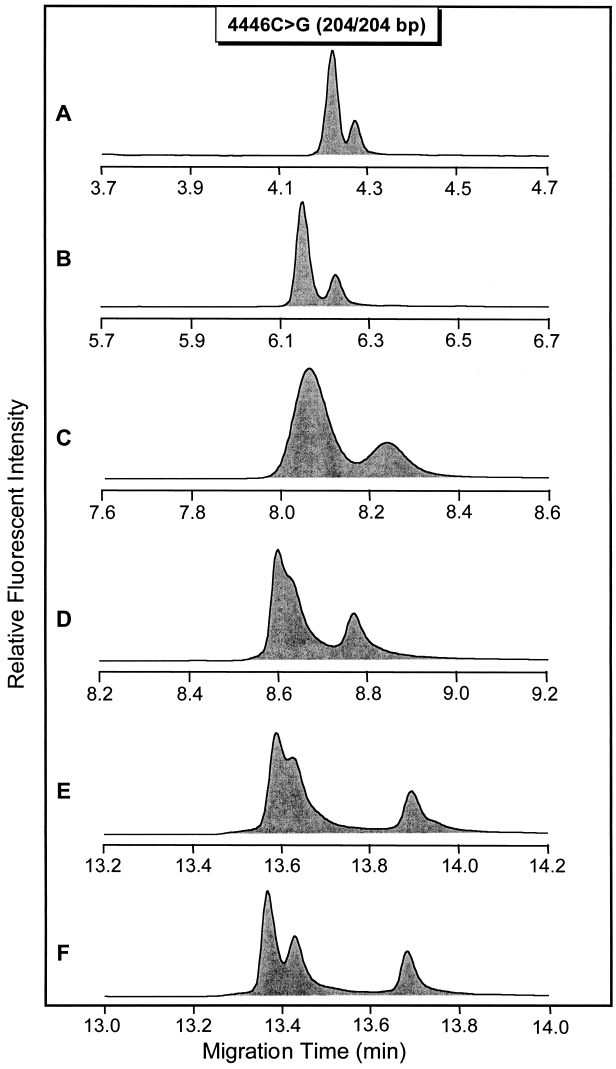

Figure 2 Effect of glycerol and urea on capillary electrophoresis-based heteroduplex analysis of *4446CG* mutant. Separation buffers (in 1 × TBE, pH 8.6): (*A*) 2.5% HEC, (*B*) 2.5% HEC containing 10% glycerol, (*C*) 4.5% HEC containing 10% glycerol, (*D*) 4.5% HEC containing 10% glycerol and 10% urea, (*E*) 4.5% HEC containing 10% glycerol, (*F*) 4.5% HEC containing 10% glycerol and 15% urea. For (*A–D*), 27-cm FC-coated capillary (effective length was 20 cm) and 370 V/cm were used; for (*E*) and (*F*) 37-cm FC-coated capillary (effective length was 30 cm) and 351 V/cm were used. The capillary was maintained at 20°C, other CE conditions were as in Fig. 1.

nating the wild type from the deletion/insertion mutations (three peaks in the duplex region with heterozygous mutants), it was inadequate for detecting the substitution mutation, *4446CG*, where only two peaks were observed in the duplex region (Fig. 2B). Improving the resolution was unsuccessful with further increases in glycerol concentration but was successful when the concentrations of glycerol, urea, and HEC in the separation solution were optimized and the length of the capillary adjusted appropriately. Detection of the *4446CG* mutation was found to be optimal with 4.5% HEC containing 10% glycerol and 15% urea, and a 37-cm FC-coated capillary. This is shown in Figure 2F.

Effect of Salt on CE-Based Heteroduplex Analysis

Because the ultimate goal of this research is to translate HDA for mutation detection to the electrophoretic microchip, differences between the two platforms must be taken into account. While PCR product analysis by CE using entangled polymer solutions is relatively immune to the high salt concentrations of PCR mixtures (Ulfelder et al. 1992; Guttman and Schwartz 1995; Pancholi et al. 1997), work by several groups (Woolley and Mathies 1994; Woolley et al. 1997; Munro et al. 1999; Shi et al. 1999) indicated that salt can degrade separations on microchips. The effect of the sample salt concentration on CE-based HDA was tested using a com-

mercial capillary with a fluorocarbon (FC) coating, which has been shown to be relatively stable and robust for DNA analysis of unpurified PCR products. Figure 3 shows the HDA results when PCR-amplified products from the *5382insC* and *5677insA* mutations were injected directly without dilution or purification (DNA in 1× PCR mixture; Fig. 3a); diluted 10 times with deionized water (DNA in 0.1× PCR mixture; Fig. 3b); purified by AutoSeq G-50 (Fig. 3c); or purified by Microcon YM-100 (DNA in deionized water; Fig. 3d). Comparing the resolution in the duplex region in Figure 3a with that in Figure 3b–3d, we can conclude that the ability to detect both mutations is reduced by dilution or purification. In contrast to previous findings (Shi et al. 1999), we found that routine desalting of the PCR products before analysis was not necessary. In fact, the resolution obtained with HDA under our conditions was found to be unaffected or better when the PCR products were used directly.

Detection Sensitivity of CE-Based Heteroduplex Analysis

In some cases, the early detection of certain genetic changes can influence the treatment outcome and/or survival rates. This is especially true in cancer, where the presence of a small amount of mutated DNA in a pool of wild-type DNA may signal the recurrence of the disease long before symptoms appear. To test the sensitivity of CE-based HDA, *6174delT* and *5677insA* mutant DNA (from heterozygous individuals) was diluted to various extents with DNA from a wild-type homozygous individual and coamplified. Figure 4 shows that the mutated DNA can be detected when presented at a concentration as low as 1% with the *6174delT* mutant (Fig. 4A) and in the 2.5%–10% range with the *5677insA* mutant (Fig. 4B). Therefore, the sensitivity for detection of the *6174delT* and *5677insA* by this method is 1% and 10%, respectively.

Heteroduplex Analysis of Six Mutations in *BRCA1* and *BRCA2* via CE

Detection of insertion and deletion mutations could be achieved by using a low-concentration entangled polymer solution, which also reduced the analysis time. This led to two sets of conditions for detecting mutations. For deletion and insertion mutations, 2.5% HEC containing 10% glycerol in 1 × TBE and a 27-cm FC-coated capillary was employed. For substitution mutations, 4.5% HEC containing 10% glycerol and 15% urea in 1 × TBE and a 37-cm FC-coated capillary were used, which is also effective for detecting the deletion and insertion mutations with higher resolution and longer analysis time. Figure 5 shows the results of CE-based HDA of two deletion (Fig. 5a,b), two insertion (Fig. 5c,d), and two substitution mutations (Fig. 5e,f). While the wild type is represented by a single peak in the duplex region (shown in Fig. 5a–f, panel A; peak identified by asterisk), the heterozygous mutants have three or four peaks in their duplex regions (shown in Fig. 5a–f, panel B; filled duplex region). By examining the different patterns in the wild-type and mutant duplex profiles, it is clear that these six mutations can be discriminated using the CE-based HDA with an analysis time of <14 min.

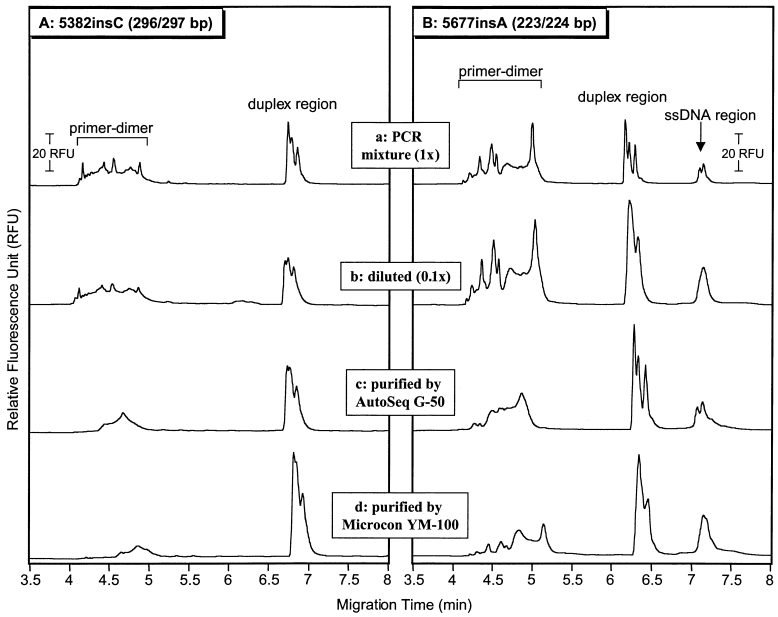

Figure 3 Effect of sample salt concentration on capillary electrophoresis-based heteroduplex analysis. Panels (*A*) and (*B*) show the heteroduplex analysis results with the mutants, *5382insC* and *5677insA*, respectively. The PCR products injected were: (*a*) PCR mixture, (*b*) PCR product diluted 10 times with deionized water, (*c*) PCR product purified by AutoSeq G-50, and (*d*) PCR product purified by Microcon YM-100. The separation buffer consisted of 2.5% HEC containing 10% glycerol in 1 × TBE (pH = 8.6). Other CE conditions were as in Fig. 1.

Heteroduplex Analysis of *BRCA1*/*BRCA2* Mutations via Microchip Electrophoresis

The same buffer systems used for detecting the deletion/insertion and substitution mutations by CE were translated to the microfabricated platform for microchip-based heteroduplex analysis. Using a microchip with a single channel (depth 20 μm, width 50 μm, effective length ~55 mm) whose surface had been covalently modified with PVP (Hofgärtner et al. 1999), each mutation could be discriminated by the heteroduplex analysis in the same manner illustrated with CE, except that analysis times were reduced to <130 sec. This represents a four- to sixfold improvement in analysis speed over CE. In the case of detecting de-

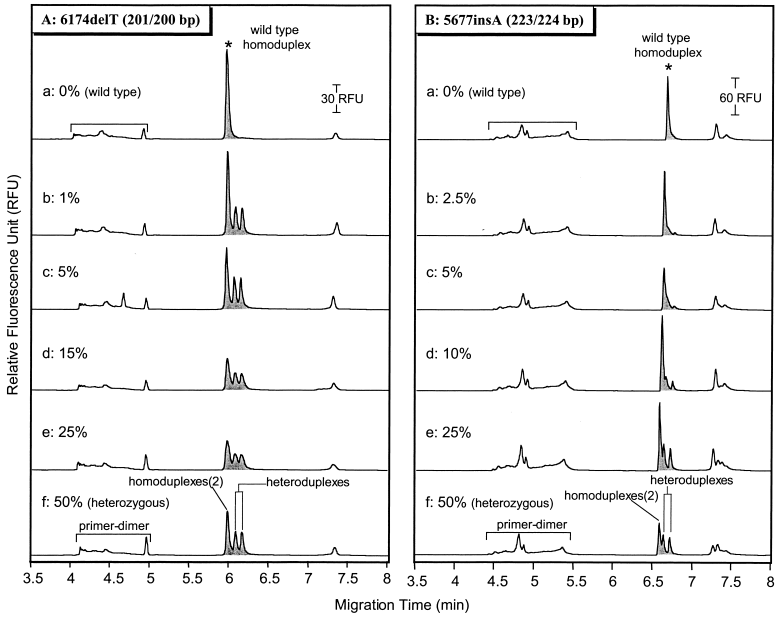

Figure 4 Detection sensitivity with capillary electrophoresis-based heteroduplex analysis. Panels (*A*) and (*B*) show the heteroduplex analysis results with the *6174delT* and *5677insA* mutants. Wild-type DNA template and the mutated DNA template were mixed together and coamplified by PCR for analysis by CE-LIF. The mixed percentages of the mutated DNA template were specified in the figure. The separation buffer was 2.5% HEC containing 10% glycerol in 1 × TBE. Other CE conditions were as in Fig. 1.

letion and insertion mutations, the resolution and the heteroduplex profiles obtained by microchip electrophoresis (Fig. 6A–D) are almost identical to those obtained by CE (Fig. 5a–5d). The exception is that resolution obtained with the microchip is slightly reduced compared to the CE profile for the 297-bp fragment when detecting the *5382insC* mutation (data not shown). With the substitution mutations, microchip-based heteroduplex analysis yielded a slight decrease in resolution (compared to CE) as shown in Figure 6E–F for the *E1250X* (*3867GT*) and *R1443G* (*4446CG*) mutations. Based on the observation that CE in a shorter capillary (27 cm) gave slightly poorer resolution for substitution mutations (shown in Fig. 2D), it is clear that the resolution on the microchip could be improved with a longer separation channel than used in this study (~5.5 cm effective length).

Detection of a Homozygous Mutation by Capillary Electrophoresis-Based Heteroduplex Analysis

After PCR amplification, HDA can be carried out with direct analysis of the PCR products for detecting mutations in the heterozygous states. This method can be easily extended to detect homozygous mutations by reannealing a mixture of the PCR products derived from the test sample and a wild type. Figure 7 shows the results for detecting wild type, the *5382insC* heterozygous allele, and the *5382insC* homozygous allele in HCC 1937 breast cancer cell line. While there was only a single peak in the duplex region with the wild-type and homozygous mutant (Fig. 7A,B), the reannealing process produced a HDA profile almost identical to that of the heterozygous allele (shown in Fig. 7C,D).

DISCUSSION

Optimizing the Conditions for CE-Based Heteroduplex Analysis

Although there have been no comprehensive studies on the effectiveness of CE-based HDA, it is well-known that several parameters can affect the sensitivity with the gel-based method (Gerrard and Dean 1998; Nataraj et al. 1999). In the gel-based HDA, the identity of the base mismatch, the additives used to create the local denaturation around a mismatched base pair, and the polymer concentrations are all related to the effectiveness of heteroduplex analysis separation. In contrast, G + C content, fragment length (between 100 and 600 bp), and the position of mismatch (centrally located vs. 50 bp from either the 5′- or 3′-end) have no effect on the sensitivity of HDA (Nataraj et al. 1999). In this report, DNA fragment size, buffer additives, and salt concentration were evaluated for their effect on CE-based HDA with insertion, deletion, and substitution mutations in *BRCA1* and *BRCA2*, while an evaluation of different polymers will be reported elsewhere (Tian et al. unpubl.). As with gel-based HDA, optimization of these parameters was found to be critical to effective mutation detection by CE-based HDA.

Because the relationship between discrimination sensitivity and the fragment size is not clear with CE-based HDA (Nataraj et al. 1999), the fragment size range that would provide optimal CE-HDA detection of the *BRCA1* and *BRCA2* mutations was determined empirically. Under the conditions employed here, there is an obvious correlation between the DNA fragment size and the ability to resolve mutant and wild-type alleles (data not shown). This result differs from slab gel–based HDA, where there is generally no size effect between 100 and 600 bp for mutation detection (Nataraj et al. 1999). Although the wild-type and mutant alleles can be distinguished using 136- and 400-bp DNA fragments for the mutation detection in our study, the functional DNA fragment size range for CE-based HDA appears to be between 200 and 300 bp, considerably narrower than in slab-gel system. The optimal length of DNA fragments was found to be in

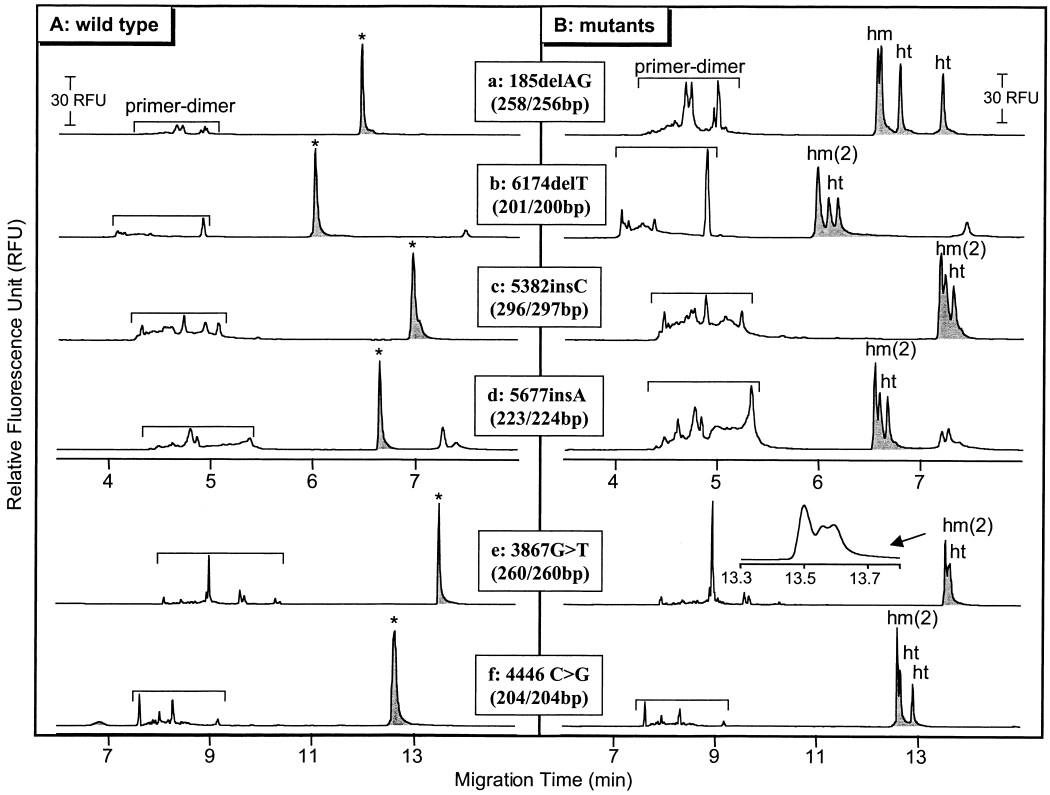

Figure 5 Capillary electrophoresis–based heteroduplex analysis of six heterozygous mutants. Panels (*A*) and (*B*) show the heteroduplex analysis results with the wild type and the mutants specified in the figure. For (*a–d*), the separation buffer consisted of 2.5% HEC containing 10% glycerol in 1 × TBE (pH = 8.6); the injection time was 20 sec at 370 V/cm, the capillary length was 27 cm (effective capillary length was 20 cm), the separation carried out at 370 V/cm with the separation temperature at 30°C. For (*e*) and (*f*), the separation buffer consisted of 4.5% HEC containing 10% glycerol and 15% urea; the injection time was 20 sec at 270 V/cm, the capillary length was 37 cm (effective capillary length was 30 cm), the separation was carried out at 351 V/cm with the separation temperature at 20°C. Other CE conditions were as in Fig. 1.

the range of 150 to 260 bp for microchip electrophoresis.

In this study, glycerol was shown to improve the detection of the *6174delT* and *5677insA* mutants, while urea also improved the resolution for detecting the substitution mutations. Adding glycerol to TBE buffer decreases the pH and has been reported to affect SSCP analysis (Gerrard and Dean 1998; Nataraj et al. 1999). Our results confirmed the pH effect on TBE buffer but show that decreasing the pH of the buffer to as low as 7.0 did not improve the resolution of HDA (data not shown). This differs from the effect on SSCP analysis, where lower pH improved the resolution by slab-gel electrophoresis (Hayashi et al. 1998). Decreasing the salt concentration in the PCR sample and/or desalting the PCR products were shown to decrease the resolution in the duplex region of the mutants. This phenomenon may be related with the effect of salt on the stability of the duplex conformation (Nakano et al. 1999; Gueron et al. 2000). It has been reported that the DNA double helix is considerably destabilized in the presence of low salt buffer (≤10 mM NaCl; Clausen-Schaumann et al. 2000). Based on the salt concentration–dependence of the resolution for HDA using capillaries or microchips, it is clear the capillary or microchannel wall must be covalently or dynamically modified in a manner that is resilient to salt in the PCR sample.

With respect to the effect of separation temperature on CE-based HDA, the results of this study show that effective HDA can be obtained with separation temperature in the 20°–40°C range (data not shown). This differs dramatically from methods described for CE-based SSCP analysis where the separation temperature was critical (Ren at al. 1997; Ren and Ueland 1999; Tian et al. 2000b).

The literature is devoid of studies that comprehensively evaluate the sensitivity of CE-based HDA. Most of the mutation-detection studies report the rate of discrimination sensitivity compared to other mutation-detection methods (Arnold et al. 1999; Gross et al. 1999; Nataraj et al. 1999). With the detection of somatic mutations (such as in tumors), it would be valuable to have the ability to detect the presence of mu-

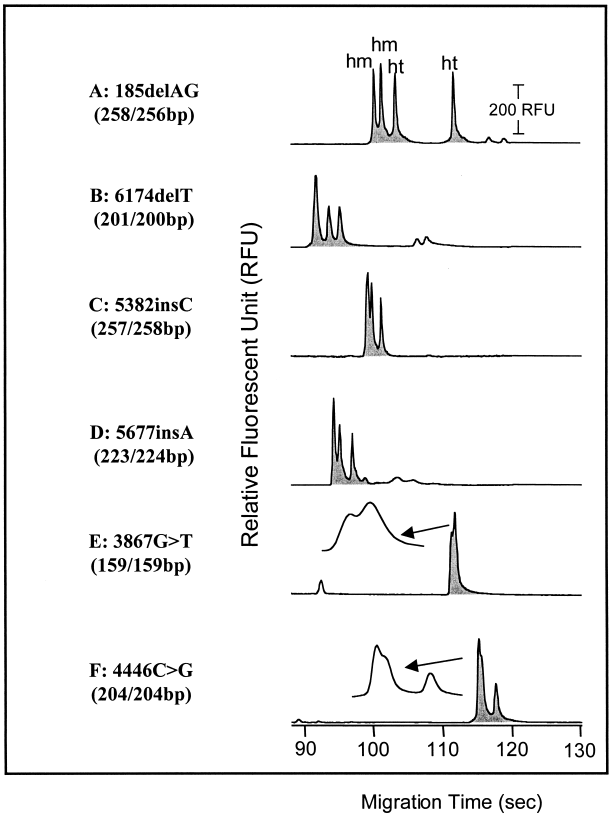

Figure 6 Fast mutation detection via heteroduplex analysis on a microfabricated electrophoretic chip. The heterozygous mutants were specified in the figure. The separation buffer was 2.5% HEC containing 10% glycerol in 1 × TBE (pH = 8.6) for (*a–d*) and 4.5% HEC containing 10% glycerol and 15% urea in 1 × TBE (pH = 8.6) for (*e–f*). The microchannel on the chip was coated with PVP (Mr 1,000,000) and detection mediated by laser-induced fluorescence (em/ex 520 nm/488 nm). The PCR products were injected into the channel for 100 sec at 333 V/cm (effective microchannel length of 5.5 cm), and the separation voltage was 573 V/cm.

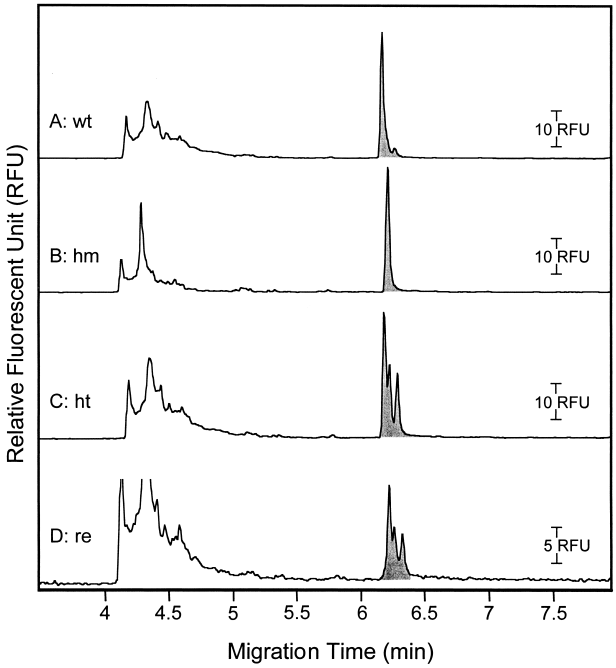

Figure 7 Detection of the homozygous mutation and two heterozygous mutations by capillary electrophoresis–based heteroduplex analysis. (*A*) wild type (wt); (*B*) HCC 1937 breast cancer cells (hm; containing *5382insC* homozygous mutation); (*C*) *5382insC* heterozygous mutation (ht); (*D*) reannealing (re) PCR amplicons from wild type and HCC 1937 (each 5 µl) were mixed, heated at 95°C for 5 min, and cooled at 0°C for 5 min before injection. The separation was carried out at 20°C, other CE conditions were as in Fig. 1.

tated DNA in the presence of wild-type DNA at a ratio of <50 : 50 (i.e., in the presence of contamination from normal tissue). In our experiments, albeit limited, CE-based HDA can detect mutated *6174delT* and *5677insA* alleles when present at concentrations as low as 1%–10%, which is in the same range as the conventional slab gel–based HDA (3%–10%; Mansukhani et al. 1997), the denaturing gradient gel electrophoresis (5%; Poncin et al. 1999), and the denaturing high-performance liquid chromatography (DHPLC; 10%–20%; Liu et al. 1998). Consequently, there is no apparent loss in sensitivity using laser-induced fluorescence detection CE for HDA.

Detecting Point Mutations by CE-Based Heteroduplex Analysis

Heteroduplex analysis relies on the conformation of duplex DNA. In the case of deletions and insertions, "bulges" form in the heteroduplexes, which generally reduces the mobility of the duplex DNA and allows for discrimination from their corresponding homoduplexes. With point mutations, the mismatched heteroduplexes form "bubbles," which are very similar to the corresponding homoduplexes (Bhattacharyya et al. 1989). The studies on slab gel–based HDA have reported that deletions and insertions are easier to be detected (Nataraj et al. 1999). The results of this study support this concept for capillary- and microchip-based electrophoresis. In contrast to the slab gel–based HDA method (Mansukhani et al. 1997), we were able to resolve the two homoduplexes in a carrier of the *185delAG* mutation by CE (partially) and by microchip electrophoresis (almost baseline resolved; Fig. 5a, panel B; Fig. 6A), which may suggest that CE and microchip electrophoresis have the potential to provide better detection efficiency with certain mutations.

While it is well-established that substitution mutations are known to be difficult to detect by HDA (Nataraj et al. 1999), White et al. (1992 and Keen et al. 1991) have defined a gel-based HDA to detect single-base substitutions in equine infectious anemia virus (EIVA) DNA using a hydrolink gel (more commonly known as mutation-detection enhancement [MDE] gel; Nataraj et al. 1999). While this method, which

identified point mutations on the basis of the presence of two bands, was effective, deletion mutations were still detected more easily. The most commonly used techniques for detecting single base substitutions are direct DNA sequencing, DGGE, and SSCP (White et al. 1992; Gerrard and Dean 1998; Hayashi et al. 1998; Cantor and Smith 1999), all of which require more manipulations than HDA.

For detecting point mutations in this study, a variety of different separation conditions were evaluated. So far, using HEC as the sieving matrix, a FC-coated capillary provided the best combination for comprehensive detection of mutations involving the deletions, insertions, or substitutions. Our results demonstrate that it is possible to detect point mutations with one base substitution by CE-based HDA with higher resolution than the gel-based HDA. While the use of this particular HEC buffer system for CE-base HDA detection of a single-base substitution mutation has not been reported previously, the only negative attribute to its use is the longer "capillary fill time" or higher pressure system (>20 psi) required to effectively pump it into the capillary (the separation system).

Diagnostic Value of Heteroduplex Analysis by CE

Following PCR amplification, HDA can be carried out directly using the PCR products without any manipulation for detecting heterozygous mutations. This method can easily be extended to detect homozygous mutations by reannealing a mixture of the PCR products derived from the homozygous allele and the wild-type allele (Fig. 7). It is expected that loss of heterozygosity can be detected in a similar fashion. The attractiveness of such a system is rooted in its simplicity, which clearly renders it amenable to automation.

A variety of mutation-detection methods, such as gel-based SSCP and polyacrylamide denaturing sequencing gel electrophoresis (Castilla et al. 1994; Struewing et al. 1995; Markoff et al 1998), matrix hybridization DNA chips (Hacia et al. 1996), allele-specific PCR, slab gel–based HDA (Gayther et al. 1996; Ozcelik et al. 1996; Mansukhani et al. 1997; Struewing et al. 1997; Hartge et al. 1999; Tong et al. 1999), and more recently, CE-based SSCP analysis (Tian et al. 2000b), have been used to detect mutations in *BRCA1* and *BRCA2*. In comparison with these methods, CE-based HDA is less complicated in that it does not require critical post-PCR manipulation of the samples and results can be obtained in a rapid and semiautomated fashion. Although the CE-based SSCP analysis was capable of detecting these mutations, strict control of denaturing conditions is required to obtain reproducible results (Tian et al. 2000b). By translating this CE-based HDA method to the microchip format, the analysis time can be decreased by sixfold. It is noteworthy that for each mutated region evaluated, 15 samples were run to confirm the accuracy and reliability of the method. Using the method described in this report, two mutations previously reported, *E1038G* (*3232AG*, missense) and *4427 C/T* (*4427CT*, polymorphism), were identified and confirmed by DNA sequencing in the samples tested (data not shown).

In conclusion, we have explored the potential of CE and microchip electrophoresis for heteroduplex analysis. The evaluation of DNA fragment length, buffer additives and pH, sample salt effect, and separation temperature, allowed for optimization of the conditions for mutation detection. The discrimination between wild type and six mutations in *BRCA1* and *BRCA2* was achieved with a reasonably high detection sensitivity (1%–10% mutated DNA present) that was not disparate with sensitivities associated with conventional methods. The total time for screening each of the six mutations by this CE-based HDA was reasonably fast, considering that DNA purification (10 min), DNA amplification by PCR (1–2 hr), and HDA by CE could be completed in ~2.5 hr. Speed, combined with the benefit of semiautomated operation, indicates that this could be a powerful system for detecting mutations. The improvement in analysis time afforded by the microchip format highlights the potential of fast separation technologies as a general strategy for screening deletion, insertion, and substitution mutations. This is particularly the case when one begins to consider a high-throughput microchip platform exploiting multiple channels on the chip (Woolley et al. 1994, 1997; Huang et al. 1999) and the use of a multicolor detection system with different dye-labeled primers such as energy-transfer fluorescent primers (Ju et al. 1995; Glazer and Mathies 1997). As several research groups make advances toward carrying out PCR in volumes conducive to the microchip (Cheng et al. 1996, 1998; Shoffner et al. 1996; Woolley et al. 1996; Waters et al. 1998; Wilding et al. 1998; Oda et al. 1998), one can expect that the "integrated molecular diagnostic system," which will seamlessly integrate DNA purification, DNA amplification by PCR, and mutation detection into a single device, will become a reality.

METHODS

Reagents

GeneAmp thin-walled PCR tubes, 10× PCR buffer, 25 mM $MgCl_2$, 100 mM dNTPs stock solutions, and Taq DNA polymerase (5 unit/μl) were from Perkin-Elmer. Boric acid, ethylenediaminetetraacetic acid tetrasodium salt (EDTA) and tris-[hydroxymethyl]aminomethane (Tris) were from Sigma Chemical. Hydroxyethylcellulose (HEC, Mr 250,000) was from Aldrich Chemical Co. PicoGreen was from Molecular Probes. μSil-Fluorocarbon polymer (FC)-coated capillaries were from J & W Scientific, Inc. Microcon YM-100 filters were from Millipore Corp. AutoSeq G-50 columns were from Amersham Pharmacia Biotech. Polyvinylpyrrolidone (PVP, Mr 1,000,000) was from Acros Organics.

Table 1. Primers Used for Heteroduplex Analysis

Position	Primers	T_a	T_m	Size
185delAG (Exon 2, *BRCA1*)	Forward: 5′GAAGTTGTCATTTTATAAACCTTT-3′ Reverse: 5′-TGTCTTTTCTTCCCTAGTATGT-3′	53 53	56	258 bp
E1250X (Exon 11, *BRCA1*)	Forward 1: 5′-TCCAACACTTGTTATTTGGT-3′ Reverse 1: 5′-CCTTTGCCAATATTACCTG-3′ Forward 2: 5′-TTTCACCCATACACATTTG-3′ Reverse 1: 5′-CCTTTGCCAATATTACCTG-3′	53 53 53 53	56 56 	159 bp 260 bp
R1443G (Exon 13, *BRCA1*)	Forward: 5′-AGCTGTGTTAGAACAGCATG-3′ Reverse: 5′-TGTTGGAGCTAGGTCCTTAC-3′	54 54	56	204 bp
5382insC (Exon 20, *BRCA1*)	Forward 1: 5′-ATATGACGTGTCTGCTCCAC-3′ Reverse 1: 5′-AGTCTTACAAAATGAAGCGG-3′ Forward 1: 5′-ATATGACGTGTCTGCTCCAC-3′ Reverse 2: 5′-CCTGTGTGAAAGTATCTAGCAC-3′ Forward 1: 5′-ATATGACGTGTCTGCTCCAC-3′ Reverse 3: 5′-GGGAATCCAAATTACACAGC-3′	56 55 56 54 56 57	58 58 58 	257 bp 296 bp 399 bp
5677insA (Exon 24, *BRCA1*)	Forward 1: 5′-GTTGGACAGTGTAGCACTCTA-3′ Reverse 1: 5′-CCACTTTGTAAGCTCATTCTT-3′ Forward 2: 5′-ATGAATTGACACTAATCTCTGC-3′ Reverse 1: 5′-CCACTTTGTAAGCTCATTCTT-3′	53 54 54 54	56 56 	136 bp 223 bp
6174delT (Exon 11, *BRCA2*)	Forward: 5′-CACCTTGTGATGTTAGTTTGGA-3′ Reverse: 5′-TGGAAAAGACTTGCTTGGTACT-3′	58 58	58	201 bp

Genomic DNA Isolation

Blood was taken by venapuncture to a glass tube containing EDTA. DNA was purified directly from the whole blood by the solid phase extraction (SPE) method described in detail elsewhere (Tian et al. 2000a). Briefly, purification of DNA involves three steps: loading, washing, and eluting. Blood was diluted with a guanine hydrochloride (GuHCl)-based loading buffer (6 M GuHCl and 1% Triton-100 as the final concentration) by 60-fold and was loaded on the silica SPE cartridge (Supelco). The cartridge was washed in 80% isopropanol (20 bed volumes). DNA was eluted by 10 mM TE at pH 8.4 (18 bed volumes) from the SPE cartridge.

Genomic DNA was isolated from lymphoblastoid cell lines obtained from the individuals heterozygous for the mutations in *BRCA1* and *BRCA2* (Coriell Cell Repositories). All were used in an anonymous fashion in the study described. The concentrations of previously purified human genomic DNA were measured by PicoGreen dsDNA quantitation assay (Singer et al. 1997) before use. The presence of *BRCA1* or *BRCA2* mutations was confirmed by fluorescent dideoxy sequencing.

Polymerase Chain Reaction

Primers used to flank the six mutations were designed based on the *BRCA1* and *BRCA2* mRNA sequences on the Genome Database (http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/query and http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/ [1999]), and the genomic sequences on the web site of the Breast Cancer Information Core (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ [1999]). The primers were evaluated by the program at http://www.williamstone.com/primers/calculator/ and the estimate annealing temperatures for each primer are listed as T_a in Table 1. Unlabeled primers were used for optimizing PCR conditions and sequencing PCR products; 6-FAM-tagged primers were used to obtain the HDA profiles (ordered from Life Technologies). The sizes of the DNA fragments amplified for detecting each mutation are listed in Table 1. PCR amplifications of *BRCA1* and *BRCA2* alleles were carried out in a Progene thermocycler (Techne) with the following reagents in 50-µl reaction mixtures: 40–80 ng of genomic DNA, 0.2 µM of the appropriate primers (one is 6-FAM tagged for HDA), 1 mM dNTPs, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, and 2.5 or 5.0 U AmpliTaq polymerase. Each PCR reaction mixture was heated for 5 min at 95°C, followed by 35 cycles of 1 min at 94°C, 0.5 min at the annealing temperature (T_m) listed in Table 1, and 0.5 min at 72°C. A final 10-min extension at 72°C was used following the final temperature cycle.

CE-Based Heteroduplex Analysis

For obtaining the HD profiles, a Beckman P/ACE 5510 system with the P/ACE LIF detector (with the excitation at 488 nm [an argon ion laser] and the emission at 520 nm) was used. Capillary electrophoresis conditions were as follows: the FC-coated capillary was 50 µm (I.D.) by 27 cm (effective length 20 cm) for deletion and insertion mutants or 37 cm (effective length 30 cm) for substitution mutants; the separation buffer was 2.5% (w/v) HEC in 1 × TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA, pH 8.6 unless specified) containing 10% glycerol for detecting deletion and insertion mutants, 4.5% (w/v) HEC in 1 × TBE buffer, containing 10% glycerol and 15% urea for detecting substitution mutants. The PCR prod-

ucts were introduced into the capillary by electrokinetical injection for 20 sec at 370 V/cm (for deletions and insertions) or 270 V/cm (for substitution). The separation was carried out at 370 V/cm (for deletions and insertions) or 351 V/cm (for substitutions) using the reversed polarity (inlet as cathode and outlet as anode), and the capillary was maintained at 30°C or 20°C (for deletions and insertions) or 20°C (for substitutions).

Microchip-Based Heteroduplex Analysis

Single-channel glass microchips were purchased from Alberta Microelectronic Corporation (AMC). The microchannel on the chip was coated with PVP following the procedures in the references (Hofgärtner et al. 1999; Munro et al. 1999). After being coated, the channel was rinsed with water before rinsing with the separation buffer, 2.5% HEC containing 10% glycerol (for deletions and insertions) or 4.5% HEC containing 10% glycerol and 15% urea (for substitutions). Sample injection on microchip was performed by applying a 400-V (333V/cm) potential across the sample and sample waste reservoirs, with the sample at ground. For separation, the sample and sample waste were grounded, −400 V was applied to the inlet and 4300 V to the outlet (573 V/cm). A fluorescence detection system, which was described elsewhere (Munro et al. 1999), was used to detect the fluorescence intensity at 520 nm with an Argon ion laser (488 nm) as the excitation source. The data were collected by a LabView program at the rate of 15 Hz.

ACKNOWLEDGMENTS

We thank Nicole J. Munro for assistance with microchip electrophoresis; Andrea Jaquins-Gerstl, Therese Liebert, Jennifer L. Profozich, Bob F. Friday, and Dr. Massimo Trucco for assistance in synthesizing oligonucleotides; Biomedical Research Support Facilities, School of Medicine, University of Pittsburgh for providing DNA sequencing services; and Beckman Instruments for the equipment and sponsored research grants. We also want to thank Dr. Saijun Fan for providing the HCC 1937 breast cancer cells and Dr. David Mao for providing the FC-coated capillaries.

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REFERENCES

Arnold, N., Gross, E., Schwarz-Boeger, U., Pfisterer, J., Jonat, W., and Kiechle, M. 1999. A highly sensitive, fast, and economical technique for mutation analysis in hereditary breast and ovarian cancers. *Hum. Mutat.* **14:** 333–339.

Bhattacharyya, A. and Lilley, D.M.J. 1989. The contrasting structures of mismatched DNA sequences containing looped-out bases (bulges) and multiple mismatches (bubbles). *Nucleic Acid Res.* **17:** 6821–6840.

Bowen, D.J., Standen, G.R., Granville, S., Bowley, S., Wood, N.A., and Bidwell, J. 1997. Genetic diagnosis of factor V Leiden using heteroduplex technology. *Thromb. Haemost.* **771:** 119–122.

Breast Cancer Information Core. 1999. http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

Cantor, C.R. and Smith, C.L. 1999. *Genomics—The science and the technology behind the Human Genome Project*, pp. 448–469, 526–568. Wiley, New York.

Castilla, L.H., Couch, F.J., Erdos, M.R., Hoskins, K.F., Calzone, K., Garber, J.E., Boyd, J., Lubin, M.B., Deshano, M.L., Brody, L.C., et al. 1994. Mutations in the *BRCA1* gene in families with early-onset breast and ovarian cancer. *Nat. Genet.* **8:** 387–391.

Cheng, J., Kasuga, T., Mitchelson, K.R., Lightly, E.R., Watson, N.D., Martin, W.J., and Atkinson, D. 1994. Polymerase chain reaction heteroduplex polymorphism analysis by entangled solution capillary electrophoresis. *J. Chromatogr. A* **677:** 169–177.

Cheng, J., Shoffner, M.A., Hvichia, G.E., Kricka, L.J., and Wilding, P. 1996. Chip PCR. II. Investigation of different PCR amplification systems in microbabricated silicon-glass chips. *Nucleic Acids Res.* **24:** 380–385.

Cheng, J., Waters, L.C., Fortina, P., Hvichia, G., Jacobson, S.C., Ramsey, J.M., Kricka, L.J., and Wilding, P. 1998. Degenerate oligonucleotide primed-polymerase chain reaction and capillary electrophoretic analysis of human DNA on microchip-based devices. *Anal. Biochem.* **257:** 101–106.

Clausen-Schaumann, H., Rief, M., Tolksdorf, C., and Gaub, H.E. 2000. Mechanical stability of single DNA molecules. *Biophys. J.* **78:** 1997–2007.

De Santis, R. and Azzi, A. 2000. Use of denaturing gradient gel electrophoresis for human polyomavirus JC sequence analysis. *J. Virol. Methods* **85:** 101–108.

Faudoa, R., Xue, Z., Lee, F., Baser, M.E., and Hung, G. 2000. Detection of novel NF2 mutations by an RNA mismatch cleavage method. *Hum. Mutat.* **15:** 474–478.

Felsenfeld, A., Peterson, J., Schloss, J., and Guyer, M. 1999. Assessing the quality of the DNA sequence from the Human Genome Project. *Genome Res.* **9:** 1–4.

Gayther, S.A., Harrington, P., Russell, P., Kharkevich, G., Garkavtseva, R.F., and Ponder, B.A. 1996. Rapid detection of regionally clustered germ-line *BRCA1* mutations by multiplex heteroduplex analysis. *Am. J. Hum. Genet.* **58:** 451–456.

Gerrard, B. and Dean, M. 1998. Single-strand conformation polymorphism and heteroduplex analysis. In *Mutation detection-a practical approach* (ed. R.G.H. Cotton, E. Edkins, and S. Forrest), pp. 25–33. Oxford University Press, New York.

Glazer, A.N. and Mathies, R.A. 1997. Energy-transfer fluorescent reagents for DNA analyses. *Curr. Opin. Biotechnol.* **81:** 94–102.

Gross, E., Arnold, N., Goette, J., Schwarz–Boeger, U., and Kiechle, M. 1999. A comparison of BRCA1 mutation analysis by direct sequencing, SSCP and DHPLC. *Hum. Genet.* **105:** 72–78.

Gueron, M., Demaret, J., and Filoche, M. 2000. A unified theory of the B-Z transition of DNA in high and low concentrations of multivalent ions. *Biophys. J.* **78:** 1070–1083.

Guttman, A. and Schwartz, J.E. 1995. Artifacts related to sample introduction in CE affecting separation performance and quantitation. *Anal. Chem.* **67:** 2279–2283.

Hacia, J.G., Brody, L.C., Chee, M.S., Fodor, S.P., and Collins, F.S. 1996. Detection of heterozygous mutations in *BRCA1* using high density oligonucleotide arrays and two-colour fluorescence analysis. *Nat. Genet.* **14:** 441–447.

Hartge, P., Struewing, J.P., Wacholder, S., Brody, L.C., and Tucker, M.A. 1999. The prevalence of common *BRCA1* and *BRCA2* mutations among Ashkenazi Jews. *Am. J. Hum. Genet.* **64:** 963–970.

Hayashi, K., Kukita, Y., Inazuka, M., and Tahira, T. 1998. Single-strand conformation polymorphism. In *Mutation detection a practical approach* (ed. R.G.H. Cotton, E. Edkins, and S. Forrest), pp. 7–23. Oxford University Press, New York.

Hofgärtner, W.T., Hühmer, A.F.R., Landers, J.P., and Kant, J.A. 1999. Rapid diagnosis of Herpes simplex encephalitis using microchip electrophoresis of PCR products. *Clin. Chem.* **45:** 2120–2128.

Hsu, I.C., Yang, Q., Kahng, M.W., and Xu, J.F. 1994. Detection of DNA point mutations with DNA mismatch repair enzymes. *Carcinogenesis* **15:** 1657–1662.

Huang, Z., Munro, N.J., and Landers, J.P. 1999. Acousto-optic-based scanning of capillary and chip arrays for multichannel fluorescence detection of DNA. *Anal. Chem.* **71:** 5309–5314.

Iannone, M.A., Taylor, J.D., Chen, J., Li, M.S., Rivers, P., Slentz-Kesler, K.A., and Weiner, M.P. 2000. Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry. *Cytometry* **39:** 131–140.

Jackson, H.A., Bowen, D.J., and Worwood, M. 1997. Rapid genetic screening for haemochromatosis using heteroduplex technology.

Br. J. Haematol. **98:** 856–859.

Ju, J, Ruan, C., Fuller, C.W., Glazer, A.N., and Mathies, R.A. 1995. Fluorescence energy transfer dye-labeled primers for DNA sequencing and analysis. *Proc. Natl. Acad. Sci.* **92:** 4347–4351.

Keen, J., Lester, D., Inglehearn, C., Curtis, A., and Bhattacharya, S. 1991. Rapid detection of single base mismatches as heteroduplexes on Hydrolink gels, *Trends Genet.* **7:** 5.

Landers, J.P. 1997. Introduction to capillary electrophoresis. In *Handbook of capillary electrophoresis* (ed. J.P. Landers), 2d ed., pp. 1–47. CRC, Boca Raton, FL.

Liu, W., Smith, D.I., Rechtzigel, K.J., Thibodeau, S.N., and James, C.D. 1998. Denaturing high performance liquid chromatography (DHPLC) used in the detection of germline and somatic mutations. *Nucleic Acids Res.* **26:** 1396–1400.

Mansukhani, M.M., Nastiuk, K.L., Hibshoosh, H., Kularatne, P., Russo, D., and Krolewski, J.J. 1997. Convenient, nonradioactive, heteroduplex-based methods for identifying recurrent mutations in the *BRCA1* and *BRCA2* genes. *Diagn. Mol. Pathol.* **6:** 229–237.

Markoff, A., Sormbroen, H., Bogdanova, N., Preisler-Adams, S., Ganev, V., Dworniczak, B., and Horst, J. 1998. Comparison of conformation-sensitive gel electrophoresis and single-strand conformation polymorphism analysis for detection of mutations in the *BRCA1* gene using optimized conformation analysis protocols. *Eur. J. Hum. Genet.* **6:** 145–150.

Munro, N.J., Snow, K., Kant, J.A., and Landers, J.P. 1999. Molecular diagnostics on microfabricated electrophoretic devices: From slab gel to capillary to microchip-based assays for T- and B-cell lymphoproliferative disorders. *Clin. Chem.* **45:** 1906–1917.

Nakano, S., Fujimoto, M., Hara, H., and Sugimoto, N. 1999. Nucleic acid duplex stability: Influence of base composition on cation effects. *Nucleic Acids Res.* **27:** 2957–2965.

Nataraj, A. J., Olivos-glander, I., Kusukawa, N., and Highsmith Jr., W.E. 1999. Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis* **20:** 1177–1185.

Oda, R.P., Strausbauch, M.A., Huhmer, A.F., Borson, N., Jurrens, S.R., Craighead, J., Wettstein, P.J., Eckloff, B., Kline, B., and Landers, J.P. 1998. Infrared-mediated thermocycling for ultrafast polymerase chain reaction amplification of DNA. *Anal. Chem.* **70:** 4361–4368.

Ozcelik, H., Antebi, Y.J., Cole, D.E.C., and Andrulis, I.L. 1996. Heteroduplex and protein truncation analysis of the *BRCA1 185delAG* mutation. *Hum. Genet.* **98:** 310–312.

Pancholi, P., Oda, R.P., Mitchell, P.S., Landers, J.P., and Persing, D.H. 1997. Diagnostic detection of herpes simplex and hepatitis C viral amplicons by capillary electrophoresis: Comparison with Southern blot detection. *Mol. Diagn.* **2:** 27–37.

Piggee, C.A., Muth, J., Carrilho, E., and Karger, B.L. 1997. Capillary electrophoresis for the detection of known point mutations by single-nucleotide primer extension and laser-induced fluorescence detection. *J. Chromatogr. A.* **781:** 367–375.

Poncin, J., Mulkens, J., Arends, J.W., and de Goeij, A. 1999. Optimizing the APC gene mutation analysis in archival colorectal tumor tissue. *Diagn. Mol. Pathol.* **8:** 11–19.

Ren, J. and Ueland, P.M. 1999. Temperature and pH effects on single-strand conformation polymorphism analysis by capillary electrophoresis. *Hum. Mutat.* **13:** 458–463.

Ren, J., Ulvik, A., Ueland, P.M., and Refsum, H. 1997. Analysis of single-strand conformation polymorphism by capillary electrophoresis with laser-induced fluorescence detection using short–chain polyacrylamide as sieving medium. *Anal. Biochem.* **245:** 79–84.

Shi, Y., Simpson, P.C., Scherer, J.R., Wexler, D., Skibola, C., Smith, M.T., and Mathies, R.A. 1999. Radial capillary array electrophoresis microplate and scanner for high-performance nucleic acid analysis. *Anal. Chem.* **71:** 5354–5361.

Shoffner, M.A., Cheng, J., Hvichia, G.E., Kricka, L.J., and Wilding, P. 1996. Chip PCR. I. Surface passivation of microfabricated silicon-glass chips for PCR. *Nucleic Acids Res.* **24:** 375–379.

Singer, V.L., Jones, L.J., Yue, S.T., and Haugland, R.P. 1997. Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. *Anal. Biochem.* **249:** 228–238.

Struewing, J.P., Brody, L.C., Erdos, M.R., Kase, R.G., Giambarresi, T.R., Smith, S.A., Collins, F.S., and Tucker, M.A. 1995. Detection of eight BRCA1 mutations in 10 breast/ovarian cancer families, including 1 family with male breast cancer. *Am. J. Hum. Genet.* **57:** 1–7.

Struewing, J.P., Hartge, P., Wacholder, S., Baker, S.M., Berlin, M., McAdams, M., Timmerman, M.M., Brody, L.C., and Tucker, M.A. 1997. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *New Engl. J. Med.* **336:** 1401–1408.

Tian, H., Hühmer, A.F.R., and Landers, J.P. 2000a. Evaluation of silica resins for direct and efficient extraction of DNA from complex biological matrices in a miniaturized format. *Anal. Biochem.* **283:** 175–191.

Tian, H., Jaquins-Gerstl, A., Munro, N.J., Trucco, M., Brody, L.C., and Landers, J.P. 2000b. Single-strand conformation polymorphism analysis by capillary and microchip electrophoresis: A fast, simple method for detection of common mutations in *BRCA1* and *BRCA2*. *Genomics* **63:** 25–34.

Toliat, M.R., Erdogan, F., Gewies, A., Fahsold, R., Buske, A., Tinschert, S., Nurnberg, P. 2000. Analysis of the NF1 gene by temperature gradient gel electrophoresis reveals a high incidence of mutations in exon 4b. *Electrophoresis* **21:** 541–544.

Tong, D., Stimpfl, M., Reinthaller, A., Vavra, N., Mullauer–Ertl, S., Leodolter, S., and Zeillinger, R. 1999. *BRCA1* gene mutations in sporadic ovarian carcinomas: Detection by PCR and reverse allele-specific oligonucleotide hybridization. *Clin. Chem.* **45:** 976–981.

Ulfelder, K.J., Schwartz, H.E., Hall, J.M., and Sunzeri, F.J. 1992. Restriction fragment length polymorphism analysis of ERBB2 oncogene by capillary electrophoresis. *Anal. Biochem.* **200:** 260–267.

Waters, L.C., Jacobson, S.C., Kroutchinina, N., Khandurina, J., Foote, R.S., and Ramsey, J.M. 1998. Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing. *Anal. Chem.* **70:** 158–162.

White, M.B., Carvalho, M., Derse, D., O'Brien, S.J., and Dean, M. 1992. Detecting single base substitutions as heteroduplex polymorphisms. *Genomics* **12:** 301–306.

Wilding, P., Kricka, L.J., Cheng, J., Hvichia, G., Shoffner, M.A., and Fortina, P. 1998. Integrated cell isolation and polymerase chain reaction analysis using silicon microfilter chambers. *Anal. Biochem.* **257:** 95–100.

Woolley, A.T., Hadley, D., Landre, P., deMello, A.J., Mathies, R.A., and Northrup, M.A. 1996. Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. *Anal. Chem.* **68:** 4081–4086.

Woolley, A.T. and Mathies, R.A. 1994. Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. *Proc. Natl. Acad. Sci.* **91:** 11348–11352.

Woolley, A.T., Sensabaugh, G.F., and Mathies, R.A. 1997. High-speed DNA genotyping using microfabricated capillary array electrophoresis chips. *Anal. Chem.* **69:** 2181–2186.

Received January 24, 2000; accepted in revised form July 12, 2000.