

Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes

(polymorphism and mutation detection/denaturing gradient gel electrophoresis/sickle-cell anemia/hemoglobin C)

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ABSTRACT Denaturing gradient gel electrophoresis (DGGE) can be used to distinguish two DNA molecules that differ by as little as a single-base substitution. This method detects ≈50% of all possible single-base changes in DNA fragments ranging from 50 to ≈1000 base pairs. To increase the number of single-base changes that can be distinguished by DGGE, we used the polymerase chain reaction to attach a 40-base-pair G+C-rich sequence, designated a GC-clamp, to one end of amplified DNA fragments that encompass regions of the mouse and human β -globin genes. We show that this GC-clamp allows the detection of mutations, including the hemoglobin sickle (HbS) and hemoglobin C (HbC) mutations within the human β -globin gene, that were previously indistinguishable by DGGE. In addition to providing an easy way to attach a GC-clamp to genomic DNA fragments, the polymerase chain reaction technique greatly increases the sensitivity of DGGE. With this approach, DNA fragments derived from <5 ng of human genomic DNA can be detected by ethidium bromide staining of the gel, obviating the need for radioactive probes. These improvements extend the applicability of DGGE for the detection of polymorphisms and mutations in genomic and cloned DNA.

Genetic linkage analysis has been severely limited in humans due to a paucity of informative polymorphic loci. The recognition that DNA sequence polymorphism can fill this void has revolutionized the field of human genetics (1, 2). Consequently, approaches that allow the detection of single-base differences in specific regions of genomic DNA have been extremely powerful for both linkage analysis and direct detection of mutations associated with human disease (3-9). The vast majority of base changes have been identified by the restriction fragment length polymorphism approach, which measures DNA sequence alterations due to a loss or gain of a restriction enzyme cleavage site or to variation in length caused by deletion or insertion (1). However, many single-base changes do not alter a restriction enzyme cleavage site and, therefore, cannot be detected by the restriction fragment length polymorphism method. One alternative method that makes it possible to detect a larger fraction of all possible base changes in a DNA fragment is denaturing gradient gel electrophoresis (DGGE; refs. 10-13).

DGGE is a gel system that separates DNA fragments according to their melting properties (10-12). When a DNA fragment is electrophoresed through a linearly increasing gradient of denaturants, the fragment remains double stranded until it reaches the concentration of denaturants equivalent to a melting temperature (t_m) that causes the lower-temperature melting domains of the fragment to melt. At this point, the branching of the molecule caused by partial

melting sharply decreases the mobility of the fragment in the gel. The lower-temperature melting domains of DNA fragments differing by as little as a single-base substitution will melt at slightly different denaturant concentrations because of differences in stacking interactions between adjacent bases in each DNA strand. These differences in melting cause two DNA fragments to begin slowing down at different levels in the gel, resulting in their separation from each other. However, DGGE will not separate DNA fragments differing by a base change in the highest temperature melting domain due to loss of sequence-dependent gel migration upon complete strand separation (14, 15). Because of this limitation, it is estimated that on average, only 50% of all the possible single-base changes in DNA fragments between 50 base pairs (bp) and several hundred base pairs long can be detected by DGGE (14-16). In a previous study, we were able to overcome this problem by cloning a high-temperature melting G+C-rich sequence, designated a GC-clamp, into a plasmid vector next to the mouse β^{major} globin promoter (14, 15). This cloned GC-clamp increased the number of mutations in the promoter detectable by DGGE from 40% of all possible single-base changes to close to 100%.

The polymerase chain reaction (PCR; refs. 17-19) is a method that uses two opposing oligonucleotides to amplify fragments of genomic DNA manyfold. The observation that short segments of DNA coding for restriction enzyme cleavage sites can be incorporated into the ends of PCR-amplified DNA fragments (18) suggested a mechanism for attaching short GC-clamps to genomic DNA fragments. Even though our initial cloned GC-clamp was 300 bp long, theoretical considerations (14, 15) and recent experimental results (E. Abrams and L.S.L., unpublished results) indicated that a GC-clamp as short as 30 bp would be sufficient to use with the DGGE system. Here, we show that 40- to 45-bp GC-clamps can be attached to amplified cloned and genomic DNA fragments by using the PCR. Using both cloned mouse β -globin promoter mutants and genomic DNA from humans with hemoglobinopathies, we show that such GC-clamps allow the separation of single-base mutations by DGGE that otherwise cannot be separated. The huge amplification of the genomic DNA fragments by the PCR also allows the signals to be detected by direct ethidium bromide staining of the gel, making the use of radioactive probes unnecessary.

MATERIALS AND METHODS

Materials. The wild-type and mutant cloned mouse β^{major} globin promoter fragments were constructed as described

Abbreviations: DGGE, denaturing gradient gel electrophoresis; PCR, polymerase chain reaction; t_m , melting temperature; nt, nucleotide(s); HbA, HbC, and HbS, normal hemoglobin and mutant hemoglobins C and sickle, respectively.

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(20). Genomic DNA samples from an individual with normal hemoglobin (HbA) alleles was prepared as described (21). Genomic DNA samples containing hemoglobin sickle (HbS) and hemoglobin C (HbC) alleles were provided by D. Higgs (Oxford) and H. Kazazian (Baltimore). Oligonucleotides were synthesized on an Applied Biosystems 480B DNA synthesizer and purified by denaturing acrylamide gel electrophoresis. The base sequences of the oligonucleotides are as follows: primer 1 (20-mer), 5'-GATTCCGTAGAGCCA-CACCC-3'; primer 2 (20-mer), 5'-AACACA ACTATGTCA-GAAGC-3'; primer 3 (60-mer), 5'-CGCCCGCCGCGCCCC-GCGCCCGTCCCGCCGCCCCGCGCGGATTCCGTA-GAGCCACACCC-3'; primer 4 (20-mer), 5'-GCTTCTGAC-ACA ACTGTGTT-3'; primer 5 (20-mer), 5'-CACCACCAA-CTTCATCCACG-3'; primer 6 (65-mer), 5'-GCGGGCGGG-GCGGGGGCACGGGGGGCGCGGGCGGGGGC-GGGGGGCTTCTGACACA ACTGTGTT-3'.

The pairs of primers 1 plus 2 or 3 plus 2 are used to amplify the mouse β^{major} globin promoter region from position -100 to position +26 relative to the cap site (20). Primer pairs 4 plus 5 or 6 plus 5 used to amplify the region of the human β -globin gene from position +6 to position +129 relative to the cap site.

PCR. The PCR conditions used were similar to those described in refs. 22 and 23. Briefly, 0.1–10 pg of cloned DNA or 50 ng of human genomic DNA was mixed with 50 pmol of each appropriate oligonucleotide primer and 75 nmol of each deoxyribonucleoside triphosphate in 50 μ l of PCR buffer [67 mM Tris·HCl, pH 8.8/6.7 mM MgCl₂/16 mM ammonium sulfate/10 mM 2-mercaptoethanol/10% (vol/vol) dimethyl sulfoxide]. One unit of *Thermus aquaticus* DNA polymerase (Perkin-Elmer/Cetus) was added and 100 μ l of mineral oil was layered over the samples. The samples were incubated at 93°C, 55°C, and 70°C for 1 min, 1 min, and 1.5 min, respectively; this cycle of incubations was manually repeated 30–40 times. The aqueous layers were transferred to another test tube, extracted with phenol, and precipitated with ethanol. The pellets were resuspended in 50 μ l of nondenaturing loading buffer [15% (vol/vol) Ficoll 400/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.1% orange G dye]. About 10% of the total amplified material was used in the agarose gel and the DGGE analysis.

DGGE. The gel apparatus and conditions for DGGE were exactly as described in ref. 13. A portion (5 μ l) of each PCR-amplified DNA fragment was electrophoresed in a 10% polyacrylamide gel with a linearly increasing gradient from 40% denaturant to 75% denaturant [100% denaturant = 7 M urea/40% (vol/vol) formamide] at 150 V for the lengths of time indicated in the figure legends. After electrophoresis, the gels were stained in ethidium bromide (2 μ g/ml) for 10 min and photographed by UV transillumination with Polaroid type 55 positive/negative film.

RESULTS

Use of the Cloned Mouse β^{major} Globin Promoter Region as a Test System. Because >135 single-base substitutions in the mouse β^{major} globin promoter region were available (20), we used this fragment as a test for the method in our initial studies. In an earlier study, a computer algorithm that uses the nucleotide sequence of a DNA fragment to predict melting behavior (24) indicated that this DNA promoter fragment melts in two distinct melting domains (14, 15). Extensive experimental analysis has confirmed that these melting predictions are accurate (14, 15, 20). A convenient way to display the predicted melting behavior of a DNA fragment is a melting map, which is a plot of t_m (the temperature at which each base pair in a molecule has a 50% chance of being helical or melted) versus the nucleotide sequence of the molecule. The melting map of the 126-bp

promoter fragment (Fig. 1A) shows that the 3' half of the promoter melts as a single domain with a t_m of $\approx 70^\circ\text{C}$ (designated domain 1), while the 5' half of the promoter melts as a single domain with a t_m of $\approx 75^\circ\text{C}$ (designated domain 2). On the basis of melting predictions, promoter fragments carrying mutations in domain 2 should not separate by DGGE; experiments with a large number of mutant promoters have confirmed these predictions (14, 15, 20). The same type of calculations performed for the promoter fragment containing a 40-bp GC-clamp at its 5' end predict that the fragment has a similar domain structure in the promoter region, but has an additional high-temperature melting domain with a t_m of 90°C at its 5' end (Fig. 1A). Thus, addition of the GC-clamp to the promoter fragment should allow the detection of mutations in domain 2 by DGGE.

To test this prediction, two sets of oligonucleotides were used to amplify the 126-bp promoter fragment from small amounts of cloned DNA. One set of oligonucleotides was comprised of two 20-mers (designated primers 1 and 2) that amplified the fragment from end to end, resulting in a 126-bp "nonclamped" fragment (Fig. 1B). The second set of oligonucleotides was comprised of one 20-mer (primer 2), the same used at the 3' end of the promoter fragment above) and a second oligonucleotide (primer 3) that is 60 nucleotides (nt) long. The 40 nt at the 5' end of primer 3 are composed of guanines and cytosines, whereas the remaining 20 nt at the 3' end are composed of the same sequence as primer 1 (Fig. 1B). In the initial round of DNA synthesis, only the 20 nt at

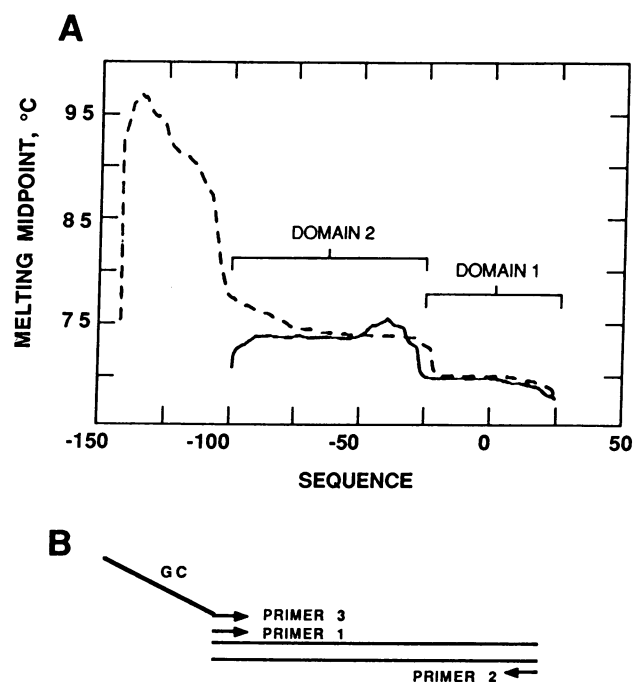


FIG. 1. Melting calculations for the mouse β^{major} globin promoter region. (A) Melting maps for the mouse promoter fragment. The t_m is plotted as a function of the nucleotide sequence position of the DNA fragment. The lines show the temperatures at which each base pair is in 50:50 equilibrium between the helical and melted configurations. At temperatures below this line, a base pair will be helical, and at temperatures above the line, it will be melted. The solid line is the melting map for the 126-bp fragment, and the dashed line is for the 126-bp fragment carrying a 40-bp GC-clamp at its 5' (position -100) end. (B) The mouse β^{major} globin fragment amplified by PCR for these studies. When primers 1 and 2 were used in the reaction, a fragment of 126 bp extending from position -100 to position +26 relative to the mRNA cap site (20) was amplified. Primers 3 and 2 amplify the same fragment, but also attach a 40-bp GC-clamp to the end at position -100, resulting in an amplified DNA fragment of 166 bp.

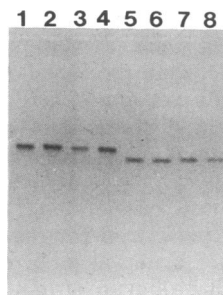


FIG. 2. PCR amplification of the mouse β^{major} globin promoter region. This figure shows a negative image of an ethidium-stained agarose gel containing the PCR-amplified products of the cloned mouse promoter region. Primers 2 and 3 amplify a fragment of 166 bp, which include the wild-type (lane 1) and mutant (lanes 2–4) promoter fragments attached to a GC-clamp. Primers 1 and 2 amplify a fragment of 126 bp, comprised of the wild-type (lane 5) and mutant (lanes 6–8) promoter fragments without a GC-clamp. Each lane contains about one-tenth of the total products from the PCR amplification. The same quantities of a single band of amplified human globin DNA fragments were obtained under similar conditions in the genomic DNA experiments, even though the amount of starting template was much lower (data not shown).

the 3' end of primer 3 were capable of annealing to the DNA sample, leaving the 40 nt at the 5' end of the G+C-rich region unpaired. After the first round of amplification, however, the entire 60 nt were incorporated into the fragments, resulting in amplified promoter fragments with the GC-clamp attached at one end. The PCR conditions that were used for this and subsequent fragments resulted in single DNA fragments of the appropriate sizes when examined by agarose gel electrophoresis (Fig. 2). In these examples, PCR amplification of the cloned mouse promoter fragments with primers 1 and 2 resulted in fragments 126 bp long (lanes 5–8), whereas amplification with primers 2 and 3 yielded fragments 166 bp long (lanes 1–4), a result of the incorporation of the additional 40-bp GC-clamp.

The PCR procedure was used with primers 1 and 2 or primers 2 and 3 to amplify the wild-type promoter fragment and several cloned DNA samples carrying single-base substitution mutations in melting domain 2. The resulting amplified DNA fragments were then subjected to electrophoresis in a denaturing gradient gel under conditions that placed the fragments in a position of the gel such that domain 2 was melted. As shown in Fig. 3A, the wild-type and mutant DNA fragments amplified with primers 1 and 2, which do not carry the GC-clamp, did not separate from the wild-type fragment in the gel. However, when the same fragments were amplified with primers 2 and 3, the mutant DNA fragments separated from the wild-type fragment and from each other (Fig. 3B). Amplification of 25 additional domain 2 mutants gave similar results (data not shown). These data indicate that the PCR procedure can be used to attach a 40-bp GC-clamp to cloned DNA fragments and that the GC-clamp allows the detection of single-base substitutions present at positions in the fragments that otherwise would not be detected.

Analysis of Single-Base Substitutions in Human Genomic DNA. We used a similar strategy to examine a region of the human β -globin gene known to carry at least two clinically significant mutations: the HbS mutation and the HbC mutation. In the melting map shown in Fig. 4A, the HbS and HbC mutations lie in the highest temperature melting region of the fragment in the absence of a GC-clamp; therefore, mutations in this region should not be resolved by DGGE. However, melting calculations predict that attachment of a 45-bp GC-clamp to the 5' end of the fragment results in a single melting domain (with a t_m of 75°C) for the β -globin portion of the fragment, and a second domain with a t_m of 95°C for the GC-clamp.

Two additional sets of oligonucleotides were synthesized to amplify the 123-bp region of DNA in the human β -globin gene around the HbS and HbC loci (Fig. 4B). One set, designated primers 4 and 5, was used to amplify the 123-bp region directly without attaching a GC-clamp, whereas the second set, designated primers 5 and 6, resulted in the amplification of the 123-bp fragment with an additional 45-bp

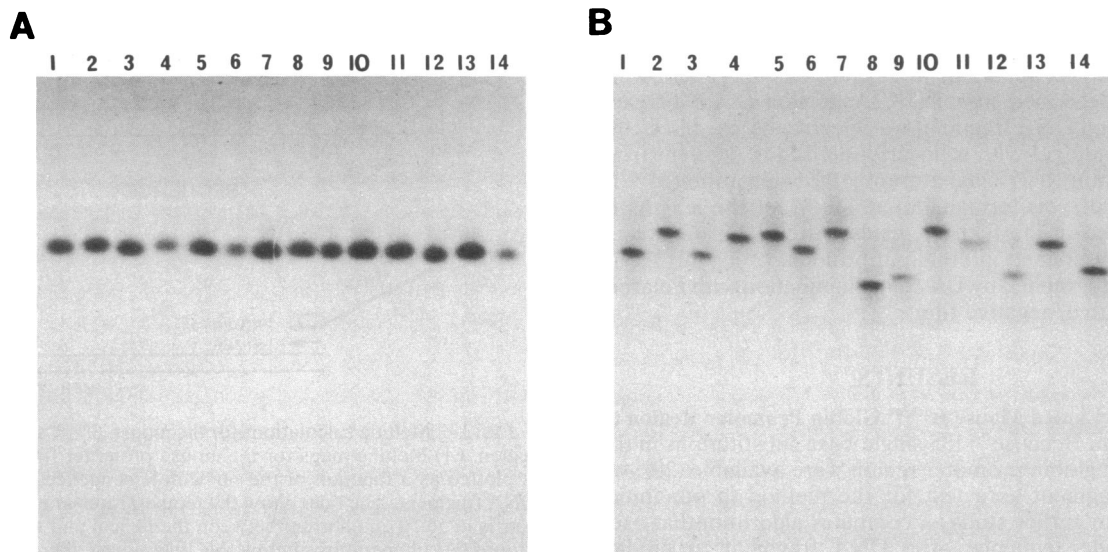


FIG. 3. DGGE analysis of amplified mouse β^{major} globin promoter fragments. (A) A negative image of an ethidium bromide-stained denaturing gradient gel containing the wild-type and 13 mutant promoter fragments PCR-amplified with primers 1 and 2, which do not attach a GC-clamp to the fragment. Electrophoresis was at 150 V for 4 hr; the final positions of the DNA fragments in the gel are equivalent to a temperature of $\approx 76^\circ\text{C}$. The mutant promoters, designated by the nucleotide substitution and the position from the mRNA cap site, are as follows. Lanes: 1, C⁻⁶⁸; 2, A⁻⁶⁵; 4, T⁻⁶³; 5, A⁻³³; 6, A⁻³²; 7, A⁻³⁹; 8, C⁻⁴⁵; 9, G⁻⁴⁸; 10, A⁻⁴⁹; 11, T⁻⁵⁴; 12, G⁻⁵⁹; 13, A⁻⁶³; and 14, C⁻⁶⁶. (B) A negative image of an ethidium bromide-stained denaturing gradient gel containing the wild-type (lane 3) and the same 13 mutant (lanes 1–2 and 4–14) promoter fragments shown in A, but PCR-amplified with primers 2 and 3, which introduce a 40-bp GC-clamp at the 5' end of the fragment. Electrophoresis was at 150 V for 5 hr; the final positions of the DNA fragments in the gel are equivalent to a temperature of $\approx 76^\circ\text{C}$. Note that the electrophoresis time for these fragments was 1 hr longer than in A since they are 40 bp longer than the nonclamped fragments.

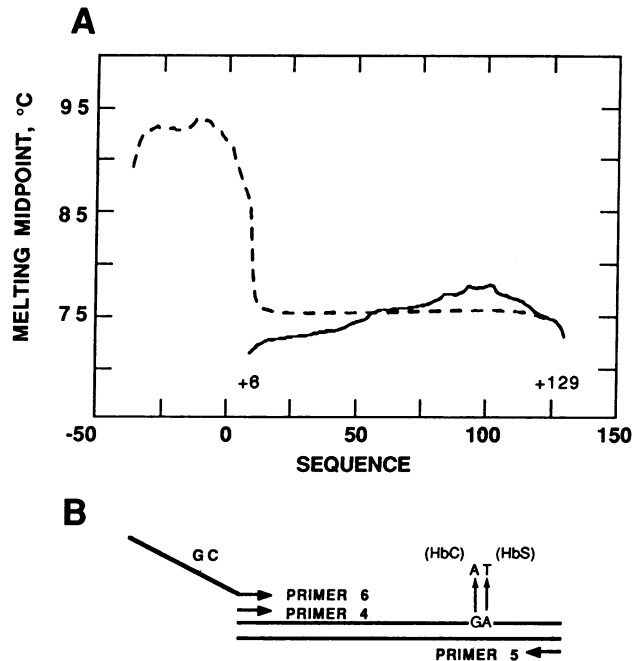


FIG. 4. Melting calculations for the human β -globin gene region containing the HbS and HbC mutations. (A) A melting map for the human β -globin gene fragment. Calculations and plotting of the melting behavior are as in Fig. 1A. The solid line is the melting map for the 123-bp human fragment in the absence of a GC-clamp, and the dashed line shows the melting behavior for the same fragment with a 45-bp GC-clamp attached to its 5' end at position +6. (B) The region of the human β -globin gene amplified by the PCR for these studies. Primers 4 and 5 amplify a fragment of 123 bp from position +6 to position +129 relative to the mRNA cap site, whereas primers 6 and 5 amplify the same fragment but also attach a 45-bp GC-clamp to its 5' end at position +6. The mutations resulting in sickle cell anemia (HbS) and HbC, which occur at positions +70 and +69, respectively, are indicated.

GC-clamp at its 5' end (Fig. 4B). The PCRs were performed with each set of primers and 50 ng of genomic DNA from four individuals. Individual 1 carried β -globin alleles of the HbA sequence; individual 2 had sickle cell anemia and was, therefore, homozygous for the HbS allele; individual 3 was homozygous for the HbC allele; and individual 4 was a compound heterozygote carrying one HbS allele and one HbC allele.

A portion of each PCR-amplified genomic DNA sample was electrophoresed in a denaturing gradient gel under conditions that placed the DNA molecules in a region of the gel such that the β -globin sequences were melted. The gel was stained with ethidium bromide and examined by UV-transillumination. As predicted, when the four DNA samples were amplified with primers 4 and 5, no separation of the fragments occurred in the gel (Fig. 5A). However, when a GC-clamp was attached to the amplified genomic DNA fragments with primers 5 and 6, separation in the gel was observed (Fig. 5B). The HbA alleles from individual 1 (lane 5) resulted in a single band in the gel that separated slightly from the single band representing the two HbS alleles from individual 2 (lane 6). The single band resulting from the two HbC alleles from individual 3 also separated from both the HbA allele and the HbS allele (lane 7). Four bands were seen on the gel when DNA from individual 4, an HbS/HbC heterozygote, was examined (lane 8). The lower band corresponds to the homoduplex DNA fragment of the HbS allele, the second from the lowest band corresponds to the homoduplex HbC allele fragment, and the two upper bands correspond to the two heteroduplex DNA fragments formed

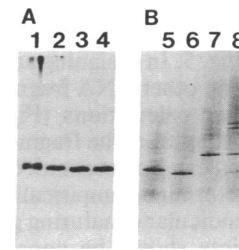


FIG. 5. Effect of a GC-clamp on DGGE analysis of the human β -globin gene region containing the HbS and HbC mutations. (A) A negative image of an ethidium bromide-stained denaturing gradient gel containing β -globin DNA fragments PCR-amplified from human genomic DNA with primers 4 and 5, which does not introduce a GC-clamp onto the fragments. Genomic DNAs from the following individuals were used. Lanes: 1, homozygous normal HbA; 2, homozygous HbS; 3, homozygous HbC; 4, heterozygous HbS/HbC. Electrophoresis was at 150 V for 5.5 hr; the final position of the fragments in the gel is equivalent to a temperature of 78°C. (B) A negative image of an ethidium bromide-stained denaturing gradient gel containing β -globin DNA fragments PCR-amplified from human genomic DNA with primers 5 and 6, which attach a 45-bp GC-clamp at the 5' end of the fragments. The same genomic DNA samples in A were used. Lanes: 5, HbA/HbA; 6, HbS/HbS; 7, HbC/HbC; 8, HbS/HbC. Electrophoresis was at 150 V for 6.5 hr; the final position of the farthest migrating fragments in the gel is equivalent to \approx 78°C.

by annealing of the strands of the HbS allele to those of the HbC allele. In agreement with earlier studies (12, 25), these heteroduplexes melt at higher positions in the gel because they are destabilized due to the single-base mismatches in the fragments. Heteroduplexes form in the PCR because the double-stranded DNA fragments are separated and reannealed during the thermal cycling of the reaction, allowing reassortment of the DNA strands from different alleles.

DISCUSSION

The results in this paper illustrate an approach for extending the usefulness of DGGE for detecting genetic variation. This approach is particularly applicable where the site of mutation or polymorphism is not known in advance and it is desirable to screen up to several hundred base pairs in a single test. Short (40- to 45-bp) GC-clamps, attached to genomic or cloned DNA fragments by the PCR, alter the melting properties of the DNA fragments such that otherwise inaccessible single-base mutations can be detected by the gel system, while the PCR amplification increases the ease and sensitivity of detection of mutations in genomic DNA. In previous studies (12) in which human genomic DNA fragments were examined by DGGE, 5–10 μ g of genomic DNA was required for each analysis, and the complex mixture of DNA fragments in genomic DNA required that radioactively labeled DNA or RNA probes be used for the detection. By contrast, the amplification by PCR presented here allows each analysis to be done with <5 ng of genomic DNA, and the results can be examined directly by ethidium bromide staining. This feature not only increases the speed of the detection of the signal, but also makes the construction of a probe unnecessary.

Both melting theory and experiments suggest that attachment of a GC-clamp to either end of almost all DNA fragments in the size range from 50 to several hundred base pairs improves the resolution of separation of most mutant DNA fragments by DGGE. In some cases, the attachment of a GC-clamp to one end of a DNA fragment alters the melting behavior of a domain in a way that simplifies the choice of denaturant conditions to use. For instance, the clamp attached to the human β -globin fragment in Fig. 4A smooths out the melting profile of the fragment into a single melting

domain; this effect should allow any base change within the DNA fragment to be detected in the same type of denaturing gradient gel used in Fig. 5. In designing an approach for using this system to examine other DNA fragments, it is useful to perform the computer calculations (Figs. 1A and 4A) to predict the melting behavior of the fragment so that optimum denaturant conditions can be determined. Alternatively, gel conditions can be determined empirically by examining the fragments in a perpendicular denaturing gradient gel (13, 20).

Several other points should be considered in designing oligonucleotides for this approach. The length of the GC-clamp is not critical; 40- and 45-bp clamps worked equally well in the present study, and it is likely that 30 bp would be effective. Although the nucleotide sequence of the GC-clamp is also not critical, it would be prudent to avoid using sequences that contain indirect repeats. In addition, because DNA synthesizers have some difficulty generating multiple adjacent guanosine residues, it is recommended that the GC-clamp portion of the oligonucleotides be composed of mostly cytidine residues, as is primer 3.

Although the sizes of the PCR-amplified DNA fragments reported here are <200 bp, it is possible to use fragments up to \approx 1000 bp with this system. DNA fragments larger than 1000 bp are difficult to examine by DGGE because they migrate very slowly in the polyacrylamide gels and because the degree of separation between mutant and wild-type fragments decreases with size due to the melting of multiple melting domains in the larger fragments. We do not know the upper limit in size of the attached DNA fragment for which a short GC-clamp will be effective. However, we have found that a similar clamp works well with a 500-bp region including the human β -globin promoter, exons 1 and 2, and intron 1 (data not shown). One potential way to maximize the resolution of mutations in a large (1000-bp) DNA fragment would be to attach GC-clamps to each end by the PCR and to digest the amplified fragment with a restriction enzyme such that two clamped DNA fragments are generated. An additional increase in resolution could be obtained by examining heteroduplexes rather than homoduplexes after PCR amplification. Heteroduplexes occur during PCR when an individual is heterozygous at the locus being examined (for example, see Fig. 5, lane 8). Alternatively, heteroduplexes between the PCR-amplified alleles and a wild-type probe can be formed (12, 23).

The overall error frequency of *Thermus aquaticus* DNA polymerase after 30 cycles of amplification by PCR is estimated to be 0.25% (26, 27). Consistent with this error rate is a background smear of bands, apparently comprised of DNA fragments with mutations introduced by the polymerase, occurring in the denaturing gradient gels in some of the experiments performed in our study (Figs. 3 and 5 and data not shown). However, this smear is not large enough to cause ambiguous interpretation of the data, even when fragments as large as 500 bp are examined. It should be noted that the smear of bands observed in DGGE after PCR amplification largely occurs in the region of the gel migrating slower than the genuine test band (for example, see Fig. 4B). This result is expected since mutant DNA fragments form heteroduplexes during the cycles of denaturation and reannealing in the PCR amplification, resulting in a majority of fragments melting at temperatures lower than the homoduplexes.

Currently, it is possible to distinguish the HbA, HbS, and HbC alleles by using several radioactively labeled or biotinylated oligonucleotides as hybridization probes with PCR-amplified DNA (28, 29). The HbS allele can also be directly detected by restriction enzyme cleavage, and often the

presence of the HbC allele can be deduced by haplotype analysis (4, 6, 8, 30). The test that we have devised here is simple, and it requires tiny amounts of genomic DNA, single lanes on a gel for each analysis, no radioactive or biotinylated probes, and no hybridization steps for detection. Thus, it may be worthwhile to consider its application in clinical tests for these specific mutations. In addition, DGGE is particularly reliable for detection of heterozygosity, since many different alleles at a locus can be distinguished unambiguously in a single test. Our results with numerous mouse mutants, the human HbS/HbC region, and additional regions in the human β -globin gene suggest that this approach will be a general one for detecting single-base changes in genomic DNA.

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1. Botstein, D., White, R., Skolnick, M. & Davis, R. (1980) *Am. J. Hum. Genet.* **32**, 314–331.
2. Solomon, E. & Bodmer, W. (1979) *Lancet* **i**, 923.
3. Flavell, R. A., Kooter, J. M., DeBoer, E., Little, P. F. R. & Williamson, R. (1978) *Cell* **15**, 25–41.
4. Geever, R. F., Wilson, L. B., Nallaseth, F. S., Milner, P. F., Bittner, M. & Wilson, J. T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5081–5085.
5. Orkin, S. H., Kazazian, H. H., Antonarakis, S. E., Goff, S. C., Boehm, C. D., Sexton, J. P., Waber, P. G. & Giardina, P. J. V. (1982) *Nature (London)* **296**, 627–631.
6. Orkin, S. H., Little, P. F. R., Kazazian, H. H. & Boehm, C. D. (1982) *N. Engl. J. Med.* **307**, 32–36.
7. Kidd, V. J., Wallace, R. B., Itakura, K. & Woo, S. L. C. (1983) *Nature (London)* **304**, 230–234.
8. Chang, J. C. & Kan, Y. W. (1982) *N. Engl. J. Med.* **307**, 30–32.
9. Pirastu, M., Kan, Y. W., Cao, A., Connor, B. J., Teplitz, R. L. & Wallace, R. B. (1983) *N. Engl. J. Med.* **309**, 284–287.
10. Fischer, S. G. & Lerman, L. S. (1979) *Cell* **16**, 191–200.
11. Fischer, S. G. & Lerman, L. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1579–1583.
12. Myers, R. M., Lumelsky, N., Lerman, L. S. & Maniatis, T. (1985) *Nature (London)* **313**, 495–498.
13. Myers, R. M., Maniatis, T. & Lerman, L. S. (1987) *Methods Enzymol.* **155**, 501–527.
14. Myers, R. M., Fischer, S. G., Maniatis, T. & Lerman, L. S. (1985) *Nucleic Acids Res.* **13**, 3111–3130.
15. Myers, R. M., Fischer, S. G., Lerman, L. S. & Maniatis, T. (1985) *Nucleic Acids Res.* **13**, 3131–3146.
16. Lerman, L. S., Silverstein, K. & Grinfeld, E. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 285–297.
17. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
18. Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. & Erlich, H. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 263–273.
19. Mullis, K. B. & Faloona, F. A. (1987) *Methods Enzymol.* **155**, 335–350.
20. Myers, R. M., Lerman, L. S. & Maniatis, T. (1985) *Science* **229**, 242–247.
21. Hofker, M. H., Wapenaar, M. C., Goor, N., Bakker, E. & Pearson, P. L. (1985) *Hum. Genet.* **70**, 148–156.
22. Kogan, S. C., Doherty, M. & Gitschier, J. (1987) *N. Engl. J. Med.* **317**, 985–990.
23. Myers, R. M., Sheffield, V. & Cox, D. R. (1988) in *Genomic Analysis: A Practical Approach*, ed. Davies, K. (IRL, Oxford), pp. 95–139.
24. Lerman, L. S. & Silverstein, K. (1987) *Methods Enzymol.* **155**, 482–501.
25. Myers, R. M. & Maniatis, T. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 275–284.
26. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
27. Paabo, S. & Wilson, A. C. (1988) *Nature (London)* **334**, 387–388.
28. Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986) *Nature (London)* **324**, 163–166.
29. Embury, S. H., Scharf, S. J., Saiki, R. K., Gholson, M. A., Golbus, M., Arnheim, N., and Erlich, H. A. (1987) *N. Engl. J. Med.* **316**, 656–661.
30. Orkin, S. H. & Kazazian, H. H. (1984) *Annu. Rev. Genet.* **18**, 131–171.