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Genetics

Mutation detection with MutH, MutL, and MutS mismatch repair proteins

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ABSTRACT ***Escherichia coli*** **methyl-directed mismatch repair is initiated by MutS-, MutL-, and ATP-dependent activation of MutH endonuclease, which cleaves at d(GATC) sites in the vicinity of a mismatch. This reaction provides an efficient method for detection of mismatches in heteroduplexes produced by hybridization of genetically distinct sequences after PCR amplification. Multiple examples of transition and transversion mutations, as well as one, two, and three nucleotide insertion/deletion mutants, have been detected in PCR heteroduplexes ranging in size from 400 bp to 2.5 kb. Background cleavage of homoduplexes is largely due to polymerase errors that occur during amplification, and the MutHLS reaction provides an estimate of the incidence of mutant sequences that arise during PCR.**

A number of molecular methods for mutation detection have been described. Several rely upon differential resolution of DNA fragments in polyacrylamide gels due to sequence heterogeneity. The single-strand conformation polymorphism procedure depends upon conformational differences between genetically distinct sequences (1), while denaturing gradient gel electrophoresis exploits the differential melting behavior of heteroduplex and homoduplex DNAs (2, 3). These methods are typically used to screen DNA fragments 200–500 bp long, and some mutations fail to induce a conformational transition that is detectable by these procedures.

Chemical approaches to mutation detection utilize reagents that modify bases in single-stranded DNA. Such reagents often react with mismatched bases due to breathing of the helix in the vicinity of mispairs (4). Carbodiimide derivatizes unpaired deoxyguanylate and deoxythymidylate, resulting in retarded electrophoretic mobility of DNA fragments containing these modifications (4). Hydroxylamine and osmium tetroxide modify deoxycytidylate, or deoxycytidylate and deoxythymidylate residues, respectively, rendering the polynucleotide backbone labile to cleavage by strong base at the position of the modified residue (5). These methods are limited by the propensity of these reagents to react with any DNA region with single-strand character. This results in high background activity in early-melting regions of the helix.

Enzymatic methods for mutation detection have also been described. Ribonuclease A cleaves DNA–RNA duplexes and RNA–RNA duplexes at mispaired ribonucleotides (6, 7). The efficacy of mismatch detection by this procedure is highly dependent upon the nature of the mispair and its sequence context; consequently, the detection sensitivity is only 30–70% (6, 8). MutY is a glycosylase that excises adenine from G·A mismatches and at much reduced efficiency from A·C mispairs (9, 10). The enzyme is highly specific and sensitive but only recognizes a subset of the possible mismatches (9–11). The mismatch binding activity of the MutS protein has also been exploited for mutation detection (12–14), but in our hands this method is limited by the relatively modest affinity of MutS for heteroduplex DNA (15, 16). The bacteriophage resolvases T4 endonuclease VII and T7 endonuclease I can cleave DNA heteroduplexes in the vicinity of a mismatch (17, 18), but these enzymes fail to recognize some mispairs and incise perfectly paired DNA yielding background signals that obscure products derived from incision at mispairs.

The *Escherichia coli* methyl-directed DNA mismatch repair system identifies and repairs base–base mispairs (19) and one, two, and three nucleotide insertion/deletion mismatches (20). Four nucleotide heterologies are weakly repaired, whereas insertion/deletion mismatches larger than four nucleotides and C·C mispairs do not appear to be recognized. Repair is initiated by binding of MutS to the mispair (21). Binding of MutL to this complex (22) results in activation of MutH, which incises the heteroduplex at d(GATC) sequences in the vicinity of the mispair (23). The data of table IV of Au *et al.* (23) demonstrate the exquisite specificity of the MutHLS initiation reaction for a mismatched base pair. For each d(GATC) cleavage provoked by a G·T mispair, less than one spurious d(GATC) cleavage occurs per 300 kb of perfectly paired Watson–Crick helix. We describe the efficacy of this system for mismatch detection in heterohybrids produced by denaturation and reannealing of PCR products derived from genetic variants.

MATERIALS AND METHODS

Plasmids Used as Templates in PCR. Wild-type and mutant *lacI* sequences were amplified from the plasmid clones of Matteson *et al.* (24). Phage f1 gene VII sequences were amplified from the plasmid templates of Ivey-Hoyle and Steege (25). In each case PCR products consisted of the gene fragments of interest as well as the surrounding vector sequence. One to three-nucleotide insertion/deletion mutants were amplified from derivatives of the replicative form of phage f1MR1 into which synthetic oligonucleotide duplexes had been inserted (26). Phage f1MR22 contains one extra nucleotide relative to phage f1MR21, phage f1MR24 contains two extra nucleotides relative to phage f1MR23, and phage f1MR26 contains three extra nucleotides relative to phage f1MR25 (see Table 1).

PCR Amplification of DNAs. Selection of PCR primers (21 nucleotides long with 5′-OH termini; Oligos Etc., Guilford, CT) was based on known template sequences. Unless otherwise noted, reactions (100 μl) contained 20 mM Tris·HCl (pH 8.2), 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 0.1% Triton X-100, 10 μg of bovine serum albumin per ml, 1 mM each dNTP (Pharmacia), 100 pmol of each primer, 5 μg of T4 gene 32 protein (Boehringer Mannheim), 100 ng template DNA, and 2.5 units of native *Pfu* DNA polymerase (Stratagene). Reactions in which synthetic products were uniformly labeled also contained 70 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; DuPont/New England Nuclear). Reactions in which synthetic products were end-labeled contained 100 pmol of the appropriate primer labeled with T4 polynucleotide kinase

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(Amersham) and [γ-^{32}P]ATP (3000 Ci/mmol, DuPont/New England Nuclear) as described (27). PCRs (15 cycles) were performed using a Perkin–Elmer Gene Amp 9600 thermocycler with incubations at 94°C for 15 sec, 60°C for 15 sec, and 72°C for 90 sec, 3 min, 4 min, or 6 min for amplification of 400 bp, 1.3 kb, 1.7 kb, and 2.5 kb, sequences respectively.

Comparative amplification with *Pfu*, Vent (New England Biolabs), and *Taq* (Amersham) polymerases (see Fig. 3*B*) used buffer conditions recommended by the manufacturer. These reactions (100 μl) contained 1× buffer supplied with each polymerase as well as 200 μM of each dNTP, 100 pmol of each primer, 5 μg T4 gene 32 protein, 15 ng template DNA, and 2.5 units of polymerase. Amplification was for 25 cycles, with each cycle consisting of 15 sec at 94°C, 15 sec at 55°C, and 30 sec at 72°C.

To avoid introduction of contaminating DNA into PCRs, buffer components were made fresh daily and reactions were assembled in a laminar flow hood using filtered pipette tips. Products were extracted with phenol and ether, precipitated with ethanol, and quantitated by an ethidium bromide dot method. Samples (0.5 μl of an appropriate dilution) and DNAs of known concentration were added to 8 μl of 1 μg ethidium bromide per ml and spotted onto plastic wrap. UV-induced fluorescence was measured using a Photometrics (Tucson, AZ) cooled charge-coupled device imager. The concentration of PCR products was determined by comparison to the fluorescence of the standards.

MutHLS Reactions. Denaturation/reannealing reactions (20 μl) contained 2.5 μg of unlabeled PCR product, 0.5 μg of uniformly ^{32}P-labeled PCR product, 10 mM NaCl, 1 mM EDTA, and 50 mM Hepes·KOH (pH 8.0). Freshly prepared 10 M NaOH (0.6 μl) was added to a final concentration of 300 mM, and the mixture was incubated at room temperature for 5 min. The solution was neutralized by addition of acetic acid to a final concentration of 300 mM, KCl to 100 mM, and potassium phosphate (pH 7.4) to 100 mM, and the DNA was hybridized at 65°C for 30 min followed by 30 min at 37°C. Reactions were then bound to a silica matrix spin column (Pierce Xtreme DNA purification columns) and eluted with distilled H_2O to remove PCR primers, dNTPs, and salts.

Reactions (10 μl) (23) contained 50 mM Hepes·KOH (pH 8.0), 20 mM KCl, 4 mM $MgCl_2$, 1 mM dithiothreitol, 50 μg bovine serum albumin per ml, 2 mM ATP, ≈10,000 cpm of PCR DNA, 250 ng MutS (21), 600 ng MutL (22), and 0.9 ng MutH (28). DNA and buffer components were preincubated at 37°C for 8 min; the reactions were initiated by adding a premixed solution of MutH, MutL, and MutS and incubation continued for 15 min at 37°C. After addition of 0.5 μl of 0.5 M EDTA and 20 μl of deionized formamide containing 0.05% bromophenol blue and 0.05% xylene cyanol, DNA products were analyzed by electrophoresis through 6% polyacrylamide in 89 mM Tris/89 mM boric acid/2 mM EDTA (final pH 8.5) and 8 M urea. DNA species were visualized by autoradiography and quantitated using a Molecular Dynamics PhosphorImager.

RESULTS

Detection of Mutations in PCR-Amplified Gene Fragments. Fig. 1 illustrates the mismatch-dependent d(GATC) cleavage assay used here for mutation detection. Regions of the *E. coli lacI* and phage f1 gene VII genes containing known point mutations were subject to 15 cycles of PCR amplification from plasmid clones. Sequences of 390 bp, 1360 bp, and 2502 bp were amplified from *lacI*-containing plasmids and 432-bp and 1169-bp sequences were amplified from f1 gene VII-containing plasmids. Heterohybrids were prepared by denaturing and reannealing a mixture of PCR products obtained from mutant and wild-type DNA (see *Materials and Methods*). Background d(GATC) cleavage not attributable to the presence of a known mispair was assessed using homohybrid control molecules prepared by denaturing and reannealing a PCR product to itself.

Four examples of each of the possible base substitution heteroduplexes were examined, and in each case MutS-dependent d(GATC) cleavage was observed (Table 1 and Fig. 2). The fraction of both heterohybrid and homohybrid molecules incised by MutH increased with increasing PCR product length. However d(GATC) cleavage of heterohybrids ranged from 17-fold (SE = 1.6, n = 8) to 40-fold (SE = 8.3, n = 8) above that observed for the corresponding homohybrids for 390-bp and 432-bp products, respectively. Signal-to-noise values decreased to 10 (SE = 0.65, n = 8), 9.1 (SE = 0.80, n = 8), and 5.7 (SE = 0.67, n = 8) as PCR products increased in length to 1169 bp, 1360 bp, and 2502 bp, respectively (Table 1).

We have also used this method to detect small insertion/deletion mutations of one, two, and three nucleotides. DNA fragments of 1750 bp were amplified from derivatives of the replicative form of phage f1MR1 (15) and from mutant phage containing one, two, and three nucleotide insertion mutations. Each of the heterohybrids containing nucleotide insertions was cleaved in a MutS-dependent fashion at its single d(GATC)

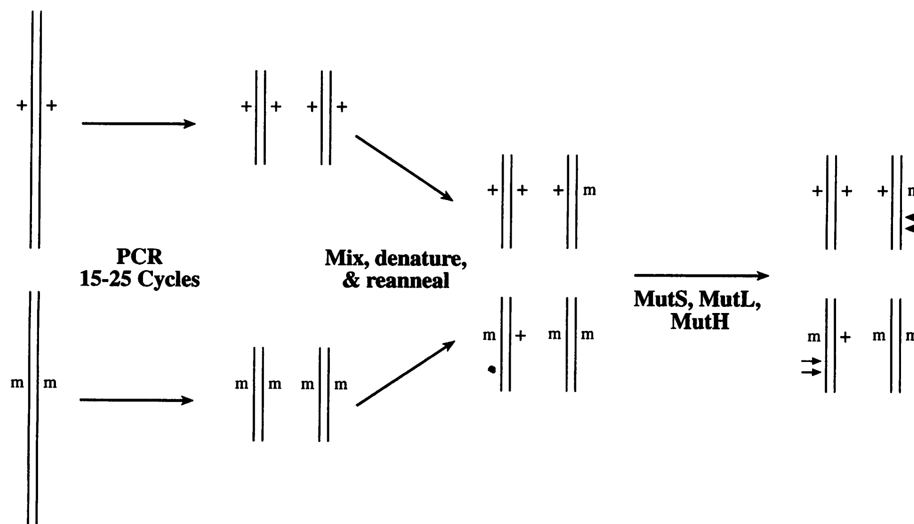

FIG. 1. Method for mutation detection using the MutH, MutL, and MutS proteins.

Table 1. MutS- and MutL-dependent MutH endonuclease activity on PCR-amplified gene fragments containing known mutations

Heteroduplexes	Gene	Sequence context	Size of PCR product, bp	% cleaved	
				Heterohybrid	Homohybrid
G/T + A/C	*lacI*	GTA**G**GCC	390	34	1.7
			1360	51	7.8
			2502	67	14
G/T + A/C	*lacI*	AAC**G**AGG	390	38	1.7
			1360	45	5.4
			2502	88	16
G/T + A/C	*lacI*	AAA**G**AGT	390	25	1.5
			1360	38	3.9
			2502	84	8.7
G/T + A/C	f1 gene VII	GTT**T**CGG	432	22	0.3
			1169	44	4.2
A/A + T/T	*lacI*	GTG**A**AAC	390	34	2.0
			1360	31	3.0
			2502	69	13
A/A + T/T	f1 gene VII	CTA**T**GTA	432	18	0.5
			1169	29	2.8
A/A + T/T	f1 gene VII	GAT**A**TCG	432	15	0.5
			1169	39	4.8
A/A + T/T	f1 gene VII	ATT**A**CGG	432	12	0.5
			1169	52	6.7
G/G + C/C	*lacI*	ATA**G**GAT	390	24	1.9
			1360	43	3.4
			2502	67	21
G/G + C/C	f1 gene VII	TTC**G**GGC	432	13	0.4
			1169	61	6.9
G/G + C/C	f1 gene VII	TAA**C**TAA	432	10	0.5
			1169	46	4.9
G/G + C/C	f1 gene VII	GGA**C**CAG	432	9.4	0.4
			1169	44	3.4
G/A + T/C	*lacI*	GTA**G**GCC	390	32	1.3
			1360	38	6.7
			2502	58	12
G/A + T/C	*lacI*	CGG**A**GGG	390	24	1.8
			1360	29	2.7
			2502	62	12
G/A + T/C	*lacI*	GTG**A**AAA	390	22	1.8
			1360	39	4.3
			2502	75	11
G/A + T/C	f1 gene VII	CTC**T**TTC	432	16	0.2
			1169	57	4.7
A, T idl	f1MR21, f1MR22	AAA/**A**\AAG	1754, 1755	24	4.9
TG, CA idl	f1MR23, f1MR24	GCC/**TG**\TGT	1754, 1756	17	2.5
CTG, CAG idl	f1MR25, f1MR26	CCT/**CTG**\GCT	1755, 1758	15	1.5

MutS- and MutL-dependent MutH endonuclease activity on products containing known mutations obtained after 15 cycles of amplification using PCR. Sequence shown is that of the sense strand with the mutation indicated in boldface type. To confirm that cleavage occurred at d(GATC) sites, hybrid molecules were digested with *Dpn* II, which cleaves at unmethylated d(GATC) sites (data not shown). Products 390 bp and 432 bp long have a single d(GATC) site ≈80 bp from the 3′ end of the sense strand (Fig. 2*B*). Products 1169 bp and 1360 bp long have seven d(GATC) sites. Point mutations are clustered in the center of these molecules flanked by three d(GATC) sites on the 5′ side and four sites on the 3′ side with respect to the sense strand. The 2502-bp products have 17 d(GATC) sites. Point mutations in these molecules are clustered in the 3′ one-third of the molecule with respect to the sense strand, with four d(GATC) sites on the 3′ side of the mutations and 13 sites on the 5′ side of the mutations.

site to a degree 5- to 10-fold greater than the homohybrid controls (Table 1).

Thus, multiple transition and transversion mutations in differing sequence contexts in PCR products of various lengths have been detected by MutS-dependent d(GATC) cleavage as have one, two, and three nucleotide insertion/deletion mutants.

Polymerase Errors During Amplification Largely Account for d(GATC) Cleavage of Homohybrid Products. During their characterization of the MutHLS reaction, Au *et al.* (23) observed low but detectable levels of MutS-dependent d(GATC) cleavage of putative homohybrid control DNAs. However, since homohybrid cleavage depended on denaturation and reannealing, this effect was attributed to DNA damage incurred during this step or to natural genetic variation within the phage DNA population used for homohybrid construction. To determine whether MutS-dependent d(GATC) cleavage of homohybrids observed here was due to damage incurred during DNA preparation or to genetic

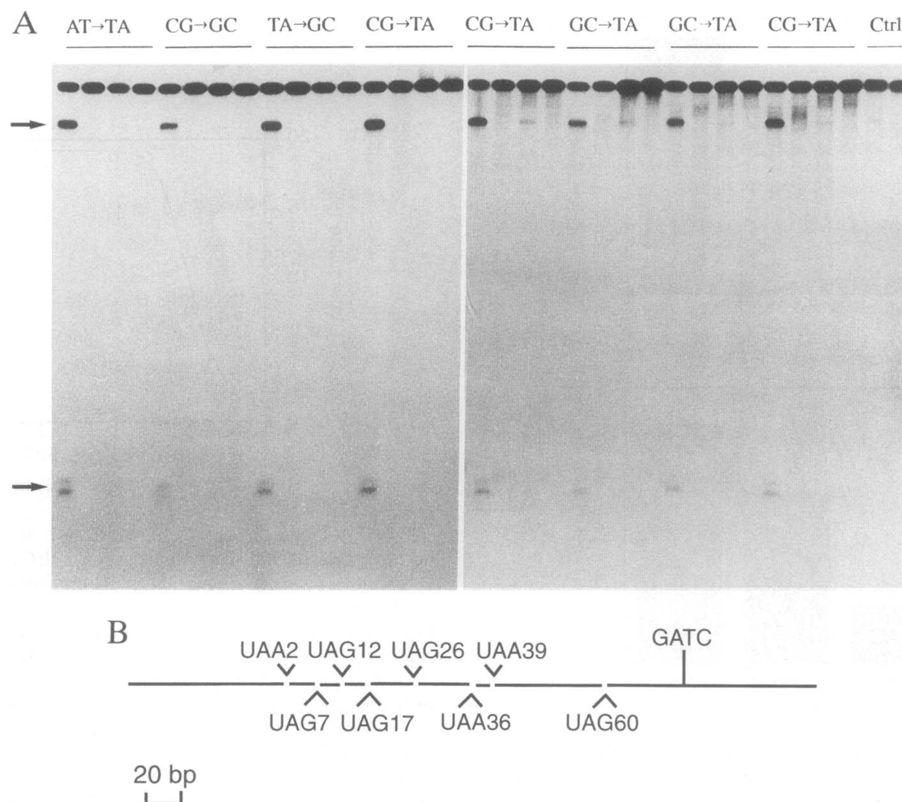

FIG. 2. Detection of mutations in the *lacI* gene. (*A*) Wild-type and mutant *lacI* gene sequences (390 bp) were amplified from plasmid templates for 15 cycles using the PCR. Unlabeled PCR products containing base substitution mutations were denatured and reannealed to radiolabeled wild-type product or to the corresponding labeled mutant product to create heterohybrids and homohybrids, respectively. Hybrids were treated with MutH, MutL, MutS, and cofactors, subjected to electrophoresis through a 6% sequencing gel, and DNA bands were visualized by autoradiography. The first two lanes in each set show products obtained from heterohybrids in the presence and absence of MutS, respectively. The third and fourth lanes in each set show products produced from homohybrids, with and without MutS, respectively. The two control lanes indicate products obtained from wild-type homohybrid sequences with and without MutS. Arrows indicate cleavage products. (*B*) Map of the PCR-amplified DNA fragments used as substrates in *A* indicating the d(GATC) site and point mutations designated by the RNA sequence of the nonsense codon followed by the number of the *lacI* codon in which they occur.

variation introduced during PCR amplification, the dependence of the level of such cleavage on PCR conditions was examined.

Phage f1 gene VII sequences of 1169 bp were amplified for 10, 20, or 30 cycles using *Pfu* polymerase, and homohybrid molecules were prepared by a denaturation and reannealing step. The fraction of homohybrids cleaved by MutH increased with the number of cycles (Fig. 3*A*), a finding consistent with either the damage or genetic variation hypothesis. However the degree of homohybrid cleavage was also found to depend on the polymerase used for PCR amplification. Thus, cleavage of amplified *lacI* homohybrids was highest with *Taq* polymerase, intermediate with Vent polymerase, and lowest with *Pfu* polymerase (Fig. 3*B*). These results parallel the error rates for these polymerases, with the lower fidelity of *Taq* polymerase due to absence of a 3′ to 5′ editing exonuclease (29–31). Although we cannot exclude a low level of template damage associated with thermal cycling, these findings indicate that the majority of the homohybrid background signal is due to polymerase errors occuring during amplification. In light of these results, we have used the high fidelity reaction conditions (4 mM $MgCl_2$, 1 mM each dNTP) of Eckert and Kunkel (32) for subsequent experiments.

Use of dNTP Pool Bias During PCR Amplification to Determine Detectability of Nucleotide Substitution Errors. A dNTP pool imbalance leads to an increased error rate during *in vitro* synthesis by DNA polymerases (33, 34). We have exploited this observation to test the utility of the MutHLS reaction for detection of PCR errors. For these experiments wild-type *lacI* sequences 1360 bp long were amplified using *Pfu*, Vent, or *Taq* polymerases under conditions of dGTP pool imbalance. As shown in Fig. 4, d(GATC) cleavage of homohybrids was dependent on the dGTP concentration bias. Homohybrids derived from amplification using *Taq* polymerase were subject to MutHLS-dependent d(GATC) cleavage to a greater degree than homohybrids amplified under the same conditions using *Pfu* and Vent polymerases. Negligible PCR product was obtained in reactions using *Taq* polymerase in which dGTP was present in a 100-fold molar excess over the other dNTPs. Since the enzyme lacks a 3′ exonuclease activity, high levels of misincorporated dNTPs induce chain termination (35). Likewise, homohybrids amplified using Vent polymerase were cleaved to a greater extent than homohybrids amplified using *Pfu* polymerase (Fig. 4). Polymerase misincorporation errors are therefore readily detectable by MutS-dependent d(GATC) cleavage.

Dependence of the Efficiency of Cleavage by Activated MutH on the Distance Between a d(GATC) Site and the End of a DNA Heterohybrid. Although highly sensitive to mismatched base pairs, the MutHLS reaction can only be used for mutation screen if the sequence of interest contains a d(GATC) site. To determine the feasibility of introducing a d(GATC) site into a PCR primer to screen sequences lacking such a site, we have evaluated the dependence of the reaction on the distance of a d(GATC) site from a DNA end. As shown in Fig. 5, the efficiency of mismatch-provoked cleavage increased with increasing distance of the d(GATC) site from the proximal end in the range of 50–150 bp reaching a maximum at the latter distance. These results suggest that a PCR primer with a d(GATC) site 50–100 nucleotides from an end would

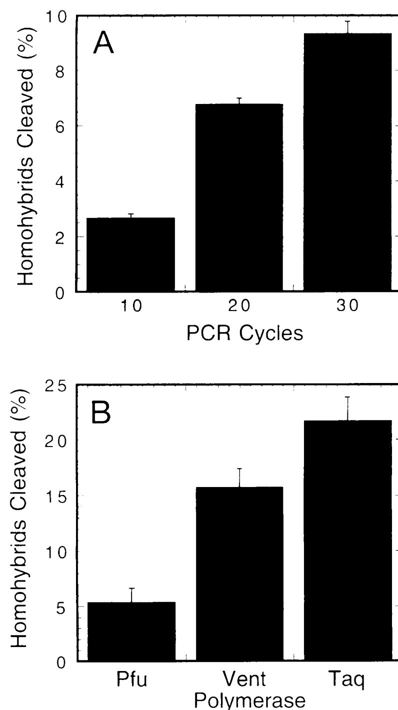

FIG. 3. Dependence of d(GATC) cleavage of homohybrid molecules on conditions used during PCR amplification. (*A*) MutHLS cleavage of 1169-bp phage f1 gene VII homohybrids obtained after 10, 20, or 30 cycles of PCR amplification using *Pfu* polymerase. (*B*) Cleavage of 1360-bp *lacI* homohybrids obtained after 25 cycles of amplification using *Taq*, Vent, or *Pfu* polymerases according to buffer conditions recommended by the manufacturer of each polymerase. Error bars indicate the standard error for four independent experiments.

prove sufficient for the purpose of amplification and subsequent mutation screen using MutH, MutL, and MutS.

DISCUSSION

Previous work has demonstrated the exquisite specificity and sensitivity of *E. coli* mismatch repair activities for recognition and repair of base substitution and small insertion/deletion

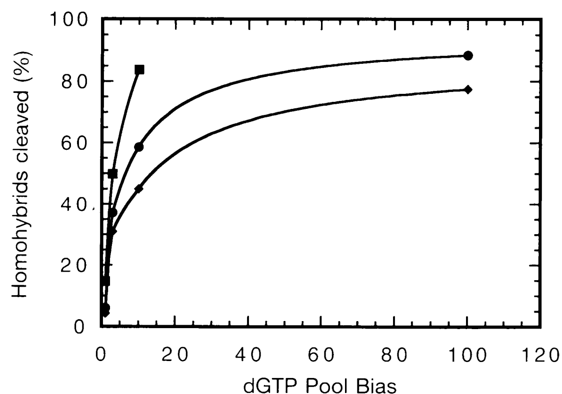

FIG. 4. Dependence of MutHLS cleavage on dNTP pool composition and polymerase used during PCR amplification. Wild-type *lacI* sequences (1360 bp) were amplified for 15 cycles under conditions of dGTP pool bias. Under equimolar conditions, each dNTP was present at 1 mM. The concentration of dGTP was 2 mM in all other reactions, and concentrations of the other three dNTPs were 667, 200, and 20 μM each. *Pfu* (◆), Vent (●), and *Taq* (■) polymerases were used as indicated. PCR products were denatured and reannealed, subjected to MutHLS cleavage, and products analyzed as described.

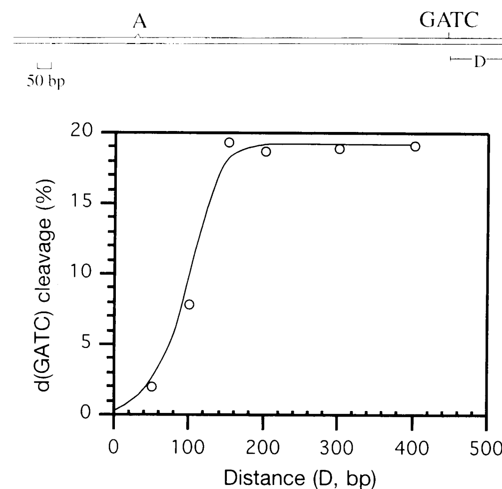

FIG. 5. Dependence of the efficiency of MutHLS cleavage of a heterohybrid on the distance between a d(GATC) site and proximal DNA end. (*Upper*) Schematic representation of heterohybrids (1470–1845 bp) with variable distance between the d(GATC) site and the DNA end. Heterohybrids were prepared after amplification of the replicative form of phage f1MR21 and f1MR22 for 15 cycles using nested reverse PCR primers and the same forward primer. Location of the single nucleotide insertion/deletion mutation is indicated as is the d(GATC) site, which are separated by about 1000 bp. (*Lower*) End-labeled PCR products were amplified, and heterohybrids prepared and analyzed as described. Maximum cleavage observed with these heterohybrids was 20%, perhaps reflecting the large (1000 bp) distance between the mutation and the d(GATC) site.

mutations (15, 19, 20, 23). We have used the mismatch-provoked MutH-, MutL-, and MutS-dependent d(GATC) cleavage reaction for *in vitro* mutation detection. This method has efficiently detected multiple examples of each of the four classes of base substitution mutation in differing sequence contexts, as well as one, two, and three nucleotide insertion/deletion mutations in PCR products ranging from 400 bp to 2.5 kb. No mismatch or small insertion/deletion mutation tested to date has failed detection by this method.

The PCR product length suitable for this procedure is limited by polymerase misincorporation during amplification. Since the probability of misincorporation during a given template copying event depends linearly on the size of the sequence being amplified, the longer the PCR product, the greater the accumulation of polymerase-induced mutations. In lieu of PCR errors, the MutHLS method should, in principle, be useful for mutation screen of molecules many kilobases in length (23). Our observations also imply that the MutHLS reaction can be used to assess the incidence of mutations introduced during PCR amplification. The frequency of polymerase errors during PCR can be estimated from equations 1 and 6 of Luria and Delbrück (36) as

$$f = 2lna,$$

where f is the expected fraction of product molecules that contain a mutation somewhere in their sequence, l is the length of the amplified segment in base pairs, n is the number of cycles, and a is the error rate for the polymerase expressed per nucleotide incorporated. Error rates for *Taq*, Vent, and *Pfu* polymerases estimated from this equation for the product obtained after 25-cycle amplification of a 1360-bp sequence (Fig. 3*B*) are 3.2×10^{-6}, 2.3×10^{-6}, and 7.9×10^{-7} for *Taq*, Vent, and *Pfu* polymerases, respectively, in reasonable agreement with previous determinations (29, 30, 32).

The reliability and applicability of molecular methods for mutation detection have been subject to technical limitations, high levels of background signal for perfectly paired DNA

sequences, and failure to detect all mutations. Methods relying upon differential resolution of DNA fragments in polyacrylamide gels are subject to severe size constraints. Chemical approaches to mutation detection are subject to background reactivity with perfectly paired sequences. Enzymatic methods have proven less robust in their sensitivity to different mutations and in some cases are subject to background signals with perfectly paired DNA. Since the MutHLS-dependent d(GATC) cleavage reaction apparently circumvents many of these limitations, this procedure may have more general application for mutation screens.

The exact location of a mutation cannot be determined with d(GATC) cleavage but it may be approximated. Multiple d(GATC) incisions in the vicinity of the mispair were observed after MutHLS reaction, with preferential cleavage of some sites. The location of the mispair may be approximated by identification of surrounding d(GATC) sites which have been incised by MutH. Molecules without a d(GATC) site may be screened with MutHLS cleavage by introducing a site into the primer used for amplification. Although MutHLS cleavage of d(GATC) sites up to 150 bp from a DNA end is less efficient than cleavage of sites further from the end, d(GATC) sites 50–100 bp from an end should be sufficient for mutation screen.

Although the majority of the DNA substrates used in this study were uniformly radiolabeled during amplification, this method should work equally well with end-labeled molecules. For example, the procedure should be compatible with the use of a fluorescent end-label, with MutHLS-dependent cleavage resulting in release of a fluorescence tag from probe sequences of interest.

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1. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2766–2770.
2. Myers, R. M., Lumelsky, N., Lerman, L. S. & Maniatis, T. (1985) *Nature (London)* **313,** 495–498.
3. Fischer, S. G. & Lerman, L. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1579–1583.
4. Novack, D. F., Casna, N. J., Fischer, S. G. & Ford, J. P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 586–590.
5. Cotton, R. G. H., Rodrigues, N. R. & Campbell, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4397–4401.
6. Myers, R. M., Larin, Z. & Maniatis, T. (1985) *Science* **230,** 1242–1246.
7. Winter, E., Yamamoto, F., Almoguera, C. & Perucho, M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7575–7579.
8. Gibbs, R. A. & Caskey, C. T. (1987) *Science* **236,** 303–305.
9. Au, K. G., Clark, S., Miller, J. H. & Modrich, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8877–8881.
10. Lu, A.-L. & Hsu, I.-C. (1992) *Genomics* **14,** 249–255.
11. Hsu, I.-C., Yang, Q., Kahng, M. W. & Xu, J.-F. (1994) *Carcinogenesis* **15,** 1657–1662.
12. Ellis, L. A., Taylor, G. R. & Baumberg, S. (1994) *Nucleic Acids Res.* **22,** 2710–2711.
13. Lishanski, A., Ostrander, E. A. & Rine, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2674–2678.
14. Wagner, R., Debbie, P. & Radman, M. (1995) *Nucleic Acids Res.* **23,** 3944–3948.
15. Su, S., Lahue, R. S., Au, K. G. & Modrich, P. (1988) *J. Biol. Chem.* **263,** 6829–6835.
16. Jiricny, J., Su, S., Wood, S. G. & Modrich, P. (1988) *Nucleic Acids Res.* **16,** 7843–7853.
17. Youil, R., Kemper, B. W. & Cotton, R. G. H. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 87–91.
18. Mashal, R. D., Koontz, J. & Sklaar, J. (1995) *Nat. Genet.* **9,** 177–183.
19. Lahue, R. S., Au, K. G. & Modrich, P. (1989) *Science* **245,** 160–164.
20. Parker, B. O. & Marinus, M. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1730–1734.
21. Su, S. & Modrich, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5057–5061.
22. Grilley, M., Welsh, K., Su, S. & Modrich, P. (1989) *J. Biol. Chem.* **264,** 1000–1004.
23. Au, K., Welsh, K. & Modrich, P. (1992) *J. Biol. Chem.* **267,** 12142–12148.
24. Matteson, R. J., Biswas, S. J. & Steege, D. A. (1991) *Nucleic Acids Res.* **19,** 3499–3506.
25. Ivey-Hoyle, M. & Steege, D. A. (1992) *J. Mol. Biol.* **224,** 1039–1054.
26. Parsons, R., Li, G., Longley, M. J., Fang, W., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B. & Modrich, P. (1993) *Cell* **75,** 1227–1236.
27. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
28. Welsh, K. M., Lu, A., Clark, S. & Modrich, P. (1987) *J. Biol. Chem.* **262,** 15624–15629.
29. Lundberg, K. S., Shoemaker, D. D., Adams, M. W. W., Short, J. M., Sorge, J. A. & Mathur, E. J. (1991) *Gene* **108,** 1–6.
30. Mattila, P., Korpela, J., Tenkanen, T. & Pitkanen, K. (1991) *Nucleic Acids Res.* **19,** 4967–4973.
31. Tindall, K. R. & Kunkel, T. A. (1988) *Biochemistry* **27,** 6008–6013.
32. Eckert, K. A. & Kunkel, T. A. (1990) *Nucleic Acids Res.* **18,** 3739–3744.
33. Kunkel, T. A. & Loeb, L. A. (1979) *J. Biol. Chem.* **254,** 5718–5725.
34. Fersht, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4946–4950.
35. Innis, M. A., Myambo, K. B., Gelfand, D. H. & Brow, M. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9436–9440.
36. Luria, S. E. & Delbrück, M. (1943) *Genetics* **28,** 491–511.