Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2815–2819, April 1991
Genetics

Coupled amplification and sequencing of genomic DNA

(DNA sequencing/PCR/homeobox cluster 2/*Taq* polymerase/polymorphism)

GUALBERTO RUANO AND KENNETH K. KIDD

Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

Communicated by Frank H. Ruddle, December 18, 1990 (received for review July 18, 1990)

ABSTRACT **Addition of dideoxyribonucleotides during the exponential phase of the PCR should result in the synthesis of two complementary sequence ladders. We have explored this hypothesis to develop coupled amplification and sequencing of genomic DNA. Coupled amplification and sequencing is a biphasic method for sequencing both strands of template as they are amplified. Stage I selects and amplifies a single target from the genomic DNA sample. Stage II accomplishes the sequencing as well as additional amplification of the target using aliquots from the stage I reaction mixed with end-labeled primer and dideoxynucleotides. We have successfully applied coupled amplification and sequencing to a 300-base-pair fragment 4 kilobases upstream from *HOX2B* directly from human whole genomic DNA.**

The cornerstone of many fundamental methods in molecular biology is the ability of polymerases to synthesize DNA from a template *in vitro* (1). DNA polymerases catalyze the addition of deoxyribonucleotides (dNTPs) to an oligonucleotide, which, by annealing to the template, serves as an extension primer. The method for sequencing DNA conceptualized by Sanger *et al.* (2) relies on polymerization directed by one such primer in the presence of chain-terminating dideoxyribonucleotides (ddNTPs). More recently, the PCR method of DNA amplification pioneered by Mullis and Faloona (3) relies on simultaneous extensions guided by two primers along both complementary strands of a defined DNA segment. Given this common mechanistic underpinning of both methods, we hypothesized that PCR could be turned into Sanger sequencing if ddNTPs were introduced during amplification. It should thereby be possible to generate simultaneously sequence ladders for each strand of a double-stranded template while sustaining some degree of amplification. This paper examines the translation of that simple concept into a useful method.

MATERIALS AND METHODS

Overall Scheme for Coupled Amplification and Sequencing (CAS) of Genomic DNA. Our hypothesis was that introduction of ddNTPs during the exponential phase of PCR should allow sequencing reactions to proceed simultaneously along both template strands without impairment by competing self-annealing of the complementary strands. Fig. 1 illustrates the general framework of the method.

Our objective requires the following: (*i*) specific amplification of a desired segment from genomic DNA to provide a sequencing template; (*ii*) progression to sequencing reactions without intervening preparation of template, without removal of primers or dNTPs and without changes in reaction buffers or polymerase enzyme; (*iii*) simultaneous sequencing of both strands of double-stranded template with the same primers used during its amplification; and (*iv*) a means of detecting

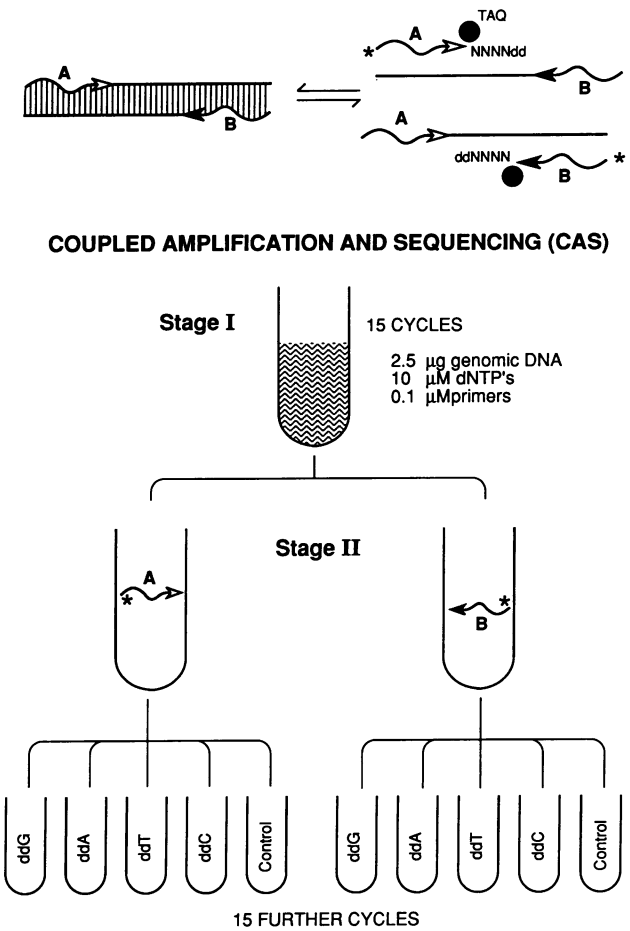

FIG. 1. Outline of CAS of genomic DNA. (*Upper*) The basis of the method is introduction of ddNTPs during PCR amplification. Both nascent strands being synthesized from double-stranded template are truncated by ddNTP incorporation. (*Lower*) Flow chart for the manual execution of the method in two stages. Selection and amplification of genomic target occur in stage I. End-labeled primer (A or B) and ddNTPs are added during stage II.

sequencing products extended from only one primer despite the presence in the reaction mix of products corresponding to two sequence ladders. Influenced by our biphasic "booster" logic developed for PCR of dilute DNA samples (4), we reasoned that a simple biphasic paradigm could in the first stage accomplish the selection and amplification of a single target from the genome and in the second stage produce the sequence ladder while allowing further amplification.

The first two requirements (*i* and *ii*) were achieved by modifying standard reaction conditions in such a manner that amplification of template proceeded to the end of, but not beyond, the exponential phase of PCR under lowered con-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAS, coupled amplification and sequencing; HOX2, homeobox cluster 2; nt, nucleotide(s); ddNTP, dideoxynucleotide.

centrations for primers and dNTPs. These modified conditions would allow ddNTPs and labeled primers to be readily added to stage I product in the same reaction buffer and without any preparative steps.

Stage I product is aliquoted into two identical volumes, each of which receives a boost of one or the other sequencing primer. As per requirement *iii*, these sequencing primers are the same as the PCR primers. Because the labeling procedure used here is simply end-labeling of either primer with [γ-^{32}P]ATP, requirement *iv* dictates that only one primer be added as a tracer for each stage II aliquot, so that only one of the two sequence ladders will be labeled in a given ampule. To ensure incorporation of the labeled primer, the ratio of labeled-to-unlabeled primer was set to 1:1 by labeling the same amount of primer present during stage I and adding it for stage II.

Each of the two aliquots boosted with the respective end-labeled primer is subaliquoted into five vials, four for sequencing and one control for regular amplification. One of the four ddNTPs at preoptimized concentrations is added to each of the sequencing reactions. The control reaction assays the extent of normal amplification without ddNTPs, as well as whether any PCR artifacts have occurred, such as amplification of nontarget segments. The resulting 10 vials are thermally cycled during stage II under conditions identical to those of stage I.

Reaction Conditions for CAS. Optimization experiments (see *Results*) led to the following protocol for sequences of ≈500 base pairs (bp).

Stage I. Human genomic DNA (2.5 μg) was amplified enzymatically in a 250-μl reaction mixture of the following reagents: 0.1 μM (10 pmol per 100 μl) of each primer, 10 μM of each dNTP (Pharmacia), 1 nM (2 units per 100 μl) *Taq* polymerase (Cetus AmpliTaq), 50 mM KCl, 0.75–3.0 mM $MgCl_2$, 10 mM Tris (pH 8.4), and 0.01% gelatin. For each template, amplification yield and specificity should be examined as a function of [$MgCl_2$] over the prescribed range to obtain a single product as seen on ethidium bromide-stained gels. Some templates may need readjustment of [$MgCl_2$] as a function of [dNTP] because nucleotides are Mg chelators. PCR was performed in a programmable thermal cycler (Perkin–Elmer) for 15 cycles each consisting of 1-min denaturation at 94°C, 1-min annealing at the lowest dissociation temperature (T_d) of either primer, and 1-min polymerization at 72°C.

Stage II. The reaction mixture obtained from stage I was divided into two 125-μl volumes. One volume received 12.5 labeled pmol of the first primer; the other received 12.5 labeled pmol of the second primer. Both primers were labeled at their 5′ ends with [γ-^{32}P]ATP (Amersham) with T4 polynucleotide kinase (New England Biolabs) to a specific activity of 2–4 μCi/pmol (1 Ci = 37 GBq). This high specific activity is essential for the autoradiography of sequencing gels because each end-labeled DNA molecule in the ladder contains only a single molecule of ^{32}P. Each 125-μl aliquot boosted with end-labeled primer was subaliquoted into 5 vol of 25 μl each—namely, one for each of the four ddNTPs and one serving as control (no ddNTP added). ddNTPs (Pharmacia) were added to their respective 25-μl ampules to final concentrations of 110 μM dideoxyguanosine (ddG), 670 μM dideoxyadenosine (ddA), 670 μM dideoxythymidine (ddT), or 400 μM dideoxycytidine (ddC). The reactions, overlaid with mineral oil, underwent 15 further cycles with the same thermal profile as in stage I.

Sample preparation for electrophoresis. At the end of stage II, the oil is extracted with chloroform and the reaction mixtures are recovered, then lyophilized, and finally reconstituted in 12.5 μl of loading dye with 90% (vol/vol) formamide. Stage II products in loading dye are boiled for 5 min. Twenty percent (2.5 μl) of each reaction is loaded in an 8% acrylamide/8 M urea gel and run at 35 W (Bio-Rad apparatus) for 2.5 hr (sufficient to run off unincorporated primer) or 5 hr. Gels are dried at 80°C for 2 hr and exposed to film (Kodak XAR-5) for 12–24 hr at room temperature without intensifying screens.

Genomic Targets for Analysis. A *Pst* I fragment 4 kilobases (kb) upstream from the human *HOX2B* homeobox domain in chromosome 17 has been fully characterized by Ferguson-Smith (5) by means of classical cloning, library screening, and M13 sequencing. From this sequence information, primers Pyg2 [5′-GCT CTA TAG GAG GCC CTG AG-3′] and Pyg3 [5′-GAG GCT GTT TAG ATG AGA CA-3′] were synthesized. From their 5′ ends, these primers delimit a 300-bp subfragment within the *Pst* I segment and served to amplify the subfragment directly from genomic DNA. No polymorphism was detected for this 300-bp stretch after an extensive population survey based on denaturing gradient electrophoresis of fragments amplified from 26 individuals of 17 ethnic groups (6). The base composition of the strand extended from Pyg2, including the primer itself, is 83 guanine, 71 adenine, 75 thymine, 71 cytosine.

Four additional templates, two in the β-globin cluster and two others in *HOX2*, have been sequenced with CAS using the reaction conditions described above. These templates are as follows: two subfragments of 630 bp (fragment A) and of 410 bp (fragment B) within a 770-bp inter-*Alu* region between human $\psi\beta$ and δ globin genes, a 320-bp fragment adjacent to the Pyg2–Pyg3 fragment in the *HOX2* cluster (fragment C), and the 630-bp segment encompassing both the 320-bp and Pyg2–Pyg3 fragments (fragment D). PCR amplification conditions and primers for these four segments, as well as the Pyg2–Pyg3 fragment, have been described (6, 7) (see *Discussion* also).

Synthesis of Oligonucleotide Primers. Oligonucleotides were prepared by the solid-phase phosphoramidite method in an automated DNA synthesizer (Applied Biosystems) and were purified by electrophoresis in a 7 M urea/20% polyacrylamide gel. When used as sequencing primers, incompletely synthesized oligonucleotides cause lane background by superimposing their misaligned reading frames in the gel.

RESULTS

Optimization of Stage I Reaction Conditions. Because a clear sequence ladder requires there be minimal amounts of undesired product, reaction conditions and cycling profile were stringently optimized for primers Pyg2 and Pyg3. Regular PCR for 30 cycles at 57°C annealing temperature, 1.5 mM $MgCl_2$, and 10 μM each of dNTP routinely synthesized only the 300-bp *HOX2* fragment at a yield of 300–500 ng per 100-μl reaction (data not shown). These reagent concentrations and this annealing temperature were adopted for CAS.

During the exponential phase of PCR, the total number of double-stranded molecules doubles during each cycle only when there is complete extension of the newly synthesized strands. Hence, the rate of increase in product during PCR could be regarded as an index for complete primer extension of both template strands. To define the cycle with the maximum rate of synthesis of product, we prepared identical reactions and removed them from the thermal cycler after different numbers of cycles. In Fig. 2*A*, yield of full-length product (denoted by arrow) was detectable after 10 cycles and rapidly increased through 20 cycles. Proportional increases began to decrease after 25 cycles until saturation was evident after 30 cycles. Hence, 15 cycles was chosen as the duration of stage I.

Products of molecular weight lower than the full-length fragment were evident as discrete bands on the sequencing gel after 15 cycles despite the absence of any chain-terminating ddNTP. These bands became more prominent

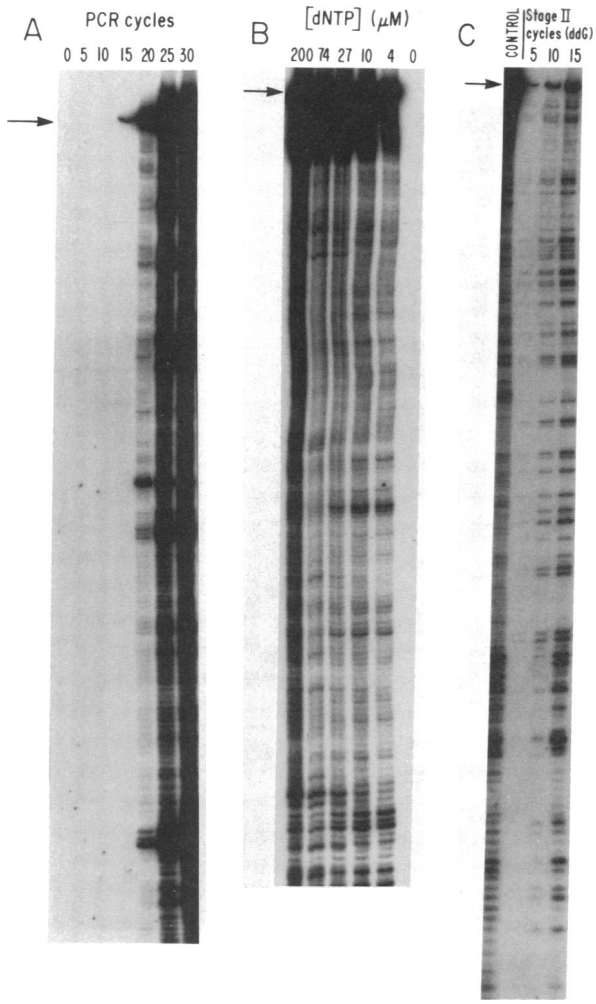

FIG. 2. Optimization of reaction conditions for the 300-bp *HOX2* fragment. Products synthesized with end-labeled primer Pyg2 were denatured and run on an 8% acrylamide sequencing gel. Arrows indicate full-length products. (*A*) Course of product synthesis during PCR as a function of amplification cycles. (*B*) Titration series for dNTPs each present at the concentration denoted for 30 cycles of PCR. (*C*) Optimization of stage II cycling profile with ddG/dG of 10. Control lane contains no ddG. End-labeled Pyg2 was present from the outset of PCR in *A* and *B*; it was added as a tracer, beginning at stage II, in *C*.

with further cycling. An experiment in which enzyme concentration was boosted 5-fold during the last 15 of 30 cycles gave the same pattern of discrete lower-molecular-weight bands (data not shown). An extension time of 5 min in the last cycle failed to reduce the low-molecular-weight bands (data not shown). Similar experiments with four other templates all produced template-specific patterns of discrete bands of molecular weight lower than full-length product.

The concentration of dNTPs, 10 μM, used in CAS is at the K_m for dNTP incorporation by *Taq* polymerase (8). The equilibrium between enzyme anchoring to template during forward extension and dissociation from the strand could favor enzyme dissociation and truncation of the nascent strand. We titrated dNTP concentration over the range 200 μM to none (Fig. 2*B*). The yield of full-length product is reduced at lower dNTP, a relationship expected to become progressively marked for longer segments. Nevertheless, at 10 μM, the yield was sufficient to synthesize roughly 400 ng of product. The same template-specific lower-molecular-weight bands are present at all dNTP concentrations.

Optimization of Stage II Reaction Conditions. For a first attempt at CAS, ddG at a 10-fold molar excess with respect to deoxyguanosine (dG) and labeled Pyg2 tracer were added to aliquots of a mixture that had undergone 15 cycles of stage I. Identical aliquots were removed from the thermal cycler after 5, 10, and 15 cycles of stage II. Fig. 2*C* presents the results from this experiment. At this ddG/dG ratio, full-length product is generated even after introduction of ddG into the reaction. The noise, as demonstrated by the control without ddG (leftmost lane), is filtered out by ddG while generating a series of clearly readable bands. The amount of full-length product increases with cycling, as does the intensity of each band in the sequence ladder, which is quite clear at 15 cycles. In comparison to the control (no ddG), ddG does reduce considerably the additional synthesis of full-length product but does not abolish it. It is evident that amplification and sequencing are occurring simultaneously during stage II.

A series of titrations examined the effects of ddNTP concentration (Fig. 3). The concentration of dNTPs was 10 μM each in all experiments during stages I and II. During stage II, end-labeled Pyg2 and ddNTPs were added to each tube over a ddNTP concentration range optimal for their incorporation by *Taq* polymerase (8). The corresponding ratios of ddNTP to dNTP (*dd/d*) range from 6 to 14 for guanine, from 29 to 100 for adenine and thymine, and from 18 to 60 for cytosine. In all four titration series, increasing amounts of ddNTP reduced the background noise to unde-

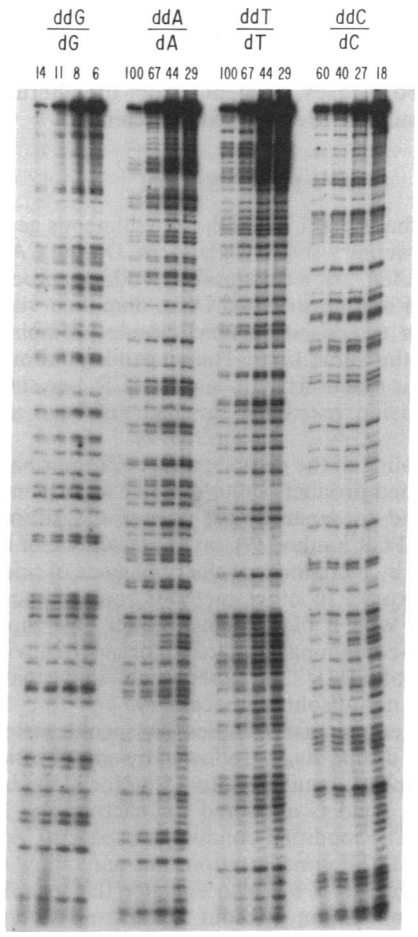

FIG. 3. Titration series to optimize the CAS ratio of each ddNTP to dNTP (dd/d) for the *HOX2* fragment. Autoradiograph of a polyacrylamide gel of heat-denatured CAS products synthesized with a supplement of end-labeled Pyg2 and ddNTPs added at the beginning of stage II. Background concentration of each dNTP is 10 μM. For ddG, the concentration range extended from 60 to 140 μM; for ddA and for ddT, it extended from 290 to 1000 μM; and for ddC, range extended from 180 to 600 μM. Bottom of the gel is 5 nucleotides (nt) from the primer.

tectable levels without impairing the clarity and strength of the signal obtained from the *bona fide* sequence ladder. The effect of [ddNTP] on the noise is most dramatic near the primer. The intensity of low-molecular-weight and high-molecular-weight bands is uniform on visual inspection of the autoradiograph at any of the ddNTP amounts tested. Increased concentrations of ddNTP reduce the synthesis of full-length product: its yield was inversely related to *dd/d* ratio. The strength of the sequencing signal decreased at *dd/d* ratios greater than the optimum. Optimal *dd/d* ratios determined from this series are: 11 for guanine, 67 for adenine and for thymine, and 40 for cytosine.

Application of CAS to Sequencing a *HOX2* Segment. The *HOX2* fragment was sequenced with CAS under the experimental conditions just delineated. The sequence ladders extended from primers Pyg2 and Pyg3 corresponding to both complementary strands of the fragment are shown in Fig. 4*A*. The sequence obtained with CAS perfectly matches that obtained by classical means (5). CAS generates simultaneously sequence for both complementary strands of a double-stranded template, as demonstrated in Fig. 4*B*. The sequence complementary to a track 80 nt from Pyg3 was found 180 bases away from Pyg2, as expected. Aligning both sequences in an antiparallel configuration, it can be seen that they are perfectly complementary. Two different parts of the sequence document the need for both sequences. In Fig. 4*A*, it can be seen that at 50 nt from Pyg3 and at 45 nt from Pyg2, stretches of four consecutive thymines are difficult to read because of common stops at the beginning or end of the stretch. In both cases, sequence from the alternate primer showed a readable stretch of four adenines.

DISCUSSION

The salient feature of CAS is the simultaneous generation of two complementary sequence ladders. During CAS, synthesis of truncated primer-extension products proceeds from both ends of double-stranded DNA amplified via the PCR. Hence, both strands of double-stranded template are sequenced at the same time. This capability follows directly from the basic reaction mechanism of PCR, two simultaneous primer-extension reactions, but had not been appreciated previously.

Self-annealing of the complementary strands has rendered PCR-generated products quite difficult to sequence without some method to separate out one of the strands. In the asymmetric PCR method (9), an excess of one of the strands is generated by including a molar imbalance of primers in the reaction mixture. In other methods, one of the strands is synthesized with a biotinylated primer and captured in a streptavidin-containing column (10) or in streptavidin-coated magnetic beads (11). Our experiments show that such maneuvers are not absolutely necessary.

CAS of genomic DNA relies on specific selection of a single target during stage I followed by amplification up to 50 fmol of template, equivalent to 15 ng for a 500-bp target (5 fmol of this yield is aliquoted for each of the 10 stage II reactions). The modified conditions used for CAS, lowered primer and dNTP concentration, not only allow direct coupling of stage I to II but also enhance the specificity of the stage I amplification without significantly compromising yield. The first step in developing a CAS strategy for a given segment is optimizing regular PCR of genomic DNA to yield a single product. If that were not possible, it should be feasible to apply stage II conditions directly to a genomic segment purified by gel electrophoresis from other products. Similarly, DNA cloned in conventional vectors should also be amenable to CAS as long as the length of the segment is within the reach of PCR. In both instances the screening and amplification afforded by stage I could be superseded by gel purification or cloning. The critical variable in these applications is adjusting template concentration to the approximate level found at the end of stage I from genomic DNA.

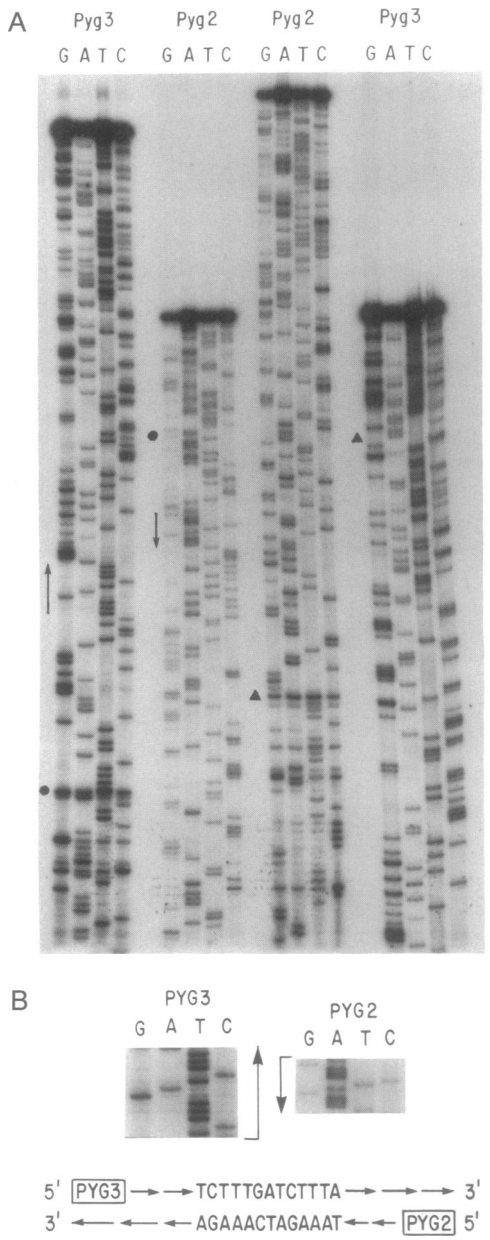

FIG. 4. Sequencing both strands of the *HOX2* fragment via CAS. (*A*) Autoradiograph is a polyacrylamide gel depicting two sequence ladders, one from primer Pyg2, another from Pyg3. The sequence ladders are complementary because they extend from opposite ends of the same fragment. Two sets of reactions are depicted for each primer. One ladder is a "short run"—i.e., electrophoresis of products for 2.5 hr. The "long run" corresponds to the same products electrophoresed for a longer time (5 hr) from an earlier load. Sites of common termination in all reactions for a given strand can be resolved by reading the complementary sequence. Circles denote one such site in sequence extended from Pyg3, which can be clarified at the complementary site in sequence extended from Pyg2. Triangles pinpoint an analogous example for a common termination site seen in the Pyg2 sequence, which is resolved by inspection of Pyg3 sequence. Arrows denote complementary sequence tracks highlighted in the next panel. (*B*) Close-up of a track of 13 bases 80 nt away from Pyg3 and 180 nt away from Pyg2. The sequences are written in an antiparallel configuration below for confirmation of sequence complementarity.

The low molecular weight bands we (Fig. 2*A*) and others (12) have observed during regular PCR persist over a wide

range of dNTP concentrations (Fig. 2*B*), *Taq* polymerase amounts, and extension times. Thus, the noise is due neither to a reduction in the driving force afforded by excess dNTP nor to limiting amounts of enzyme. The spurious bands may represent sites of preferential transient interaction of the two complementary strands, either full-length or truncated, and hence sites of difficult read-through for the polymerase. At these sites misincorporation of dNTPs or ddNTPs may occur promiscuously. Alternatively, the 5′–3′ exonuclease activity of *Taq* polymerase may cleave the strand domain annealed to the template being read by the enzyme. The hypothesis of partial annealing could also explain the selective removal of PCR noise over sequence signal by higher [ddNTP] during CAS (Fig. 3). High [ddNTP] impairs the synthesis of full-length product and, hence, should reduce considerably the postulated bimolecular annealing interactions. In contrast, given the molar excess of primers, sequencing itself could be considered a first-order process. Additionally, degradation of extension products induced by thermal cycling could also account for some noise. It is to be hoped that newer thermostable polymerases or genetically engineered versions of the *Taq* enzyme may not be prone to such artifacts.

Obtaining a complete ladder requires that the probability of incorporating a ddNTP be sufficiently low to allow, for some strands, sequential incorporation of a dNTP instead of a ddNTP at all possible sites in the sequence. This assures a more uniform distribution of signal in the ladder and also assures continued synthesis of full-length template beyond that originally amplified during stage I, thereby further increasing signal. The ratios given here yield readable sequences in the range 300–500 bp but may have to be lowered for longer templates or slightly increased for templates <200 bp. We have successfully used CAS for a total of six independent sequences ≈400 bp long: both strands of three different targets, two targets in HOX2 and one in the β-globin cluster. All sequences gave results similar to the one illustrated. Additional CAS of two targets ≈650 bp long bore out the length dependence: the sequence was clearly readable but required three times longer autoradiographic exposure. In templates of very biased GC or AT content, the effective "length" of the molecule could be much longer for the predominant bases than for the others. The ratios may have to be decreased for the frequent bases and increased for the infrequent ones.

Although many protocols utilize nested primers and nonthermostable polymerases for sequencing PCR products (13, 14), in CAS the same primers, enzyme, and buffer are used for amplification and sequencing. The cycling of sequencing reactions has previously been found to enhance band intensity (15, 16). CAS not only benefits from this arithmetic amplification but also from enhancement of sequence signal by continued increase in template amounts during stage II. Misincorporation of bases by *Taq* polymerase will not compromise the reliability of CAS for two reasons. (*i*) At optimized Mg concentrations, the error rate of the enzyme is quite low, 10^{-4} or less (17). (*ii*) The vast number of template copies used in CAS, 7.5×10^5 in 2.5 μg of human genomic DNA, renders negligible any random artifact caused by misreading a particular template molecule.

Patients or normal humans as well as animals and plants important in agriculture or species preservation are analyzed for DNA sequence variation usually via secondary typing methods. These methods include restriction fragment length polymorphisms, chemical cleavage, and denaturing gradient electrophoresis (18). However, an exhaustive search for mutations and polymorphisms is only achieved by sequencing of DNA amplified from each organism (19–21). CAS is likely to accelerate such searches. CAS could also corroborate the fidelity of amplified "sequence tagged sites" that define chromosomal domains (22) and be useful for sequencing products of inverse PCR (23) and *Alu* PCR (24). Furthermore, CAS is a feasible extension to existing technology for automated DNA sequencing based on fluorescent tagging of primers (25).

Because CAS simultaneously produces for each template two distinct but complementary sequence ladders that are electrophoresed together, it is inherently a multiplex sequencing process (26). In principle, it should be possible to amplify and sequence simultaneously both complementary strands of several templates. Electrophoresis of the composite CAS products followed by electroblotting of sequencing gels and hybridization to strand-specific probes should render CAS amenable to significant and widespread use in large-scale sequencing projects.

We are extremely grateful to W. Fenton (Yale University) for sharing his biochemical insight during the development of the method. We are thankful to B. Strauss, S. Zullo (Yale University), and R. E. Kouri (Bios, New Haven, CT) for invaluable collaboration as well as to D. H. Gelfand (Cetus) for his careful reading of the manuscript. This research was supported by National Institutes of Health Grant GM-40883 and National Science Foundation Grant BNS 8813234 awarded to K.K.K. G.R. is the recipient of a Dissertation Fellowship from the Ford Foundation.

1. Kornberg, A. (1969) *Science* **163,** 1410–1418.
2. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
3. Mullis, K. B. & Faloona, F. A. (1987) *Methods Enzymol.* **155,** 335–350.
4. Ruano, G., Fenton, W. & Kidd, K. K. (1989) *Nucleic Acids Res.* **17,** 5407.
5. Ferguson-Smith, A. C. (1989) Ph.D. thesis (Yale Univ., New Haven, CT).
6. Ruano, G., Gray, M. R., Miki, T., Ferguson-Smith, A. C., Ruddle, F. H. & Kidd, K. K. (1990) *Nucleic Acids Res.* **18,** 1314.
7. Ruano, G., Kidd, K. K. & Stephens, J. C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6296–6300.
8. Innis, M. A., Myambo, K. B., Gelfand, D. H. & Brow, M. A. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9436–9440.
9. Gyllensten, U. B. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7652–7656.
10. Mitchell, L. G. & Merril, C. R. (1989) *Anal. Biochem.* **178,** 239–242.
11. Hultman, T., Stahl, S., Hornes, E. & Uhlen, M. (1989) *Nucleic Acids Res.* **17,** 4937–4946.
12. Olsen, D. B. & Eckstein, F. (1989) *Nucleic Acids Res.* **17,** 9613–9620.
13. Wong, C., Dowling, C. E., Saiki, R. K., Higuchi, R. G., Erlich, H. A. & Kazazian, H. H. (1987) *Nature (London)* **330,** 384–386.
14. Engelke, D. R., Hoener, P. A. & Collins, F. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 544–548.
15. Carothers, A. M., Urlaub, G., Mucha, J., Grunberger, D. & Chasin, L. A. (1989) *BioTechniques* **7,** 494–499.
16. Murray, V. (1989) *Nucleic Acids Res.* **17,** 8889.
17. Keohavong, P. & Thilly, W. G. (1989) in *Polymerase Chain Reaction* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 19–23.
18. Rossiter, B. J. F. & Caskey, C. T. (1990) *J. Biol. Chem.* **265,** 12753–12756.
19. Yandell, D. W. & Dryja, T. P. (1989) *Am. J. Hum. Genet.* **45,** 547–555.
20. Paabo, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1939–1943.
21. Cavalli-Sforza, L. C. (1990) *Am. J. Hum. Genet.* **46,** 649–651.
22. Olson, M., Hood, L., Cantor, C. & Botstein, D. (1989) *Science* **245,** 1434–1435.
23. Ochman, H., Gerber, A. S. & Hartl, D. L. (1988) *Genetics* **120,** 621–623.
24. Nelson, D. L., Ledbetter, S. A., Corbo, L., Victoria, M., Ramariz-Solis, R., Webster, T. D. & Caskey, C. T. (1989) *Proc. Natl Acad. Sci USA* **85,** 6686–6690.
25. Gibbs, R. A., Nguyen, P. N., Edwards, A., Civitello, A. B. & Caskey, C. T. (1990) *Genomics* **7,** 235–244.
26. Church, G. M. & Kieffer-Higgins, S. (1988) *Science* **240,** 185–188.