Optimization of the Polymerase Chain Reaction with Regard to Fidelity: Modified T7, *Taq*, and Vent DNA Polymerases

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The fidelity of DNA polymerases used in the polymerase chain reaction (PCR) can be influenced by many factors in the reaction mixture. To maximize the fidelity of DNA polymerases in the PCR, pH, concentrations of deoxynucleoside triphosphates, and magnesium ion were varied. Denaturing gradient gel electrophoresis was used to separate the polymerase-induced mutants from wild-type DNA sequences. Thermolabile modified T7 DNA polymerase, thermostable *Taq*, and Vent DNA polymerases were studied. Fidelity of all three DNA polymerases was sensitive to concentrations of deoxynucleoside triphosphates, magnesium ion, and pH. Within conditions that permitted efficient amplification, optimization with regard to these three factors yielded an average error rate in error/base pair incorporated of 7.2×10^{-5} for *Taq*, 4.5×10^{-5} for Vent, and 4.4×10^{-5} for modified T7 (Sequenase) DNA polymerases.

DNA amplification by the polymerase chain reaction (PCR)⁽¹⁻³⁾ can be accomplished by DNA polymerases from many sources.^(1,2,4) Specific DNA polymerases have been reported to amplify with different efficiencies (i.e., yield of sequence amplification per cycle) and fidelities (frequency of polymerase-induced errors), with the kind and rate of error depending on the specific DNA polymerase and PCR conditions.⁽⁴⁻⁹⁾ Each error, once initiated, is amplified along with the original wild-type sequences, increasing the fraction of polymerase-induced mutant sequences linearly with the number of amplification cycles. Under low-fidelity conditions, this mutant fraction can become significant: 40% of the amplification products are mutant when a 200-bp fragment is amplified one million-fold by a DNA polymerase with an error rate of 10^{-4} ,⁽⁴⁾ such as Klenow fragment.⁽¹⁰⁾ For some purposes such as sequencing a wild-type gene, the polymerase-induced mutations are generally distributed over the sequence of interest so that an accurate consensus sequence is usually obtained.^(11,12) For studies of mutants within wild-type populations, it is vital that the polymerase-induced mutant sequences do not mask the preexisting mutant sequences.^(11,13,14)

Due to its thermostability, *Taq* DNA polymerase has been the most widely used DNA polymerase. It does not have to be added every cycle, as was necessary for the thermolabile

Klenow fragment referred to in the original descriptions of DNA amplification.^(1,3,10) However, the fidelity of *Taq* has been reported to be 2×10^{-4} error/bp per duplication,^(4,7-10) which renders it unsuitable for studies requiring both low noise and high amplification. Studies requiring a higher fidelity of amplification have used DNA polymerases such as modified T7 DNA polymerase (Sequenase) or T4 DNA polymerase,^(4,13,14) which permitted amplification with error rates of about 3.4×10^{-5} and 0.3×10^{-5} error/bp per duplication, respectively.⁽⁴⁾ However, both T4 and modified T7 DNA polymerases are thermolabile.

Recently, a new thermostable DNA polymerase called Vent DNA polymerase was isolated and made commercially available in a cloned form by New England Biolabs (Beverly, MA). Vent was reported to have a 3'→5' proofreading exonuclease.⁽¹⁵⁾ Our laboratory has been screening all available thermostable polymerases and has found that this new thermostable DNA polymerase, under conditions described for primer extension, permitted amplification with a useful degree of fidelity. Reaction conditions such as pH, concentrations of dNTP, and magnesium ion can greatly affect the fidelity of DNA polymerases.^(5,7,9,16-20) Eckert and Kunkel used the M13mp2 fidelity assay and found that conditions of low pH with low and equimolar concentrations of dNTP to Mg^{2+} increased the fidelity of *Taq*.^(7,9) We have ex-

tended their observations using denaturing gradient gel electrophoresis (DGGE) to separate and enumerate all polymerase-induced mutant sequences from the correctly amplified sequences. To maximize the fidelity of *Taq*, Vent, and Sequenase, varied concentrations of dNTPs, pH, and magnesium were tested iteratively, and the optimal conditions with regard to these variables have been defined. We have confirmed the finding of Eckert and Kunkel^(7,9) and defined conditions in which the *Taq* polymerase operates with a three-fold improvement in fidelity during amplification in PCR relative to the most widely used reaction conditions.⁽⁴⁾ More importantly, we find that the thermostable Vent polymerase under optimal conditions yielded a PCR amplification with both high fidelity and high efficiency which should be of general use to researchers using PCR.

MATERIALS AND METHODS

Materials

Taq polymerase was obtained from Perkin Elmer Cetus (Norwalk, CT). Vent polymerase, purified from *Thermococcus litoralis*, and its recombinant form cloned in *Escherichia coli* (designated Rec-Vent in this paper) were obtained from New England Biolabs (Beverly, MA). Sequenase (modified T7 DNA polymerase) was obtained from United States Biochemicals (Cleveland, OH). 2'-deoxynucleoside 5'-triphosphates (dNTPs) were obtained as 100 mM solution from Pharmacia (Piscataway, NJ). Primer P1, 5'-CATATATTAATATACTCAC-3'; primer P2, 5'-TCCTGATTTATTTCTGTA-3'; and primer P4, 5'-GACTGACGTCTTGCTCGAG-3' were obtained from Synthetic Genetics (San Diego, CA). [γ -³²P]ATP (6000 Ci/mmol) was from New England Nuclear (Boston, MA) and T4 polynucleotide kinase was from New England Biolabs (Beverly, MA). The reagents in the 5' DNA terminus labeling system were from Bethesda Research Lab (Gaithersburg, MD).

Procedure

The stock of DNA template for PCR was obtained by amplification from human genomic DNA, followed by purification with denaturing gradient

gel to ensure a homogeneous population of the desired fragment of human HPRT exon 3 and flanking intron 2. This template was amplified from 10⁵ copies to about 10¹² copies and polyacrylamide gel purified. An aliquot (10¹⁰ copies) was further amplified 100-fold, under identical conditions with primer P4 end-labeled by T4 polynucleotide kinase.⁽¹³⁾ The number of DNA copies generated was measured at different cycles to permit calculation of efficiency. Conditions that maintained exponential increases in DNA per cycle were used throughout this work. The ³²P-end-labeled DNA of the correct size was then purified from polyacrylamide gel, boiled, and reannealed so that each strand of any PCR-generated mutant homoduplexes could be hybridized to the complementary strand of the excess wild-type products to form heteroduplexes. These heteroduplexes were then separated from the wild-type homoduplexes by denaturing gradient gel electrophoresis. For each PCR condition, the total heteroduplex and the correctly amplified homoduplex fractions were measured for the calculation of fidelity in error/base pair incorporated per duplication.

PCR Procedure

pH, dNTP, and Mg²⁺ concentrations were experimental variables. MgSO₄ was used for *Taq* and Vent, while MgCl₂ was used for Sequenase. For Vent and *Taq*, the PCR reaction mixture was 10 mM KCl, 10 mM (NH₄)₂SO₄, 0.1% Triton X-100, 0.1 μg/μl BSA, 1 μM each primer and 20 mM Tris buffer. Four units of enzyme were added for every 100 μl of reaction volume, with 3 more units of *Taq* polymerase added after 30 cycles. The amplification cycle consisted of 94°C for 1 min, 53°C for 2 min, and 70°C for 2 min. For Sequenase, the reaction mixture was 3 μM each primer, and 10 mM Tris buffer. The amplification cycle consisted of boiling for 1 min, cooling down to 37°C (1 minute), adding 1 unit (1 μl) of Sequenase, and incubation at 37°C for 2 min.⁽⁴⁾

Efficiency

To determine the efficiency of amplification at the exponential phase of DNA synthesis, a 5-μl aliquot of the

PCR reaction mixture was removed and electrophoresed on 6% polyacrylamide gel together with a series of authentic standards whose numbers of molecules are known. The gel was stained with ethidium bromide and the PCR products of the correct size were measured by comparing the yield of the ultraviolet-induced fluorescence emitted by the ethidium bromide molecules intercalated into the DNA of the samples of DNA of the standard. The error of this method of quantitation is about twofold. However, this would affect the efficiency only slightly. For instance, a calculated efficiency of 70% due to 10⁷-fold amplification after 30 PCR cycles, would be 75% if the DNA was actually twice as much. The efficiency of amplification was calculated from the formula:

$$(1 + Y)^n = \frac{\text{Final DNA copy number}}{\text{Initial DNA copy number}}$$

where *Y* is the efficiency per cycle and *n* is the number of PCR cycles under conditions of exponential amplification.^(1,2,14)

Denaturing Gradient Gel Electrophoresis (DGGE)

To separate DNA duplexes containing only homologous wild-type sequences (WT homoduplexes) from the mutant/wild-type heteroduplexes, denaturing gradient gel electrophoresis was employed.⁽⁴⁾ DNA was dissolved in a 30-μl solution of 400 mM NaCl, 10 mM Tris buffer at pH 7.5, 2 mM EDTA, boiled for 5 min, and reannealed for 5 hr at 65°C. The DNA was ethanol precipitated and separated on a denaturing gradient gel. The gel consists of a 12.5% polyacrylamide gel (acrylamide/bis-acrylamide = 37.5:1), containing a linearly increasing gradient of denaturant, parallel to the direction of electrophoresis, of 19–29% (vol/vol) where 100% denaturant is 7 M urea/40% formamide.⁽²¹⁾ The gel was run for 15 hr at 150 volts while submerged in TAE buffer (40 mM Tris/20 mM acetic acid/2 mM EDTA, pH 8.3) at 60°C, dried, autoradiographed, and recorded on a phosphorimager screen (Molecular Dynamics, Sunnyvale, CA).

Determination of Fidelity

Fidelity of amplification was deter-

mined by analyzing the denaturing gradient gel exposed to the phosphor-imager screen. The amount of excitation from the radioactive bands in the heteroduplex region (from the origin of the gel to the WT homoduplex band) and the band of WT homoduplex were measured. The background noise, such as nonspecific DNA binding in the gel, was measured from control lanes loaded with only DGGE purified wild-type DNA homoduplex sequences that had been subjected to the identical treatment of boiling, reannealing, and ethanol precipitation. The background control samples were run on the same denaturing gradient gels as the experimental samples. The heteroduplex fraction (HeF) due to polymerase amplification was calculated as: $\text{HeF} = (\text{Total heteroduplex counts} - \text{background heteroduplex counts}) / \text{total counts}$.

The fidelity was calculated⁽⁴⁾ as:

$$f = \frac{\text{HeF}}{b d}$$

where f is the error rate (errors/base pair incorporated duplication); HeF is heteroduplex fraction; b is the length of the single-strand low melting domain in which mutants can be detected (104 bp); and d is the number of DNA duplications (30).

RESULTS

Table 1 shows the values of pH, dNTPs, and magnesium with their corresponding efficiencies and fidelities that were observed in the studies with Sequenase, *Taq*, and Vent. Table 1A

shows the efficiencies and error rates using conditions commonly used in PCR for *Taq* and Sequenase,⁽⁴⁾ or in the case of Vent, those suggested by the supplier to be successful in primer extension. Table 1B shows the improvement brought about by the optimization effort.

Figure 1 is an autoradiogram of a denaturing gradient gel comparing the polymerization-induced mutations by Sequenase, *Taq*, and Vent. The band of wild-type homoduplex indicated by an arrow in the figure has been confirmed by sequencing. The positions of the mutant/WT type heteroduplexes of Vent are very similar to *Taq*, with a few exceptions. As conditions were adjusted for the improvement of fidelity, the positions of these heteroduplexes remained the same with changes only in their intensity relative to the WT homoduplex. The specific *Taq* and Sequenase point mutations corresponding to these bands have been previously reported.⁽⁴⁾

Optimization began with the conditions of Table 1A and iteratively examined the effects of dNTP concentration, pH, and then magnesium ion concentration. The effects of dNTP concentration on fidelity are shown in Figure 2. In a first round of experiments, the dNTP concentrations that permitted each polymerase efficiency to exceed 35% were defined. Within this range, *Taq* displayed some improvement in fidelity by either increasing or decreasing dNTP concentrations. The fidelity of Vent was somewhat improved by increasing dNTP as was that of Sequenase. Within the ranges

FIGURE 1 Analysis by DGGE of the PCR products after 10^9 -fold amplification by Sequenase, *Taq*, and Vent DNA polymerases. The arrow points to the position where correctly amplified wild-type homoduplex focus.

shown on Figure 2, efficiencies were invariant for all polymerases, ~90% for Sequenase, ~70% for *Taq* and Vent. Based on these observations, 0.05 mM, 0.5 mM, and 3.5 mM dNTP for *Taq*, Vent, and Sequenase, respectively, were used in the next stage, study of pH effects.

The efficiencies of Vent and Sequenase were relatively invariant in the pH range of 7–9, but the efficiency of *Taq* decreased markedly below pH 8. As shown in Figure 3, within these ranges, *Taq* showed an improved fidelity when the pH was reduced to 8. Vent showed no change in fidelity from pH 8 to 9, but fidelity decreased at pH 7. The fidelity of Sequenase was invariant over the pH range of 7 to 9. Based on these observations, pH 8.0 was used for all three polymerases for the next step of the optimization procedure.

TABLE 1 Summary of the PCR Conditions

DNA polymerase	dNTP (mM)	pH	Mg (mM)	Efficiency (%)	Error rate (error/bp incorporated)
A. Initial conditions					
Sequenase	2.55	8.0	5	90	5.4×10^{-5}
<i>Taq</i>	0.2	8.8	2	70	2.0×10^{-4}
Vent	0.2	8.8	2	70	6.6×10^{-5}
B. Improved conditions					
Sequenase	3.5	8.0	2.5	90	4.4×10^{-5}
<i>Taq</i>	0.5–1.5	8.0	5	36	7.2×10^{-5}
Vent (and Rec-Vent)	0.5–1.5	8.5	7.5	70	4.5×10^{-5}

FIGURE 2 Fidelity of three DNA polymerases as a function of equimolar dNTP concentrations: (▲) *Taq* (pH 8.8, 2 mM MgSO_4), (□) *Vent* (pH 8.8, 2 mM MgSO_4), and (○) *Sequenase* (pH 8.0, 5 mM MgCl_2). The efficiency was ~90% for *Sequenase*, ~70% for *Taq*, and 70% for *Vent*, for all dNTP concentrations shown. The arrows indicate the initial concentration of dNTP.

The next factor studied was the concentration of magnesium ion. Two separate batches of *Taq* (Perkin Elmer Cetus) were used. One batch of *Taq* showed acceptable amplification (~35%) only from 5 to 10 mM Mg . As shown in Figure 4, with the magnesium concentrations that permitted acceptably efficient amplification, the fidelity of *Taq* was improved both by increasing MgSO_4 to 10 mM or decreasing it to 0.5 mM. *Vent* polymerase and its bacterially produced cloned version, Rec-*Vent*, were found to have similar efficiency and fidelity. Their efficiencies were insensitive to MgSO_4 concentrations over the range studied, but below 2 mM MgSO_4 , the fidelity of *Vent* and Rec-*Vent* DNA polymerase decreased drastically. A small but useful improvement in *Sequenase* fidelity occurred when MgCl_2 was decreased from 5 mM (Table 1A) to 2.5 mM.

At this point conditions had been defined which yielded fidelities about 4.5×10^{-5} for both *Vent* and *Sequenase* and as low as about 1.2×10^{-4} for *Taq* polymerase, each representing improvement of fidelity over that obtained using the original conditions of Table 1A.

To test for covariance of effects on fidelity among the variables studied, the lowest error rate conditions from Figure 4 were then probed in reverse order with regard to the effects of pH and then dNTP using the two thermostable polymerases, *Vent* (Rec-*Vent*) and *Taq* polymerases. As may be seen

by comparing Figure 5 with Figure 3, the fidelity of *Taq* as a function of pH was not changed by the alterations in dNTP or MgSO_4 concentrations. The best fidelity of *Taq* was still obtained at pH 8 and efficiency dropped off below pH 8. However, the shift in dNTP and MgSO_4 concentrations did eliminate the increased error rate of *Vent* polymerase observed at pH 7 in Figure 3. Now a difference between native *Vent* and recombinant *Vent* polymerase was found: The efficiency of Rec-*Vent* dropped precipitously below pH 7.5.

Figure 6 shows the fidelity of *Taq*, *Vent*, and Rec-*Vent* polymerases as

dNTP concentrations were varied at the pH and MgSO_4 concentrations that optimized fidelity. As seen by comparison with Figure 2, *Taq* was now found to amplify at dNTP concentrations above 0.5 mM, albeit with low efficiency (~36%). Remarkably, at pH 8 and 5 mM MgSO_4 , the error rate of *Taq* dropped toward that seen previously for *Vent* and *Sequenase*, reaching a maximum fidelity at or above 0.5 mM dNTP. This effect was not found with the first round of optimizations (Figs. 2, 3, and 4), and emphasized the practical importance of reexamining the effect of the first two factors, namely pH and dNTP (Figs. 5 and 6).

The fidelity of both *Vent* and Rec-*Vent* was relatively invariant with dNTP concentration (Fig. 6), but once again a difference between *Vent* and Rec-*Vent* preparations was found in that only *Vent* showed efficient amplification below 0.5 mM dNTP. Because only Rec-*Vent* will be commercially available, except upon special request from the manufacturer, the Rec-*Vent* results should be noted in standard PCR applications.

DISCUSSION

The error frequency of different DNA polymerases has been measured by a variety of assays and ranged from 10^{-4} to 10^{-7} error/base pair incorporated.⁽⁴⁻⁷⁾ In this study, a combination of PCR and DGGE provides a sensitive and reliable assay to measure the wide

FIGURE 3 Fidelity of *Sequenase*, *Taq*, and *Vent* DNA polymerases as a function of pH: (▲) *Taq* (0.05 mM each dNTP, 2 mM MgSO_4), (□) *Vent* (0.5 mM each dNTP, 2 mM MgSO_4), (○) *Sequenase* (3.5 mM each dNTP, 5 mM MgCl_2). The efficiency was ~90% for *Sequenase*, ~70% for *Taq*, and *Vent*, except for *Vent* at pH 9 where it was ~45%. The arrows indicate the initial pH.

FIGURE 4 Fidelity of Sequenase, *Taq*, Vent, and Rec-Vent as a function of Mg^{2+} concentrations: (▲) *Taq* (0.05 mM each dNTP), (□) Vent, (×) Rec-Vent (both 0.5 mM each dNTP), and (○) Sequenase (3.5 mM each dNTP). All reactions were at pH 8.0, using $MgCl_2$ for Sequenase and $MgSO_4$ for *Taq*, Vent, and Rec-Vent. The efficiency was 90% for Sequenase, ~70% for *Taq* Lot 3813 (▲), except at ≤ 1 mM Mg^{2+} (36%), 40–63% for *Taq* Lot 3964 (▲), and ~70% for Rec-Vent and Vent, except at 0.5 mM Mg^{2+} (60%). The arrows indicate the initial Mg^{2+} concentration.

range of fidelity of different DNA polymerases over a variety of sequence contexts in the 104-bp target studied.⁽⁴⁾ In its present form, error rates as low as 5×10^{-6} errors/base pair incorporated can be measured if care is taken to begin with a sufficiently large number of denaturing gradient gel-purified templates to eliminate jackpot effects. Another advantage is that DGGE provides a fast and accurate way to measure polymerase-induced mutants in virtually any sequence of interest to the investigators. All base-pair substitutions, small additions, and deletions are detected. The nature of the sequence changes can be determined by isolating and sequencing these DNA fragments.^(4,13,21) However, if such information is not required, relative changes in the nature of polymerase-induced errors can be inferred from the pattern on the denaturing gradient gel alone.

The fidelity of DNA synthesis depends on the ability of DNA polymerases to discriminate against an incorrect nucleotide being ligated onto a primer-template, to extend from the mispair, and to correct the mispair by 3'→5' exonucleolytic reversal of polymerization. This last ability is possible only for DNA polymerases with active 3'→5' proofreading exonuclease. Among the three enzymes studied here, Vent is the only one known to

have an active 3'→5' exonuclease activity. However, its fidelity was matched by Sequenase and was 1.6-fold better than *Taq* polymerase, both of which are reported to be devoid of exonuclease activity.^(8,22)

The fidelity of all three enzymes, Sequenase, *Taq*, and Vent, was shown to be sensitive to changes in pH, concentrations of dNTP, and Mg^{2+} ions. The fidelities of *Taq* and Sequenase were shown to have a different dependence on dNTP concentration than Vent. Previous studies of *Taq* had concluded that its fidelity is good at low

equimolar concentration of total dNTP to Mg^{2+} concentration and that, in general, it is improved by lowering dNTP concentration, even in excess of Mg^{2+} .^(7,9) Our results confirm these reports but also show that for both *Taq* and Sequenase, in conditions where the Mg^{2+} concentration is high and the total dNTP concentration is less than that of magnesium, fidelity is optimal at higher dNTP concentration. However, the fidelity of Vent, though invariant over a wide range of dNTP concentration, slightly decreased at high dNTP concentration. This may be due to diminished proofreading ability by high dNTP concentrations that has been observed in many DNA polymerases with 3'→5' exonuclease activity such as *E. coli* Pol I and T4 polymerase.^(6,17,23,24) High concentrations of dNTP are postulated to increase the rate of polymerization of the next correct nucleotide following the mispair, and thus decrease the probability of excision by the exonuclease.^(23,25)

The effect of pH again showed no common trend on the fidelity of the three DNA polymerases tested. There was only a slight effect on the fidelity of Sequenase. The fidelity of Vent decreased at low pH only in conditions of low equimolar concentrations of dNTP to Mg^{2+} , whereas the fidelity of *Taq* progressively decreased as pH was raised. This supports the results of previous studies on *Taq*,^(7,9) that at all Mg^{2+} and dNTP concentrations, the fidelity of *Taq* can be improved by

FIGURE 5 Fidelity of *Taq*, Vent, and Rec-Vent as a function of pH: (▲) *Taq* (0.05 mM each dNTP, 5 mM $MgSO_4$), (□) Vent and (×) Rec-Vent (both 0.5 mM each dNTP, 7.5 mM $MgSO_4$). The efficiency was ~40% for *Taq*, ~70% for Vent, and Rec-Vent (57% at pH 9).

FIGURE 6 Fidelity of *Taq*, *Vent*, and *Rec-Vent* as a function of dNTP concentrations: (▲) *Taq* (5 mM MgSO_4 , pH 8.0), (□) *Vent*, and (×) *Rec-Vent* (both 7.5 mM MgSO_4 , pH 8.5). The efficiency was 34–38% for *Taq*, except at 0.5 and 1.5 mM dNTP (~59%), and ~70% for *Vent* and *Rec-Vent*.

lowering the pH. Mg^{2+} is used in this study as the activating divalent metal ion for DNA polymerization. It is considered a physiological activator because it is found in cells.⁽⁵⁾ Depending on its concentration, Mg^{2+} has been shown to increase or decrease polymerase fidelity.^(7,9,18,19) Fidelity has been shown to decrease by increasing the concentration of free Mg^{2+} .^(7,9) Indeed, the fidelities of *Sequenase* and *Taq* were very slightly decreased by high concentrations of Mg^{2+} . The high fidelity of *Vent* was mainly invariant and decreased only at very low Mg^{2+} concentration. Other studies on DNA polymerases with 3'→5' exonuclease activity such as *E. coli* Pol I have similarly noted that there was no effect of Mg^{2+} concentration on their fidelity,⁽¹⁸⁾ except at inhibiting concentration.⁽¹⁹⁾

As adjustments in the PCR conditions were made, the intensities of all of the heteroduplexes relative to the WT homoduplex changed. However, there was no observable change in the bands of the heteroduplexes in the denaturing gradient gel. This indicates that hotspots for polymerase-induced errors were similar regardless of changes in the PCR conditions employed. The specific polymerase-induced mutants of *Sequenase* and *Taq* have been previously reported.⁽⁴⁾ The positions of the heteroduplexes in the denaturing gradient gel are very similar between *Taq* and *Vent*, with a few exceptions. These bands have been isola-

ted and are being sequenced.

The fidelity of *Sequenase*, *Taq*, and *Vent* can be usefully improved by appropriate changes in pH, concentrations of dNTP, and Mg^{2+} ion relative to conditions previously employed in PCR. It is probable that there are factors other than dNTP, pH, and Mg^{2+} that are also important for their fidelity in which overall mutant fractions of less than 10% are required for 100-bp sequences amplified a million-fold. The conditions represented in the results of this study were limited to those that allowed efficient amplification of a specific template, HPRT exon 3. It is probable that fidelity and efficiency are functions of the DNA sequence being amplified. The variations in the fidelity and efficiency of different lots of DNA polymerases is of practical importance in experimental procedure and should be noted. Thus, it is important to optimize the PCR conditions based on the DNA sequence of interest and the purpose of the study. The major improvement in the fidelity of *Taq* was achieved at very low amplification efficiency (~36%) whereas *Vent* (~70%) and *Sequenase* (~90%) consistently showed efficient amplification. Our results showed that *Vent*, a thermostable DNA polymerase, is as accurate in DNA synthesis as *Sequenase* and should be of use to researchers generating PCR products.

ACKNOWLEDGMENTS

This work was supported by grants

from the U.S. Department of Energy (DE-FG02-86ER60448) and the National Institute of Environmental Health Sciences (P30-ES02109, 2P01-ES03926, 5P01-ES01640, and 5P42-ES04675).

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Received May 1, 1991; accepted in revised form July 2, 1991.

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L L Ling, P Keohavong, C Dias, et al.

Genome Res. 1991 1: 63-69

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