GoTaq™ DNA Polymerase: A New Enzyme Formulation for Amplifying DNA Fragments

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Introduction

PCR[a] or RT-PCR[a] is the tool of choice for answering many biological questions, making amplification almost ubiquitous in life science research. Commonly, the mode of analysis used post-PCR is agarose gel electrophoresis. The usual protocol is to set up the reaction, run the amplification profile on a thermal cycler, remove an aliquot, mix with gel loading solution, load onto a gel and separate the sample by electrophoresis. The workhorse of amplification is *Taq* DNA polymerase. Promega is a global leader in providing *Taq* DNA Polymerase[a], and we have developed a new formulation called GoTaq™ DNA Polymerase[a,b]. Reactions set up with GoTaq™ DNA Polymerase and the supplied Green Reaction Buffer can go directly from thermal cycler to gel analysis. There is no need to add a gel loading solution.

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GoTaq™ DNA Polymerase contains native *Taq* DNA Polymerase in a formulation based on the popular PCR Master Mix[a,b]. The enzyme is supplied with 5X Green GoTaq™ Reaction Buffer. The 1X Green Buffer has sufficient density to sink into a well of an agarose gel and, upon electrophoresis, separates into a blue dye and a yellow dye. The blue dye runs at 3–5kb, and the yellow dye runs below 50bp on a 1% agarose gel. GoTaq™ DNA Polymerase is also provided with a 5X Colorless GoTaq™ Reaction Buffer in case you need to perform absorbance or fluorescence measurements without first purifying the amplimer. Both 5X buffers are pH 8.5 and contain 7.5mM $MgCl_2$, giving a final $MgCl_2$ concentration of 1.5mM in the 1X buffer. Both 5X buffers have the same composition with the exception of the dyes. This article presents information on the characteristics of the enzyme and reaction buffers, explains reasons to choose one buffer over the other, and compares the characteristics of GoTaq™ DNA Polymerase with the amplification properties of our other *Taq* DNA Polymerase formulations. Also, the article reports compatibility of GoTaq™ DNA Polymerase with RT-PCR, PCR enhancing agents and downstream applications such as T-vector cloning.

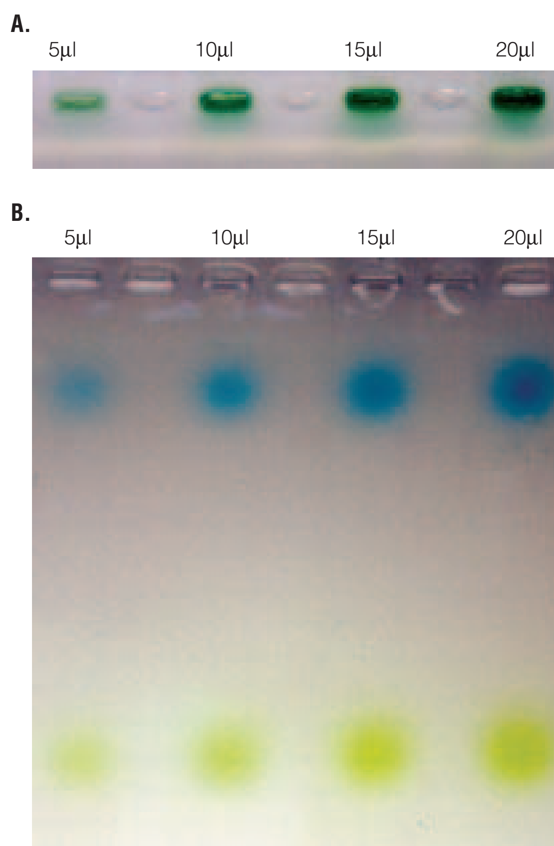

Figure 1. Amplification reactions using GoTaq™ DNA Polymerase with Green GoTaq™ Reaction Buffer. Panel A shows loaded wells of an agarose gel. **Panel B** shows the blue and yellow dyes after electrophoresis. Volumes of 5, 10, 15, and 20µl of the amplification reactions were loaded onto a 1% agarose gel with TBE buffer and subjected to electrophoresis.

Green GoTaq™ Reaction Buffer: Direct Loading on Agarose Gels

The proprietary 5X Green GoTaq™ Reaction Buffer eliminates the need to add loading buffers/dyes to amplification samples before loading them on a gel. With the Green GoTaq™ Reaction Buffer, a wide range of reaction volumes may be easily visualized when loaded into a gel (Figure 1, Panel A). In addition, sample migration can be monitored with the two dyes (Figure 1, Panel B). During electrophoresis, the blue dye comigrates at the same rate as a 3–5kb fragment in a 1% agarose gel. This dye migrates at approximately the same rate as the commonly used loading dye, xylene cyanol. The yellow dye migrates at a rate faster than the amplification primers (<50bp), making it an easy marker to ensure that the DNA fragments of interest remain in the gel. The dyes do not interfere with migration of the DNA in agarose gels; the

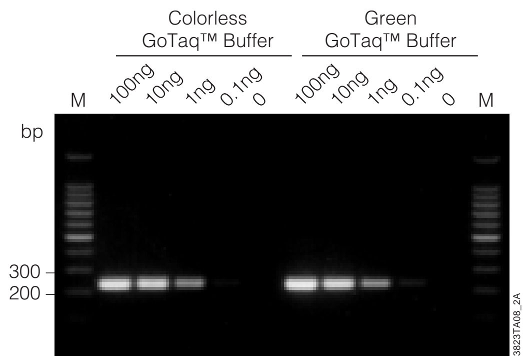

Figure 2. Detection of a Factor V fragment from human neuroblastoma SH-SY5Y DNA using GoTaq™ DNA Polymerase with either Colorless GoTaq™ Reaction Buffer or Green GoTaq™ Reaction Buffer. A 250bp fragment of the Factor V gene was amplified using the indicated amounts of template DNA. Lane M, 100bp DNA Ladder (Cat.# G2101). Genomic DNA was isolated with the Wizard® SV Genomic DNA Purification System (Cat.# A2360).

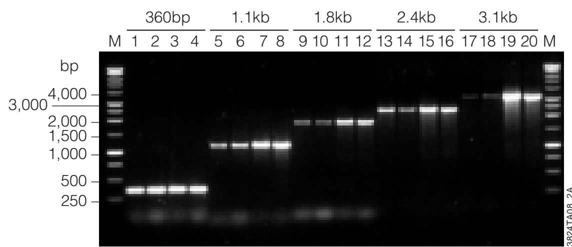

Figure 3. Comparison of amplification reactions using *Taq* DNA Polymerase in Storage Buffer B, *Taq* DNA Polymerase in Storage Buffer A and GoTaq™ DNA Polymerase. A 360bp α-1 antitrypsin fragment from 3.3ng Human Genomic DNA (Cat.# G3041), a 1.1kb IL-1β fragment from 1ng Mouse Genomic DNA (Cat.# G3091), a 1.8kb APC fragment from 3.3ng Human Genomic DNA, a 2.4kb APC fragment from 33ng Human Genomic DNA and a 3.1kb APC fragment from 75ng Human Genomic DNA were amplified using indicated amounts of template DNA. Amplifications were performed with *Taq* DNA Polymerase in Storage Buffer B (lanes 1, 5, 9, 13, 17) in Thermophilic DNA Polymerase Reaction Buffer, with *Taq* DNA Polymerase in Storage Buffer A (lanes 2, 6, 10, 14, 18) in Thermophilic DNA Polymerase Reaction Buffer, with GoTaq™ DNA Polymerase (lanes 3, 7, 11, 15, 19) in Colorless GoTaq™ Reaction Buffer, or with GoTaq™ DNA Polymerase (lanes 4, 8, 12, 16, 20) in Green GoTaq™ Reaction Buffer. Lane M, BenchTop 1kb DNA Ladder (Cat.# G7541).

DNA fragments migrate the same distance as corresponding DNA markers when the dyes are present. DNA fragments that comigrate with the blue dye are not masked by the dye if a volume ≤20µl is loaded.

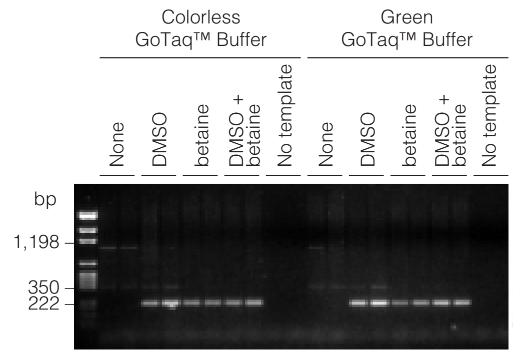

Figure 4. Amplification of a fragment of the human retinoblastoma gene using GoTaq™ DNA Polymerase with Colorless GoTaq™ Reaction Buffer or Green GoTaq™ Reaction Buffer with and without the addition of enhancing agents, DMSO and betaine. Amplifications contained 500ng Human Genomic DNA, 0.8µM of each primer and 1.25u GoTaq™ DNA Polymerase in 50µl reactions. As indicated, reactions contained no additives, 5% DMSO, 1M betaine, or 5% DMSO + 1M betaine. No-template control reactions were included. Amplification primers and cycling conditions were as given in reference 1. Lane M, pGEM® DNA Markers (Cat.# G1741).

Which Reaction Buffer Should I Use?

The 5X Green GoTaq™ Reaction Buffer is not recommended for downstream applications that require fluorescence or absorbance measurements. The dyes in the reaction buffer absorb light between 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. The dyes also have excitation peaks at 488nm and 600–700nm that correspond to the excitation wavelengths of commonly used fluorescence detection instrumentation. Although the yellow dye has the same excitation wavelength as that used by many fluorescent scanners (488nm), there is minimal interference because of the rapid migration of the yellow dye in the gel. Gels scanned with a 488nm scanner will have a light gray dye front below the primers that corresponds to the yellow dye front.

The 5X Colorless GoTaq™ Reaction Buffer is recommended for any application where absorbance or fluorescence measurements will be taken without prior clean-up. However, the 5X Green GoTaq™ buffer can still be used if the DNA fragments are purified first. Common PCR clean-up systems such as the Wizard® SV Gel and PCR Clean-Up System[(c)] (Cat.# A9281), Wizard® PCR Preps DNA Purification System[(d)] (Cat.# A2180) and Wizard® MagneSil™ PCR Clean-Up System[(e)] (Cat.# A1930) will easily separate the blue and yellow dyes from the amplified DNA fragment. Alternatively, you can separate the dyes from the DNA fragment by agarose gel electrophoresis followed by excision of the DNA fragment from the gel.

Table 1. Buffer Compatibility with Various Applications.

Application	Compatibility with Green GoTaq™ Buffer	Compatibility with Colorless GoTaq™ Buffer
Amplifying fragment from cDNA generated by the Reverse Transcription System	+	+
Amplifying fragment from cDNA generated by the ImProm-II™ Reverse Transcription System	+	+
T-vector cloning	+	+
TNT® T7 Quick for PCR DNA	+	+

Amplification

The 5X Green and 5X Colorless GoTaq™ Reaction Buffers give approximately equivalent amplification yield and sensitivity for most reactions that we have tested. To illustrate this, a 250bp fragment of the Factor V gene was amplified using SH-SY5Y neuroblastoma cell genomic DNA as the template. GoTaq™ DNA Polymerase with either 5X Green GoTaq™ Reaction Buffer or 5X Colorless GoTaq™ Reaction Buffer was used for amplification. Yield and sensitivity were similar for both buffers (Figure 2). In the few cases where a difference between the two buffers was noted, the difference could be eliminated if the reactions were optimized for each buffer.

Promega now offers three different formulations of *Taq* DNA Polymerase: GoTaq™ DNA Polymerase, *Taq* DNA Polymerase in Storage Buffer A (Cat.# M1861), and *Taq* DNA Polymerase in Storage Buffer B (Cat.# M1661). We compared the ability of our *Taq* DNA Polymerases to amplify five different targets. In this comparison, we used the reaction buffer provided with each enzyme (Thermophilic DNA Polymerase Buffer was used for *Taq* DNA Polymerase in Storage Buffer A and B). For amplification of some fragments, a higher $MgCl_2$ concentration was needed. In these cases, the $MgCl_2$ was adjusted to the required concentration using a 25mM $MgCl_2$ stock (Cat.# A3511). We found that the GoTaq™ DNA Polymerase in either the Green or the Colorless Buffer worked as well as our other *Taq* DNA Polymerase formulations (Figure 3). In some cases, reactions performed with GoTaq™ DNA Polymerase gave superior results. In addition, GoTaq™ DNA Polymerase used in conjunction with the Green or the Colorless GoTaq™ Reaction Buffer can amplify fragments of a wide size range (Figure 3).

Compatibility with PCR Enhancing Agents

Occasionally amplification of a specific DNA region proves to be difficult, especially with targets that can form a secondary structure or have a high GC content. For these situations, a number of PCR-enhancing agents can be used to allow amplification of these difficult regions (1–4). Two of the most popular additives are dimethyl sulfoxide (DMSO) and betaine. We tested the compatibility of DMSO and betaine with GoTaq™ DNA Polymerase using Green GoTaq™ Reaction Buffer and Colorless GoTaq™ Reaction Buffer. For this experiment, we amplified a 180bp fragment of the human retinoblastoma gene; this fragment spans the exon-intron 1 junction and requires the addition of DMSO or betaine (1,5) for amplification. We found that both DMSO and betaine are compatible with GoTaq™ DNA Polymerase using either Green GoTaq™ Reaction Buffer or Colorless GoTaq™ Reaction Buffer (Figure 4). Without additive, the 180bp fragment is not amplified, and only non-specific fragments are observed. When either or both of the enhancing agents are added to the PCR, the 180bp fragment is amplified. In addition, DMSO and betaine have no adverse effect on the blue and yellow dyes. Both dyes retain their color and migrate as expected in an agarose gel during electrophoresis.

Compatibility with Applications

We have tested compatibility of the DNA fragments generated with GoTaq™ DNA Polymerase in Green GoTaq™ Reaction Buffer and Colorless GoTaq™ Reaction Buffer in several applications. We have found that fragments generated were compatible with several upstream and downstream applications (Table 1). GoTaq™ DNA Polymerase can be used for PCR after generation of cDNA. In addition, amplification products created with GoTaq™ DNA Polymerase have the characteristic A-overhang so they can be cloned into pGEM®-T Vectors[f,g] (Cat.# A3600, A3610). Finally, we found that fragments generated using GoTaq™ DNA Polymerase can be used with TNT® T7 Quick for PCR DNA[h,i,j] (Cat.# L5540) for direct transcription and translation.

Summary

PCR products generated using our newly formulated GoTaq™ DNA Polymerase and 5X Green GoTaq™ Reaction Buffer can be directly loaded into agarose gels. This eliminates the need to add loading dyes/buffers to amplification samples, streamlining experiments where gel analysis is used. GoTaq™ DNA Polymerase is also provided with a Colorless Reaction Buffer, for experiments where absorbance or fluorescence measurements are necessary without first purifying the PCR product of interest. Using GoTaq™ DNA Polymerase along with the Green or Colorless GoTaq™ Reaction Buffers, we have amplified fragments ranging in size from 180–250bp to 3.1kb. The enzyme and buffers are compatible with the PCR enhancing agents, DMSO and betaine. Besides gel analysis, DNA fragments generated with GoTaq™ DNA Polymerase can be used in several downstream applications as well as for PCR after generation of cDNA from RNA using reverse transcription systems.

Acknowledgments

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References

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Ordering Information

Product	Size	Cat.#
GoTaq™ DNA Polymerase	100u	M3001
	500u	M3005
	2,500u	M3008

For Laboratory Use.

[a]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for person that either have a license to perform PCR or are not required to obtain a license.
[b]U.S. Pat. No. 6,242,235 has been issued to Promega Corporation for polymerase stabilization by polyethoxylated amine surfactants. Other patents are pending.
[c]U.S. Pat. Nos. 5,658,548, 5,808,041 and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents are pending.
[d]Licensed under U.S. Pat. No. 5,075,430.
[e]U.S. Pat. Nos. 6,027,945, 6,368,800, Australian Pat. No. 732756, and Japanese Pat. No. 3253638 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles. Other patents are pending.
[f]U.S. Pat. No. 4,766,072.
[g]Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.
[h]U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.
[i]U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. No. 3009458 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.
[j]U.S. Pat. Nos. 4,966,964, 5,019,556, 5,266,687, Australian Pat. Nos. 616881, 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor are exclusively licensed to Promega Corporation.

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