The "Go-To" Choice: GoTaq™ DNA Polymerase

Introducing GoTaq™ DNA Polymerase: Improved Amplification With a Choice of Buffers

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Abstract

GoTaq™ DNA Polymerase, Promega's new formulation of Taq *DNA Polymerase, is supplied with two buffers—Green GoTaq™ Reaction Buffer and Colorless GoTaq™ Reaction Buffer. DNA amplified using GoTaq™ DNA Polymerase with Green GoTaq™ Reaction Buffer can be loaded directly onto agarose gels, eliminating the need to add loading dye or buffer to the PCR sample before electrophoresis. When PCR samples are not loaded onto a gel or when the dyes in the Green GoTaq™ Reaction Buffer would interfere with downstream applications, the Colorless GoTaq™ Reaction Buffer can be used.*

This buffer, when used with GoTaq™ DNA Polymerase for amplification, allows the researcher to go directly from thermal cycler to gel without having to mix the sample with gel-loading buffer.

Introduction

PCR[a] and RT-PCR are common techniques used in biological research, and *Taq* DNA polymerase is the most common thermostable DNA polymerase used in these applications. Promega has developed a new formulation of this popular enzyme, GoTaq™ DNA Polymerase[a,b], as well as two new reaction buffers for use with this enzyme.

Once the researcher has amplified a DNA fragment, he or she frequently will analyze the fragment using agarose gel electrophoresis. To streamline this process we developed the Green GoTaq™ Reaction Buffer. This buffer, when used with GoTaq™ DNA Polymerase for amplification, allows the researcher to go directly from thermal cycler to gel without having to mix the sample with gel-loading buffer.

GoTaq™ DNA Polymerase contains native *Taq* DNA polymerase in a proprietary formulation and is supplied with both a 5X Green GoTaq™ Reaction Buffer and a 5X Colorless GoTaq™ Reaction Buffer. When diluted to 1X, the Green GoTaq™ Reaction Buffer has sufficient density to sink in the wells of an agarose gel or nondenaturing TBE polyacrylamide gel. The buffer contains two dyes that separate upon electrophoresis to yield a blue dye and a yellow dye, which can be used to monitor the progress of electrophoresis.

The 5X Colorless GoTaq™ Reaction Buffer is recommended for use with GoTaq™ DNA Polymerase when performing absorbance or fluorescence measurements without prior purification of the amplimer.

The Colorless GoTaq™ Reaction Buffer has the same composition as the 5X Green GoTaq™ Reaction Buffer, without the two dyes. Both 5X GoTaq™ Reaction Buffers contain 7.5mM $MgCl_2$ at pH 8.5, providing 1.5mM $MgCl_2$ in the 1X buffer.

In this article, we describe the properties of both the enzyme and the reaction buffers, explore the characteristics of the dyes in the Green GoTaq™ Reaction Buffer and examine some considerations in choosing the proper buffer. In addition, we compare the amplification properties of GoTaq™ DNA Polymerase with those of other Promega *Taq* DNA polymerase formulations. Finally, we report on the compatibility of GoTaq™ DNA Polymerase with RT-PCR, T-vector cloning and other applications.

Green GoTaq™ Reaction Buffer

The proprietary 5X Green GoTaq™ Reaction Buffer eliminates the need to add loading buffers and dyes to amplification samples before electrophoresis. With this buffer, a wide range of reaction volumes may be loaded into the wells (Figure 1, Panel A). Migration progress can be monitored by tracking the two dyes in the buffer (Figure 1, Panel B). During electrophoresis, the blue dye migrates at the same rate as a 3–5kb DNA 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 that of the primers used in the amplifications (<50bp), making it easy to ensure that the DNA fragments of interest remain in the gel. This dye also runs faster than the orange electrophoresis marker found in Promega's 6X Blue/Orange Loading Dye (Cat.# G1881). The dyes do not interfere with migration of the DNA in agarose gels; fragments migrate the same distance as corresponding markers when the dyes are present. Also, DNA fragments that comigrate with the blue dye are not masked by the dye when ≤20µl is loaded.

Choice of Reaction Buffer

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 and 300nm, making standard A_{260} determination of DNA concentration unreliable. The dyes also have excitation peaks at 488nm and 600–700nm, which correspond to the excitation wavelengths used in common fluorescence detection instruments. Although the yellow dye has the

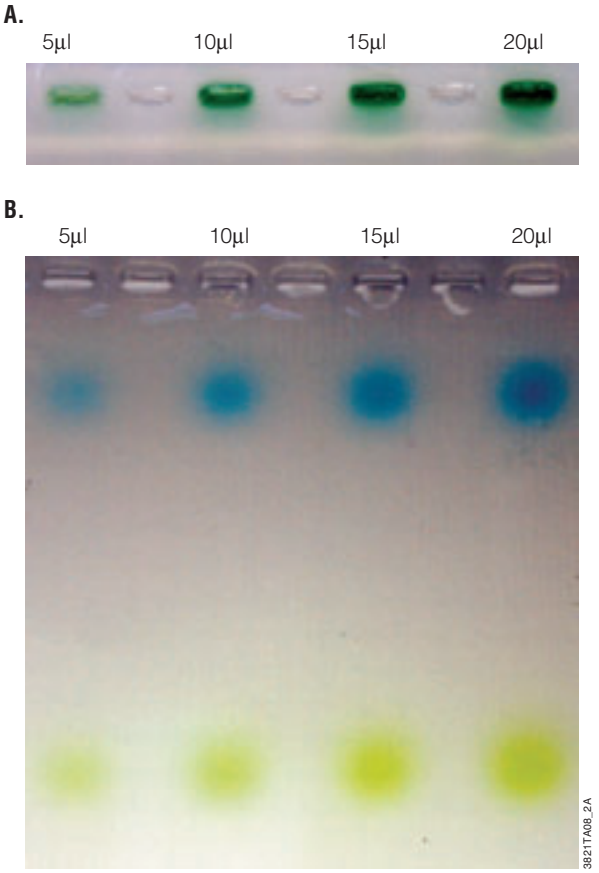

Figure 1. Amplification reactions using GoTaq™ DNA Polymerase with Green GoTaq™ Reaction Buffer. Panel A. Loaded wells of an agarose gel. **Panel B.** Blue and yellow dyes after electrophoresis. Volumes of 5, 10, 15 and 20µl of the amplification reactions were loaded into a 1% agarose gel with TBE buffer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

same excitation wavelength as that used by many fluorescent gel scanners (488nm), it minimally interferes because of its rapid migration 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.

The 5X Colorless GoTaq™ Reaction Buffer is recommended for applications in which absorbance or fluorescence measurements will be made without prior clean-up steps. However, the 5X Green GoTaq™ Reaction Buffer may 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 remove the blue and yellow dyes from amplified DNA. Alternatively, dyes can be removed from the amplified DNA by either ethanol precipitation or agarose gel electrophoresis followed by excision of the DNA fragment from the gel.

PCR Performance

The 5X Green and 5X Colorless GoTaq™ Reaction Buffers provide approximately equivalent yield and sensitivity for most amplifications that we have tested. To illustrate this, we amplified a 1.2kb fragment of the α-1 antitrypsin gene using Human Genomic DNA (Cat.# G3041) as the template. We used GoTaq™ DNA Polymerase with either 5X Green GoTaq™ Reaction Buffer or 5X Colorless GoTaq™ Reaction Buffer for the amplifications. Yield and sensitivity were similar for both buffers (Figure 2).

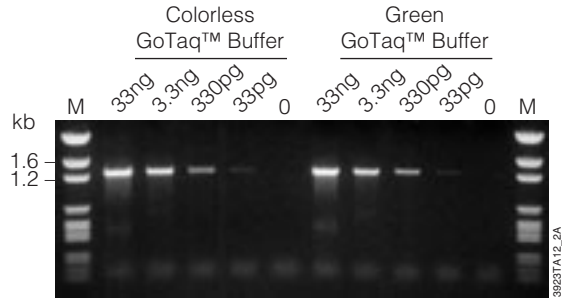

Figure 2. Detection of an α-1 antitrypsin fragment from human genomic DNA using GoTaq™ DNA Polymerase with either Colorless GoTaq™ Reaction Buffer or Green GoTaq™ Reaction Buffer. A 1.2kb fragment of the α-1 antitrypsin gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lane M, BenchTop pGEM® DNA Markers (Cat.# G7521).

Promega now offers three different formulations of *Taq* DNA polymerase: GoTaq™ DNA Polymerase, *Taq* DNA Polymerase in Storage Buffer A[(a)] (Cat.# M1861), and *Taq* DNA Polymerase in Storage Buffer B[(a)] (Cat.# M1661). We compared the amplification ability of these enzyme formulations using 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, we needed higher $MgCl_2$ concentration. In these cases, we adjusted the $MgCl_2$ to the required concentration using a 25mM $MgCl_2$ stock (Cat.# A3511). The GoTaq™ DNA Polymerase in either the green or the colorless buffer worked as well as the 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 with the Green and Colorless GoTaq™ Buffers successfully amplified fragments of a wide size range (Figure 3).

Use in RT-PCR

GoTaq™ DNA Polymerase and either GoTaq™ Reaction Buffer can be used for PCR after generation of cDNA in uncoupled RT-PCR. We tested the ability of GoTaq™ DNA Polymerase with either Green or Colorless GoTaq™ Reaction Buffer to amplify a 275bp β-actin target from a cDNA template generated using the ImProm-II™ Reverse

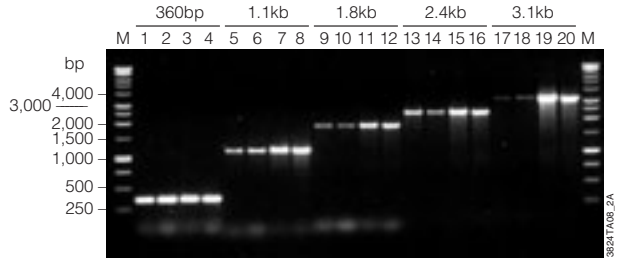

Figure 3. Comparison of amplification reactions using *Taq* DNA Polymerase in Storage Buffer B, *Taq* DNA Polymerase in Storage Buffer A and GoTaq™ DNA Polymerase. A 360bp α-1 antitrypsin fragment from 3.3ng Human Genomic DNA (Cat.# G3041), a 1.1kb IL-1β fragment from 1ng Mouse Genomic DNA (Cat.# G3091), 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 using either *Taq* DNA Polymerase in Storage Buffer B (lanes 1, 5, 9, 13, 17) with Thermophilic DNA Polymerase Buffer (Cat.# M1901), *Taq* DNA Polymerase in Storage Buffer A (lanes 2, 6, 10, 14, 18) with Thermophilic DNA Polymerase Buffer, GoTaq™ DNA Polymerase (lanes 3, 7, 11, 15, 19) with Colorless GoTaq™ Reaction Buffer, or GoTaq™ DNA Polymerase (lanes 4, 8, 12, 16, 20) with Green GoTaq™ Reaction Buffer. Lane M, BenchTop 1kb DNA Ladder (Cat.# G7541). All amplifications were perfomed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

Transcription System (Cat.# A3800). GoTaq™ DNA Polymerase successfully amplified the target, and the yield and sensitivity were similar for both GoTaq™ buffers (Figure 4). Figure 4 illustrates the use of 20µl of the ImProm-II™ cDNA reactions for the template; however, we also found that 1µl of the cDNA reactions can be used (data not shown) as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* #TM236 (1).

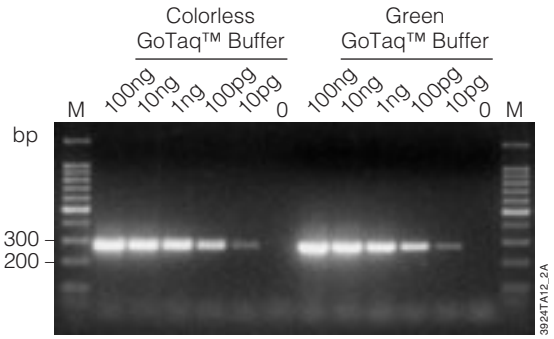

Figure 4. Detection of a β-actin fragment from total RNA. Indicated amounts of total RNA from Jurkat cells isolated using the SV Total RNA Isolation System[f,g] (Cat.# Z3500) served as a template in the 20µl cDNA synthesis reactions. cDNA was generated as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* (1) using GoTaq™ DNA Polymerase with either Colorless or Green GoTaq™ Buffer. PCR was performed using Oligo(dT) primer. A 275bp β-actin fragment was amplified using 20µl of the cDNA synthesis reactions in 100µl PCR amplifications as directed in the *ImProm-II™ Reverse Transcription Technical Manual* (1). Lane M, 100bp DNA Ladder (Cat.# G2101). All amplifications were perfomed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

Table 1. Compatibility of GoTaq™ DNA Polymerase with Various Applications.

Application	Green GoTaq™ Reaction Buffer	Colorless GoTaq™ Reaction Buffer
Uncoupled RT-PCR:		
Amplifying fragment from cDNA generated by the ImProm-II™ Reverse Transcription System	Yes	Yes
Amplifying fragment from cDNA generated by the Reverse Transcription System (AMV RT)	Yes	Yes
Additions to PCR Amplifications:		
Addition of PCR enhancing agents DMSO and betaine	Yes	Yes
Use of anti-*Taq* DNA polymerase antibodies for "hot-start" PCR	Yes	Yes
Downstream Applications:		
T-vector cloning	Yes	Yes
TnT® T7 Quick for PCR DNA[h,i,m]	Yes	Yes
Automated DNA sequencing after PCR amplimer clean-up	Yes	Yes

We have determined that GoTaq™ DNA Polymerase and either GoTaq™ Reaction Buffer can be used for PCR after cDNA generation using the Reverse Transcription System[h,i] (Cat.# A3500) (Table 1; specific data not shown). As with any uncoupled RT-PCR, carryover concentrations of magnesium chloride, dNTPs and buffer must be taken into account when planning the PCR. One note of caution—when calculating the amount of magnesium chloride to be added to the amplifications, remember that GoTaq™ Reaction Buffers, unlike many buffers, contain 1.5mM magnesium chloride.

T-Vector Cloning

Amplification products created with GoTaq™ DNA Polymerase have an A-overhang for cloning into T-vectors. To illustrate this, we generated a 542bp fragment using GoTaq™ DNA Polymerase with either Green or Colorless GoTaq™ Reaction Buffer. We cleaned up the amplification products using the Wizard® MagneSil™ PCR Clean-Up System (Cat.# A1930) and cloned them into the pGEM®-T Easy Vector System II[j,k] (Cat.# A1380), following the directions given in the technical literature (2, 3). We found that the percentage of recombinant colonies (white colonies ÷ total colonies × 100) was 82% for the Green GoTaq™ Reaction Buffer and 83% for the Colorless GoTaq™ Reaction Buffer. The Control Insert provided with the pGEM®-T Easy System yielded 89% recombinant colonies. (A no-insert control yielded <1%.). This indicates that inserts generated using GoTaq™ DNA Polymerase with the GoTaq™ Reaction Buffers have A-overhangs and can be cloned into T-vectors. In addition, fragments generated using GoTaq™ DNA Polymerase and either GoTaq™ Reaction Buffer can be cloned into the pTARGET™ Mammalian Expression Vector System[k,l] (Cat.# A1410) (data not shown).

Conclusion

PCR products generated using Promega's newly formulated GoTaq™ DNA Polymerase and 5X Green GoTaq™ Reaction Buffer can be loaded directly into agarose and nondenaturing TBE polyacrylamide gels. This eliminates the need to add loading dyes and buffers to amplification samples, thereby streamlining experiments where gel analysis is used.

GoTaq™ DNA Polymerase also includes 5X Colorless GoTaq™ Reaction Buffer for experiments in which absorbance or fluorescence measurements are necessary, eliminating the need to purify the PCR product of interest before analysis.

Using GoTaq™ DNA Polymerase we have amplified fragments ranging from 180bp to 3.1kb. In addition to the benefits in gel analysis, GoTaq™ DNA Polymerase will generate DNA fragments that can be cloned into T-vectors and used in other applications (Table 1). GoTaq™ DNA Polymerase and either of the GoTaq™ Reaction Buffers can be used for amplification of cDNA using reverse transcription systems.

Acknowledgments

We thank Susan Fly, Tracy Worzella, Natalie Betz and Katharine Miller for the applications testing information to generate Table 1.

References

1. *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation.
2. *Wizard® MagneSil™ PCR Clean-Up System Technical Bulletin* #TB290, Promega Corporation.
3. *pGEM®-T and pGEM®-T Easy Vector Systems Technical Manual* #TM042, Promega Corporation.

Protocols

- *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation. (www.promega.com/tbs/tm236/tm236.html)
- *Wizard® MagneSil™ PCR Clean-Up System Technical Bulletin* #TB290, Promega Corporation. (www.promega.com/tbs/tb290/tb290.html)
- *pGEM®-T and pGEM®-T Easy Vector Systems Technical Manual* #TM042, Promega Corporation. (www.promega.com/tbs/tm042/tm042.html)

Not Pictured

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Ordering Information

Product	Size	Cat#
GoTaq™ DNA Polymerase[a,b]	100 units	M3001
	500 units	M3005
	2,500 units	M3008
5X Green GoTaq™ Reaction Buffer	20ml	M7911
5X Colorless GoTaq™ Reaction Buffer	20ml	M7921

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 persons 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 and 5,808,041, Australian Pat. No. 689815 and other patents pending.

[d] Licensed under U.S. Pat. No. 5,075,430.

[e] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.

[f] U.S. Pat. No. 6,218,531 and other patents pending.

[g] Australian Pat. No. 730718 and other patents and patents pending.

[h] U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

[i] U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

[j] U.S. Pat. No. 4,766,072.

[k] Licensed under one or more of U.S. Pat. Nos. 5,487,993 and 5,827,657 and European Pat. No. 0 550 693.

[l] The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

[m] 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.