GoTaq® Green Master Mix: From Amplification to Analysis

By Laura Flanagan, B.S., Sarah Wheeler, B.S., Mark Koeff, B.S., and Kimberly Knoche, Ph.D., Promega Corporation.

Abstract

GoTaq® Green Master Mix, the latest in the Promega line of amplification products, features convenient and fast gel loading with fewer manipulations and less likelihood of contamination. All pertinent components are included in the master mix so the researcher simply adds his or her preferred amplification primers, template and water. GoTaq® Green Master Mix combines the convenience of a master mix with the popular direct-to-gel loading that the GoTaq® Green Reaction Buffer offers.

In addition to the benefits of a master mix format, GoTaq® Green Master Mix offers the convenience of loading samples directly into agarose gels.

Introduction

PCR and RT-PCR[a] are two common techniques used in biological research. Analysis of DNA fragments by gel electrophoresis is quite common, and convenience is an important factor in selecting an amplification system. The ability to directly load the PCR sample into an agarose gel without adding gel loading dye or buffer to each sample is highly desirable. GoTaq® Green Master Mix[a] allows amplification reactions to be loaded directly into a gel, because the reaction buffer contains dyes and has sufficient density to sink in the wells of agarose or nondenaturing TBE polyacrylamide gels. GoTaq® Green Master Mix contains two dyes (blue and yellow) that separate during electrophoresis, allowing you to monitor the progress of samples on the gel (Figure 1).

In this article, we describe the properties and characteristics of GoTaq® Green Master Mix. We demonstrate the sensitivity and compare amplification performance of *Taq* DNA Polymerase[b] to the GoTaq® Green Master Mix using a variety of targets. Also, amplifications are performed using different DNA template sources including DNA isolated using commercially available kits and crude DNA preparations. We discuss the use of GoTaq® Green Master Mix in RT-PCR and mention it in other applications.

PCR Performance

GoTaq® Green Master Mix amplifies target DNA with good yield and sensitivity. To illustrate this, we amplified a 1.2kb / -1-antitrypsin fragment from a Human Genomic DNA template (Cat.# G3041) (Figure 2). We have achieved sensitivity to 33pg.

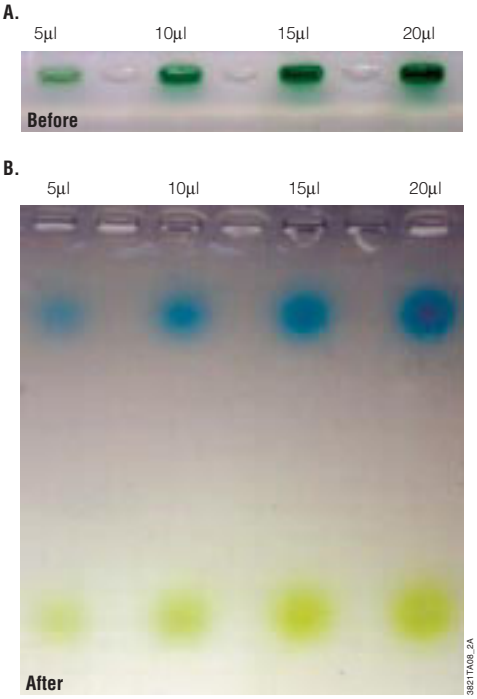

Figure 1. Separation of blue and yellow dyes used in GoTaq® Green Master Mix before and after electrophoresis. Panel A. Loaded wells of an agarose gel. **Panel B.** Blue and yellow dyes after electrophoresis. Volumes of 5, 10, 15 and 20µl of the amplification reactions were loaded into a 1% agarose gel with TBE buffer and subjected to electrophoresis.

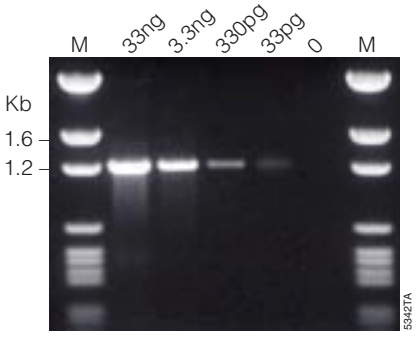

Figure 2. Detection of an / -1-antitrypsin fragment from human genomic DNA using GoTaq® Green Master Mix. A 1.2kb fragment of the / -1-antitrypsin gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lane M, BenchTop pGEM® DNA Markers (Cat.# G7521).

The GoTaq® Green Master Mix performance was similar to or better than standard *Taq* DNA Polymerase in the amplification reactions described here. We compared the ability of the two enzymes to amplify seven DNA targets. For the GoTaq® Green Master Mix amplifications, the provided buffer contained $MgCl_2$ (1.5mM final concentration) and dNTPs (200µM each, final concentration). For *Taq* DNA Polymerase, the buffer provided with the enzyme was used, and $MgCl_2$ and dNTPs were added to final concentrations equal to those found in the GoTaq® Green Master Mix. We obtained better yield for a number of targets using the GoTaq® Green Master Mix when compared to standard *Taq* DNA Polymerase (Figure 3). In addition, we saw increased sensitivity with these targets (data not shown). We successfully amplified targets from 143bp to 4.1kb (data not shown) from a genomic DNA template using GoTaq® Green Master Mix (Table 1).

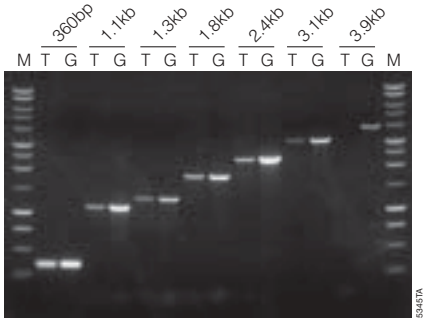

Figure 3. Comparison of amplification reactions using GoTaq® Green Master Mix and *Taq* DNA Polymerase. The following fragments were amplified using standard *Taq* DNA Polymerase (Lanes T) or GoTaq® Green Master Mix (Cat.# M7122; Lanes G). A 360bp ⁄ -1-antitrypsin fragment from 3.3ng Human Genomic DNA (Cat.# G3041); 1.1kb IL-1€fragment from 10ng Mouse Genomic DNA; 1.3kb €globin fragment from 330pg Human Genomic DNA; 1.8kb APC fragment from 3.3ng Human Genomic DNA; 2.4kb APC fragment from 3.3ng Human Genomic DNA; 3.1kb APC fragment from 3.3ng Human Genomic DNA; 3.9kb APC fragment from 3.3ng Human Genomic DNA. Lane M, BenchTop 1kb DNA Ladder (Cat.# G7541).

Different Template DNA Sources

Researchers frequently amplify template DNA isolated using crude DNA preparations or commercially available kits. We demonstrate the ability of GoTaq® Green Master Mix to amplify template DNA from both crude preparations and commercially available systems (Figure 4). GoTaq® Green Master Mix amplified crude DNA templates including genomic mouse toe DNA (1) (Figure 4, Panel A) and bacterial colony DNA from bacteria harboring a T-vector plasmid (pTARGET™ Mammalian Expression Vector, Cat.# A1410) containing an insert (Figure 4, Panel D). The Master Mix also amplified DNA templates isolated using commercially available systems including human DNA isolated from blood using the MagneSil® Genomic, Large Volume System (Cat.# A4080) (Figure 4, Panel B) and corn DNA isolated using Wizard® Magnetic 96 DNA Plant System (Cat.# FF3760) (Figure 4, Panel C).

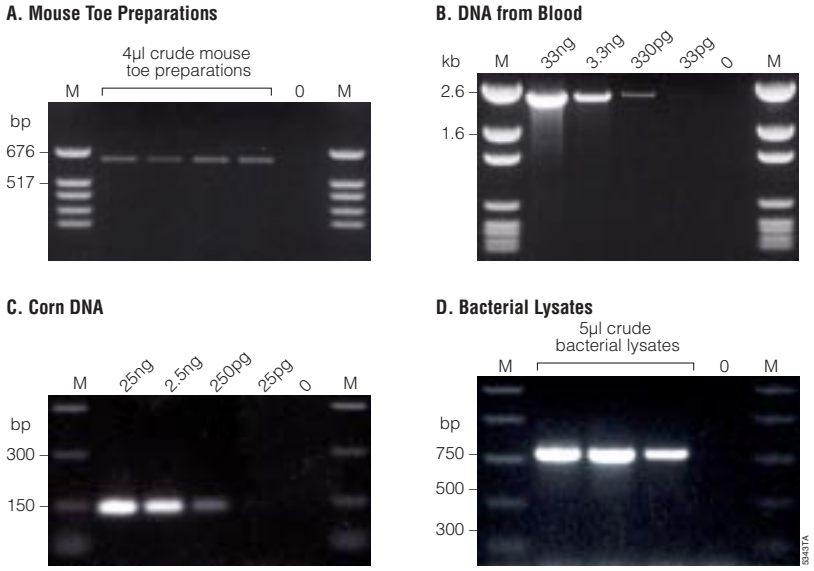

Figure 4. Amplification of DNA from different sources using GoTaq® Green Master Mix. Panel A. Amplification of a 670bp MIN (multiple intestinal neoplasia) fragment from crude mouse toe DNA preparations (1,2). Four microliters of DNA from four different crude mouse toe preparations was added to PCR amplifications. Lane 0, no-template control. Lane M, Bench Top pGEM® DNA Markers (Cat.# G7521). **Panel B.** Amplification of a 2.4kb APC fragment of human genomic DNA from blood purified using the MagneSil® Genomic, Large Volume System (Cat.# A4080). The indicated amount of DNA was amplified using GoTaq® Green Master Mix. Lane M, BenchTop pGEM® DNA Markers (Cat.# G7521). **Panel C.** Amplification of a 143bp EST AW067275 fragment from corn DNA isolated using Wizard® Magnetic 96 DNA Plant System (Cat.# FF3760). The DNA was amplified using GoTaq® Green Master Mix using the indicated amounts of corn DNA as a template (3). Lane M, BenchTop PCR Markers (Cat.# G7531). **Panel D.** Amplification of a 542bp plasmid insert fragment from crude bacterial colony DNA preparations. Bacterial colonies were suspended in 50µl of sterile water, boiled for 10 minutes, centrifuged for 5 minutes, and 30µl of the supernatant was removed. The supernatant was diluted 1:10,000, and 5µl of the diluted crude bacterial DNA from 3 colonies was amplified. Lane 0, no-template control. Lane M, BenchTop PCR Markers (Cat.# G7531).

Table 1. Compatibility of GoTaq® Green Master Mix with Various Applications.

Application	GoTaq® Green Master Mix
PCR	
Amplify targets from 143bp to 4.1kb in length	Yes
PCR Enhancer	
DMSO (5%)	Yes
Betaine (1M)	Yes
Uncoupled RT-PCR	
Amplify fragment from cDNA generated by the ImProm-II™ Reverse Transcription System	Yes
Amplify fragment from cDNA generated by the Reverse Transcription System (AMV RT)	Yes
Direct Gel Loading	
Agarose or nondenaturing TBE polyacrylamide gel	Yes
Cloning	
Compatible with T-vector cloning	Yes

The GoTaq® Green Master Mix gave good yield and sensitivity in these amplifications. The use of GoTaq® Green Master Mix can streamline commonly used techniques such as PCR screening methods to determine the genotype of organisms from crude DNA preparations and purified DNA from plants, bacteria and animals.

Use in RT-PCR

When doing uncoupled RT-PCR, the cDNA is generated by the reverse transcriptase, then all or part of this reaction is used for PCR. The technical literature for our two popular reverse transcription systems, (ImProm-II™ Reverse Transcription System[c,d] (Cat.# A3800) and the Reverse Transcription System[c] (Cat.# A3500), give protocols for using stand-alone enzymes for PCR. However, GoTaq® Green Master Mix can be used for PCR after generation of cDNA in uncoupled RT-PCR. We tested this by generating cDNA from total mouse liver RNA using the ImProm-II™ Reverse Transcription System (4) (Cat.# A3800) and then amplifying a 540bp, €actin target using GoTaq® Green Master Mix. We used 1µl of the reverse transcription reaction for the data generated in Figure 5, and we achieved sensitivity to 5pg.

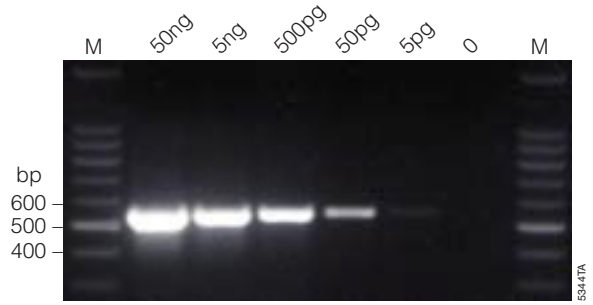

Figure 5. Detection of a €actin fragment from total mouse liver RNA. Twenty-microliter cDNA synthesis reactions were performed using the indicated amounts of total RNA from mouse liver isolated using RNAgents® Total RNA Isolation System (Cat.# Z5110). The cDNA was synthesized as directed in the ImProm-II™ Reverse Transcription System Technical Manual (4) using the Oligo(dT)$_{15}$ Primer (Cat.# C1101). A 540bp €actin fragment was amplified using 1µl of the cDNA synthesis reactions and 50µl of GoTaq® Green Master Mix in 100µl PCR amplifications. Lane M, 100bp DNA Ladder (Cat.# G8291).

In additional experiments we amplified the 540bp €actin fragment using a 20µl ImProm-II™ Reverse Transcription reaction in 100µl amplifications (data not shown). We found that we could use either 40µl or 50µl GoTaq® Green Master Mix in the 100µl amplifications with equal yield and sensitivity. However, for some targets there may be critical, specific conditions that might be sensitive to carryover of reaction components (e.g., $MgCl_2$) from the reverse transcription reactions. In these cases we recommend the use of GoTaq® Flexi DNA Polymerase (Cat.# M8291). In addition we found that GoTaq® Green Master Mix can also be used for PCR after generation of cDNA by the Reverse Transcription System (Cat.#A3500; Table 1, data not shown).

Additional Applications

Some targets are difficult to amplify due to secondary structure or high GC content. Frequently, the addition of PCR-enhancing agents such as dimethyl sulfoxide (DMSO) or betaine enable successful amplification (5–8). We performed amplifications containing DMSO or betaine and found that both are compatible with GoTaq® Green Master Mix (Table 1, specific data not shown). In addition, neither enhancing agent had an adverse effect on the blue or yellow dyes. Both dyes retained their color and migrated as expected in agarose gels.

Fragments generated using GoTaq® Green Master Mix can be cloned into T-vectors (Table 1, data not shown). This indicates that GoTaq® DNA Polymerase, like standard *Taq* DNA Polymerase, adds a single deoxyadenosine in a template-independent manner to the 3´ end of amplified fragments (9,10).

Direct Gel Loading

GoTaq® Green Master Mix eliminates the need to add loading buffers and dyes to amplification samples prior to electrophoresis. The 1X Master Mix has sufficient density to sink into the wells of a gel, and migration can be monitored using the two dyes (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 (approximately the same rate as the commonly used loading dye, xylene cyanol). The yellow dye migrates at a rate faster than the primers used in the amplification reactions (<50bp), making it easy to ensure that the DNA fragments of interest remain in the gel. The dyes do not interfere with the migration of DNA in agarose gels; fragments migrate the same distance whether the dyes are present or absent. Also, DNA fragments that co-migrate with the blue dye are not masked by the dye during UV transillumination when A20µl is loaded.

Removal of Dyes for Direct Measurement

The blue and yellow dyes of the GoTaq® Green Master Mix can be removed from amplification reactions with the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) to below detectable levels. The dyes in the GoTaq® Green Master Mix 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. Dyes can also be removed by simple ethanol precipitation.

Conclusion

GoTaq® Green Master Mix offers fast and convenient PCR amplification and gel loading. In addition to the benefits of a master mix format, it allows the convenience of loading samples directly into an agarose gel prior to electrophoresis. We found that the GoTaq® Green Master Mix can be used to amplify a wide range of target sizes and that the yield and sensitivity are similar to or better than standard *Taq* DNA Polymerase for many different targets. In addition, this product can be used in the PCR step of uncoupled or two-step RT-PCR when either the ImProm-II™ Reverse Transcription System or the Reverse Transcription System is used to synthesize the cDNA. Finally, it also generates fragments amenable to T-vector cloning and works well with PCR-enhancing agents.

Acknowledgments

We would like to thank Richard Halberg and the William Dove Lab (Grant R37 CA63677) at McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, for crude mouse toe DNA preparations and the MIN (multiple intestinal neoplasia) amplification procedure. We would like to thank Susan Koller, Terri Grunst, Brian Andersen, Susan Fly and Amanda Glebs from Promega for experimental materials and protocols. In addition, we would like to thank Cindy Sprecher and Doug Storts from Promega for their ideas and guidance for these experiments.

References

1. Dove, W. *et al.* DNA Isolation Protocol Grant R37 CA63677.
2. Dietrich W.F. *et al.* (1993) *Cell* **75**, 631–9.
3. Wang, M.L. *et al.* (2005) *Plant Genetic Resources* **3**, 45–57
4. *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation.
5. Frackman, S. *et al.* (1998) *Promega Notes* **65**, 27–9.
6. Baskaran, N. *et al.* (1996) *Genome Res.* **6**, 633–8.
7. Weissensteiner, T. and Lanchbury, J.S. (1996) *Biotechniques* **21**, 1102–8.
8. Hengen, P.N. (1997) *Trends in Biochemical Sciences* **22**, 225–6.
9. Clark, J.M. (1988) *Nucl. Acids Res.* **16**, 9677–86.
10. Newton, C.R. and Graham, A. (1994) In: *PCR*, BIOS Scientific Publishers, Ltd., Oxford, UK, 13.

Ordering Information

Product	Size	Cat.#
GoTaq® Green Master Mix	100 reactions	M7122
	1,000 reactions	M7123
ImProm-II™ Reverse Transcription System*	100 reactions	A3800
Reverse Transcription System*	100 reactions	A3500

*For Laboratory Use.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

(a) The PCR process, which is the subject of European Pat. Nos. 201,184 and 200,362 owned by Hoffmann-LaRoche*, 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.

* The above primary European Pat. Nos. 201,184 and 200,362 will expire on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

(b) Certain applications of this product are covered by patents issued to parties other than Promega and applicable in certain countries. Because purchase of this product does not include a license to perform any of these patented applications, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

(c) U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

(d) 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.