GoTaq® Green Master Mix for Quick and Easy Two-Step RT-PCR

ABSTRACT

Here we describe the use of GoTaq® Green Master Mix in reverse transcription PCR (RT-PCR) using two different reverse transcription systems available from Promega. GoTaq® Green Master Mix allows the researcher to move directly from the reverse transcription reaction to PCR and then directly to agarose gel analysis, enabling rapid screening of many samples.

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Introduction

Convenience is an important factor when selecting an amplification system. Thermostable DNA polymerases can be used for one-step (coupled) or two-step (uncoupled) RT-PCR. *Taq* DNA polymerase is the most commonly used thermostable polymerase for PCR amplification of first-strand cDNA synthesized by a reverse transcriptase in two-step RT-PCR experiments(1) . Previous work shows that the GoTaq® DNA Polymerase performs as well as or better than standard *Taq* DNA polymerase(2) and GoTaq® Green Master Mix performs well when small volumes of the reverse transcription reaction are used for template in PCR(3) . Some RT-PCR targets are highly dependent on precise concentrations of magnesium, dNTPs and buffer components, while other RT-PCR targets are not. For targets that are in the latter category, GoTaq® Green Master Mix may be a quick, convenient option for the PCR step. Here we demonstrate the flexibility of the GoTaq® Green Master Mix in the PCR step of RT-PCR experiments by using larger volumes of the reverse transcription reaction and using two different RT systems from Promega, the Reverse Transcription System (Cat.# A3500) and the ImProm-II™ Reverse Transcription System (Cat.# A3800).

The GoTaq® Green Master Mix is a premixed, ready-to-use solution containing GoTaq® DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer. The master mix offers enhanced performance over conventional *Taq* DNA polymerase through a special formulation of enzyme and buffer. GoTaq® Green Master Mix contains yellow and blue dyes that separate during electrophoresis, acting as a tracking dye, and an agent that increases the density; this allows you to load reactions performed with GoTaq® Green Master Mix directly onto agarose gels without adding loading dye/buffer.

Using GoTaq® Green Master Mix to Amplify First-Strand cDNA Synthesized Using the Reverse Transcription System

The Reverse Transcription System provides reagents to efficiently reverse transcribe RNA into cDNA in 15 minutes. The cDNA prepared from each reaction using this system may be used directly in multiple PCR amplifications using GoTaq® DNA Polymerase.

The Reverse Transcription System was used to synthesize cDNA from the Kanamycin Positive Control RNA Template included with the system. This template is predicted to yield a 1.0kb fragment after reverse transcription and amplification. Reverse transcription (RT) was performed using Oligo(dT)$_{15}$ Primers (Cat.# C1101) as described in the *Reverse Transcription*

System Technical Bulletin #TB099(4) . The 20µl RT reaction was brought to a total volume of 100µl as described in the manual. We used 20µl of the diluted RT reaction as the template for PCR using GoTaq® Green Master Mix. The final 100µl PCR amplification consisted of 20µl diluted RT reaction; 50µl GoTaq® Green Master Mix (final working concentration 1X), target-specific primers and Nuclease-Free Water. Figure 1 below depicts amplification of an approximately 1kb fragment from as little as 0.5 zeptomoles initial RNA input for the RT reaction.

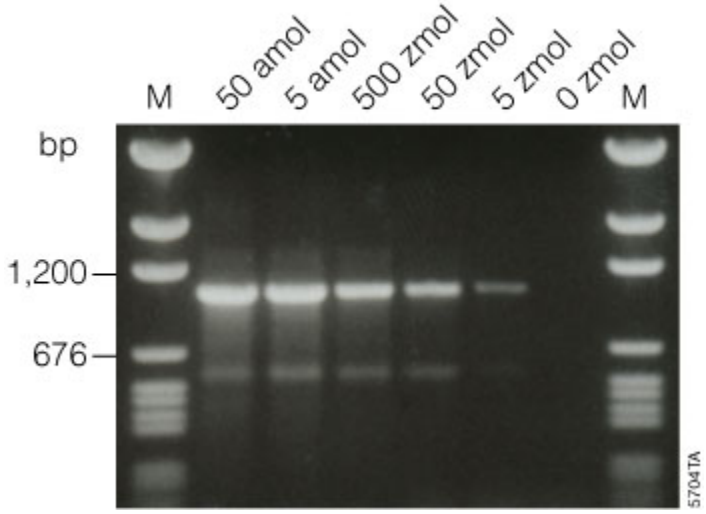

Figure 1. Amplification of the Kanamycin Positive Control Template from the Reverse Transcription System by two-step RT-PCR. Quantities of 0.05zmol to 50amol template were used for reverse transcription reactions. The Reverse Transcription System (Cat.# A3500) along with the Oligo(dT)15 Primers (Cat.# C1101) were used as described in Technical Bulletin #TB099 for 20µl reactions. The 20µl RT reaction was diluted to 100µl total volume. Twenty microliters of the diluted RT reaction, 50µl GoTaq® Green Master Mix (Cat.# M7122) and target-specific primers were used in a 100µl PCR. Lane M. Benchtop pGEM® Markers (Cat.# G7521).

Using GoTaq® Green Master Mix to Amplify First-Strand cDNA Synthesized Using the ImProm-II™ Reverse Transcription System

We next used GoTaq® Green Master Mix in the PCR amplification of two-step RT-PCR with the ImProm-II™ Reverse Transcription System (Cat.# A3800). In these experiments we amplified a 540bp ß-actin fragment from total mouse liver RNA. The reverse transcription reaction was performed as described in the *ImProm-II™ Reverse Transcription System Technical Manual* #TM236(5) using the Oligo(dT)15 Primer (Cat.# C1101). The 20µl RT reaction was added to 50µl GoTaq® Green Master Mix and target-specific primers, and the PCR amplification volume was brought to a total of 100µl. Figure 2 shows that we were able to amplify the 540bp fragment when less than 100pg of RNA was used in the initial RT reaction.

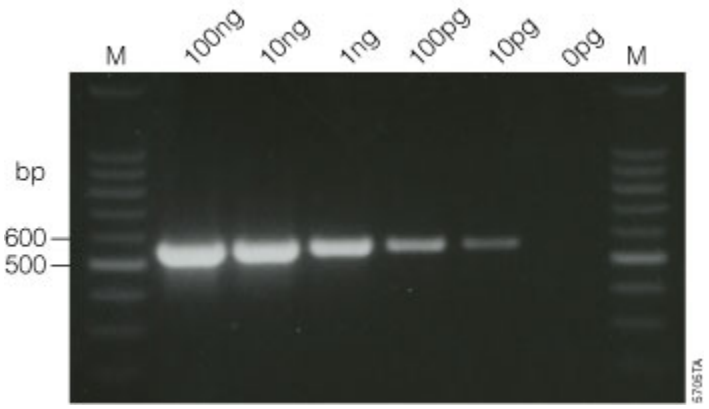

Figure 2. Amplification of 540bp β-actin fragment by two-step RT-PCR using ImProm-II™ Reverse Transcription System and 50µl GoTaq® Green Master Mix. Quantities of 0.05zmol to 50amol template were used for reverse transcription reactions. The Reverse Transcription System (Cat.# A3500) along with the Oligo(dT)15 Primers (Cat.# C1101) were used as described in Technical Bulletin #TB099 for 20µl reactions. The 20µl RT reaction was diluted to 100µl total volume. Twenty microliters of the diluted RT reaction, 50µl GoTaq® Green Master Mix (Cat.# M7122) and target-specific primers were used in a 100µl PCR. Lane M. Benchtop pGEM® Markers (Cat.# G7521).

One concern with two-step RT-PCR is carryover of components such as nucleotides, $MgCl_2$ and other buffer components from the RT reaction to the PCR. We repeated the RT-PCR amplification of the 540bp ß-actin fragment using the ImProm-II™ Reverse Transcription System and GoTaq® Green Master Mix, but this time we only used 40µl GoTaq® Green Master Mix in the 100µl PCR amplification. This reduces the amount of *Taq* DNA polymerase in the reaction, but accounts somewhat for carryover of magnesium and other components from the RT reaction. Figure 3 shows that we successfully amplified the ß-actin fragment even when as little as 10pg total RNA was used in the initial RT reaction.

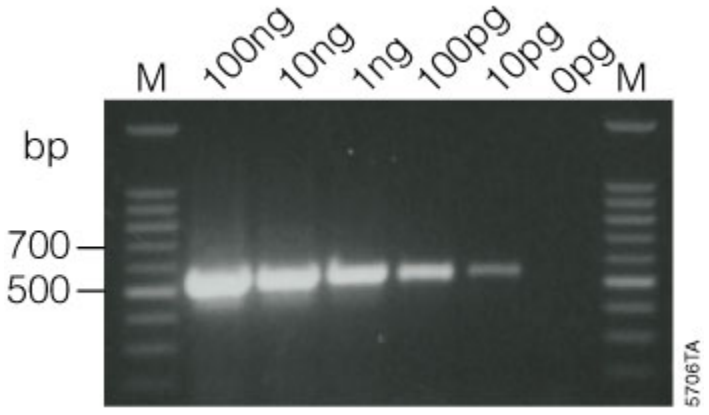

Figure 3. Amplification of 540bp β-actin fragment by two-step RT-PCR using ImProm-II™ Reverse Transcription System and 50µl GoTaq® Green Master Mix. RNA was isolated from mouse liver tissue using the RNAgents® Total RNA Isolation System. Quantities ranging from 10pg to 100ng total RNA were used as template for reverse transcription. Reverse Transcription reactions were performed as described in the *ImProm-II™ Reverse Transcription System Technical Manual* #TM236 with Oligo(dT)15 Primers (Cat.# C1101). The 20µl undiluted RT reaction was added to 50µl of GoTaq® Green Master Mix and target-specific primers, and the total PCR volume was brought to 100µl with Nuclease-Free Water. Lane M. BenchTop 100bp DNA Ladder (Cat.# G8291).

When the amplification of a template is highly dependent on precise concentrations of salts and other reaction components, we recommend using GoTaq® Flexi DNA Polymerase, which allows you to optimize the magnesium, dNTP and buffer concentrations in your reactions(6) . Both the 5X Green GoTaq® Flexi Buffer and the 5X Colorless GoTaq® Flexi Buffer are provided, allowing you to choose to load directly to gel or perform absorbance readings on your amplified DNA.

Summary

The GoTaq® Green Master Mix provides the convenience and flexibility you need to screen multiple samples using RT-PCR. You can go directly from your reverse transcription reaction (1–20µl volume) to PCR and then load samples directly from PCR onto an agarose gel for analysis. If you have an amplimer that requires specific magnesium concentrations, you can choose GoTaq® Flexi DNA Polymerase to design the reaction that works for your experimental system.

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