TECHNICAL MANUAL

GoTaq® Long PCR Master Mix

Instructions for Use of Product
M4021

Revised 5/17
TM359

GoTaq® Long PCR Master Mix

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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

GoTaq® Long PCR Master Mix[a] is a ready-to-use solution for long PCR targets. The master mix contains everything needed to perform long PCR—optimized buffer, dNTPs, $MgCl_2$ and an optimized hot-start enzyme blend for long PCR. Only the template and primers need to be added to amplify your target. The GoTaq® Long PCR Master Mix requires little or no reagent optimization and can amplify up to 30kb of genomic DNA and 40kb of lower-complexity targets such as plasmid and lambda DNA. Genomic targets over 20kb may require additional optimization.

The GoTaq® Long PCR Master Mix uses a blend of hot-start, recombinant *Taq* DNA polymerase and a recombinant proofreading DNA polymerase. The recombinant *Taq* DNA polymerase is bound by proprietary antibodies that inhibit activity at lower temperatures. This allows reactions to be set up at room temperature. The polymerase activity is restored after an initial denaturation cycle at high temperature (1). In addition to *Taq* DNA polymerase, the GoTaq® Long PCR Master Mix contains a small amount of DNA polymerase with 3´→ 5´ exonuclease (i.e., proofreading) activity.

1. Description (continued)

Both enzymes are necessary for amplifying long targets. Alone, *Taq* DNA polymerase is inefficient at amplifying fragments larger than 3–5kb due to its inability to repair nucleotide mismatches following misincorporation. Misincorporation of nucleotides leads to truncated products that cannot be amplified in subsequent cycles. The addition of a small quantity of proofreading enzyme allows mismatches to be repaired and extension to continue, resulting in the amplification of long amplicons with high yield (2).

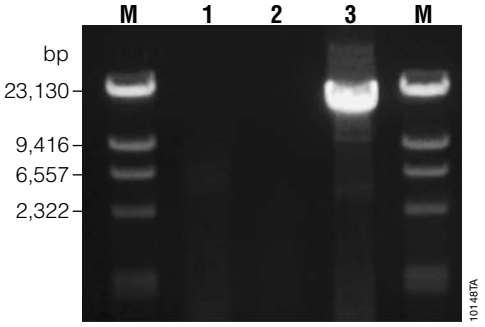

Figure 1. Amplification of a 17.5kb β-globin fragment from human genomic DNA using GoTaq® Long PCR Master Mix. GoTaq® Hot Start DNA Polymerase alone (lane 1) or proofreading DNA polymerase alone (lane 2) were unable to amplify the 17.5kb fragment. When the two polymerases are combined in GoTaq® Long PCR Master Mix (lane 3), the 17.5kb fragment is easily amplified. Each lane contains 10µl of the amplification reaction. Lane M, Lambda DNA/HindIII Markers (Cat.# G1711).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
GoTaq® Long PCR Master Mix	**100 reactions**	**M4021**

Each system contains sufficient reagents for 100 50µl reactions. Includes:

- 2 × 1.25ml GoTaq® Long PCR Master Mix, 2X
- 13ml Nuclease-Free Water
- 50µl Long PCR Control Primer Pair
- 30µg Human Genomic DNA

Storage Conditions: Store all components at –30 to –10°C. Alternatively, the Human Genomic DNA can be stored at 4°C. See product label for expiration date.

3. General Considerations

Amplicon Length: GoTaq® Long PCR Master Mix is designed for PCR targets greater than 5kb. It is not optimized for amplifying shorter amplicons. Human genomic DNA targets up to 30kb in size and lambda DNA targets up to 40kb in size have been amplified.

Template Integrity: Successful amplification of long amplicons depends on the template integrity. Use of poor-quality templates may lead to low yield. For very long targets (>15kb), use high-quality intact template DNA (3). It may be necessary to increase the amount of template in the reaction for very long amplicons.

Hot-Start Reaction Setup: GoTaq® Long PCR Master Mix is a hot-start reagent. Reactions can be set up at room temperature. Reactions incubated at room temperature from 90 minutes up to 6 hours performed similarly to reactions cycled immediately after setup when evaluated by gel electrophoresis.

4. PCR Protocol

Materials to Be Supplied by the User

- template DNA
- upstream primer
- downstream primer
- nuclease-free water
- $MgCl_2$ (optional)
- mineral oil (optional)

These are guidelines for starting your PCR experiments; however, for best results we recommend optimizing your amplification conditions for each target. Please refer to Section 5 for guidance in optimizing your PCR. We recommend performing positive and no-template controls, as they may be critical for confirming your PCR results as well as for troubleshooting. Set up your reactions carefully, and use aerosol-resistant pipette tips to minimize cross-contamination. Ideally, you should use separate areas for pre-and post-amplification handling and setup. If you are working with multiple samples, you can prepare a large reaction mix without the template.

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4.A. Amplification

1. Thaw all reagents. Vortex the GoTaq® Long PCR Master Mix to ensure proper mixing and prevent formation of magnesium gradients.
2. Combine the components listed in Table 1 in a sterile tube using aerosol-resistant pipette tips. Scale up reaction mix volumes if you are preparing more than one reaction.

Table 1. GoTaq® Long PCR Master Mix Reaction Assembly.

Component	Component Volume	Final Concentration
GoTaq® Long PCR Master Mix, 2X	25µl	1X
upstream primer	5–50pmol	0.1–1.0µM
downstream primer	5–50pmol	0.1–1.0µM
template DNA	0.1–0.5µg[1]	<0.5µg/50µl
Nuclease-Free Water to a final volume of	50µl	—

[1]Use 0.1–0.5µg human genomic DNA. Use 0.25–2.5ng for less complex templates such as plasmids and lambda DNA.

3. Mix components well, and centrifuge the reaction mixtures. If you are using a thermal cycler without a heated lid, overlay the reactions with 1–2 drops of mineral oil to prevent evaporation.
4. Place the tubes in the thermal cycler. The heat block may start at room temperature if desired.
5. Begin the thermal cycling program. If your primer melting temperatures (T_m) are above 60°C, use the cycling profile given in Table 2 as a guideline. If your primer melting temperatures are below 60°C, use the cycling profile given in Table 3 as a guideline. Optimizing the cycling profile is necessary for each primer/target combination.

Table 2. Thermal Cycling Guidelines for GoTaq® Long PCR Master Mix Using 2-Step PCR: Primer Melting Temperatures Above 60ºC.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94–95ºC	2 minutes[1]	1 cycle
Denaturation	92–94ºC	10–30 seconds[2]	
Annealing/Extension	65ºC[3]	1 minute/kb	25–35 cycles
Final Extension	72ºC	10 minutes	1 cycle
Soak	4ºC	Indefinite	1 cycle

[1]A two-minute denaturation time at 94–95ºC is necessary to dissociate the antibodies from *Taq* DNA Polymerase and initiate the hot-start PCR. Incubation at these high temperatures for longer periods of time will decrease enzyme activity.

[2]Denaturation time and temperature depend on the thermal cycler used.

[3]Annealing/extension at 65ºC works for most targets. However, reactions may need to be tested at different temperatures in increments of 1–2°C above and below 65°C to find the optimal conditions for your target.

Table 3. Thermal Cycling Guidelines for GoTaq® Long PCR Master Mix Using 3-Step PCR: Primer Melting Temperature Below 60ºC.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94–95ºC	2 minutes[1]	1 cycle
Denaturation	92–94ºC	10–30 seconds[2]	
Annealing	5º below T_m	15–30 seconds	
Extension	65–72ºC[3]	1 minute/kb	25–35 cycles
Final Extension	72ºC	10 minutes	1 cycle
Soak	4ºC	Indefinite	1 cycle

[1]A two-minute denaturation time at 94–95ºC is necessary to dissociate the antibodies from *Taq* DNA Polymerase and initiate the hot-start PCR. Incubation at these high temperatures for longer periods of time will decrease enzyme activity.

[2]Denaturation time and temperature depend on the thermal cycler used.

[3]Extension at 65ºC works for most targets. However, reactions may need to be tested at temperatures in 1–2°C increments starting at 65°C and going up to 72°C to find the optimal conditions.

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4.B. Gel Analysis

Analyze the PCR products by agarose gel electrophoresis. The PCR products are easily visible by UV illumination following ethidium-bromide staining.

Store PCR products at –20°C until needed. After amplification, a majority of PCR products will contain a 3´-overhang. In addition, some blunt-end fragments also will be present. The PCR products can be further purified using a number of procedures including the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

5. Optimization

Template Denaturation: Depurination of the template can occur at high temperatures during PCR. Minimize the time spent at high temperatures during PCR cycling by optimizing your denaturation time and temperature for your specific cycler. Decreasing the amount of time spent at high temperatures and/or decreasing the denaturation temperature will improve long PCR performance (2–4). Please note that the 2-minute initial denaturation cycle at 94–95°C is necessary to dissociate the antibodies and activate the GoTaq® Hot Start Polymerase.

Annealing Temperature: For each primer pair and target, an optimal annealing temperature must be chosen to gain both specificity and high yield in your PCR. The use of primers with similar T_ms over 60°C allows for 2-step PCR cycling. We recommend testing reactions starting with 65°C and try additional reactions at temperatures above and below 65°C in increments of 1–2°C to determine the optimal temperature. We have found that 65°C works well with many primer pairs and targets for 2-step PCR. For primers with T_ms below 60°C, we recommend 3-step PCR. Optimize the annealing temperature by starting 5°C below the T_m of the primers. Increasing the annealing temperature in increments of 1–2°C for individual reactions will help find the optimal annealing temperature.

Extension Temperature: Extension temperatures 68°C or lower have been found to work best in long PCR (2,5). We have successfully amplified some targets at 72°C; however, we recommend using 65°C as a starting point when using GoTaq® Long PCR Master Mix.

Extension Time: Use approximately 1 minute/kb of target for extension time. If the extension time is not long enough, the polymerase may not be able to complete extension of the fragment, leading to truncated products or no visible product. For very long targets (over 15kb), adding 10–20 seconds per cycle to the extension time after the first 10–15 cycles can increase yield. Many thermal cyclers have an auto-segment extension program option that can be used to add the extra extension time.

Magnesium Concentration: GoTaq® Long PCR Master Mix includes $MgCl_2$ at a concentration of 2.5mM per 1X reaction. If your PCR target requires more magnesium, titrate additional quantities in increments of 0.5mM $MgCl_2$ up to 4mM.

6. Control

6.A. Amplification of 17.5kb β-Globin Fragment from Human Genomic DNA

1. Thaw the reagents. Vortex the GoTaq® Long PCR Master Mix to ensure proper mixing and prevent formation of magnesium gradients.
2. Combine components listed in Table 4 in a sterile, 0.5ml thin-walled microcentrifuge tube using aerosol-resistant pipette tips.

Table 4. Long PCR Control Reaction Assembly: 17.5kb β-Globin Fragment.

Component	Component Volume	Final Concentration
GoTaq® Long PCR Master Mix, 2X	25µl	1X
Long PCR Control Primer Pair	1µl	0.2µM each primer
Human Genomic DNA	200ng	4ng/µl
Nuclease-Free Water to a final volume of	50µl	—

3. Mix components well, and centrifuge reaction mixtures briefly. If you are using a thermal cycler without a heated lid, overlay the reactions with 1–2 drops of mineral oil to prevent evaporation.
4. Place the reactions in a thermal cycler with cycling conditions given in Table 5.

Table 5. Thermal Cycling Guidelines for GoTaq® Long PCR Master Mix Amplification of the 17.5kb β-Globin Fragment.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95ºC	2 minutes	1 cycle
Denaturation	92ºC	30 seconds	
Annealing/Extension	65ºC	15 minutes	30 cycles
Final Extension	72ºC	10 minutes	1 cycle
Soak	4ºC	Indefinite	1 cycle

6.B. Gel Analysis

Analyze the PCR products by agarose gel electrophoresis. The PCR products are easily visible by UV illumination following ethidium-bromide staining.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low yield or no amplification	Template degradation. Template integrity is very important for long PCR. Verify template integrity by gel electrophoresis.
	Suboptimal cycling conditions: • Use 94–95°C for 2 minutes during the initial denaturation step. • Verify programming and functionality of the thermal cycler. • Try 2-step PCR cycling protocol with the anneal/extension step at 65°C. Lower temperatures may be attempted in 1–2°C increments below 65°C down to 60°C. • For 3-step PCR, decrease anneal temperature and/or test an extension temperature gradient of 60–68°C. • Use extension times ≥1minute/kb or add more cycles.
	Inhibitor was present in template. Use a smaller volume of template in reaction or clean up the template DNA.
	Primer design. Check primer specificity and verify that the primers are not self-complementary or complementary to each other.
	Reaction conditions. Optimize primer and magnesium concentration.
Nonspecific amplification products	Suboptimal reaction conditions: • Decrease the amount of template used. • Increase annealing temperature. • Adjust cycling conditions (see Section 5 for details about optimization).
	Problem with the primers. Primers used may have secondary priming sites; redesign primers for greater specificity.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Smearing of amplification products on a gel	Template integrity is very important for long PCR. Verify the integrity of the template DNA by gel electrophoresis. Template DNA should be of high molecular weight.
	Suboptimal reaction conditions: • Decrease number of cycles. • Adjust annealing temperature or extension temperature. • Use 1 minute/kb of fragment for extension to decrease synthesis of truncated PCR products. • Decrease number of cycles. • Optimize the denaturation step. You may need to increase time or temperature especially for a human genomic template. • Decrease amount of template. • Decrease amount of primers used.
	Carryover from previous PCRs. Separate preamplification area from post-amplification area.

8. References

1. Knoche, K., Ensenberger, M. and Sprecher, C. (2008) Get the convenience of hot-start PCR with the new GoTaq® Hot Start Polymerase. *Promega Notes* **99**, 8–11.
2. Barnes, W.M. (1994) PCR amplification of up to 35-kb DNA with high fidelity and high yield from λ bacteriophage templates. *Proc. Natl. Acad. Sci. USA* **91**, 2216–20.
3. Cheng, S. *et al.* (1995) Template integrity is essential for PCR amplification of 20- to 30-kb sequences from genomic DNA. *PCR Methods Appl.* **4**, 294–8.
4. Cheng, S. *et al.* (1994) Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc. Natl. Acad. Sci. USA* **91**, 5695–9.
5. Barnes, W.M. (2003) In: *Tips for long and accurate PCR. PCR Primer A Laboratory Manual.* Dieffenback, C.W. and Dveksler, D.S., eds., Cold Spring Harbor Press, 53–60.

9. Related Products

Product	Size	Cat.#
GoTaq® 2-Step RT-qPCR System	50 RT reactions + 200 qPCR reactions	A6010
GoTaq® qPCR Master Mix	200 reactions	A6001
	1,000 reactions	A6002
GoTaq® Hot Start Polymerase	100 units	M5001
	500 units	M5005
	2,500 units	M5006
	10,000 units	M5008
Wizard® SV Gel and PCR Clean-Up System	50 reactions	A9281
	250 reactions	A9282
	1,000 reactions	A9285

10. Summary of Change

The following change was made to the 5/17 revision of this document:

1. Corrected the final concentration value for Long PCR Control Primer Pair in Table 4.