

The New MasterAmp™ High Fidelity RT-PCR Kit for Accurate and Flexible RT-PCR

Judith T. Schanke, EPICENTRE Technologies

Introduction

Reliable and accurate amplification of RNA templates is crucial for research based on the cloning and expression of new messages. Reverse transcription and subsequent PCR amplification (RT-PCR) are both error-prone enzymatic reactions.^{1,2} The use of a high-fidelity enzyme blend containing a thermostable DNA polymerase with 3' → 5' exonuclease (proofreading) activity is critical when the accuracy of the amplification product is important.

Here we introduce the MasterAmp™ High Fidelity RT-PCR Kit, the most convenient high-fidelity RT-PCR system available. The RT or first-strand cDNA synthesis is catalyzed by Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) Plus, the most reliable enzyme for full-length cDNA synthesis.³ Subsequent PCR uses MasterAmp TAQurate™ DNA Polymerase Mix, a unique blend of thermostable DNA polymerases that dramatically reduces the error rate of PCR amplification. The kit also includes a convenient MasterAmp 2X RT-PCR PreMix containing reaction buffer, dNTPs and MgCl₂ that has been optimized for both reverse transcription and PCR amplification.

The MasterAmp High Fidelity RT-PCR Kit provides flexibility when performing RT-PCR reactions as either a one-step or two-step procedure (Figure 1). The one-step procedure is designed for performing first-strand synthesis and PCR in one tube using specific primers supplied by the user. Since reverse transcription and PCR are carried

out successively without opening the tube lid, this protocol minimizes sample handling and the possible introduction of Rnases.

In the two-step procedure first-strand synthesis is performed using either random nonamers or oligo d(T)₁₈ primers supplied in the kit. Subsequent PCR is achieved in one of two ways. The modified two-step procedure, shown in Figure 1B, allows the addition of specific primers and TAQurate DNA Polymerase Mix directly into the reaction mix after the RT step, eliminating the need to set up a separate PCR reaction. The standard two-step RT-PCR can also be performed. In this protocol, a small amount (5-20%) of the RT reaction is removed for subsequent PCR. This variation can be useful when several different primer pairs are used to analyze the same cDNA template.

In this article we demonstrate amplification from a variety of different RNA templates, including multiple targets, using the MasterAmp High Fidelity RT-PCR Kit. We also show RNA amplification using non-specific RT primers and amplification of long RNA templates.

Materials and Methods

The standard one-step RT-PCR reactions contained 100-500 ng total cellular or viral RNA, 12.5 pmoles of each specific primer, 1X RT-PCR PreMix, 40 Units of MMLV-RT Plus, and 1 U of TAQurate DNA Polymerase Mix in a total volume of 50 µl. Reverse transcription was performed at 37-50°C for 30-60 minutes, depending on the secondary structure and length of the RNA template. The reverse transcription was immediately followed by a denaturation step at 95°C for 1 minute, then 30 cycles of 92°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds to 6 minutes, depending on the length of the expected product. The annealing step for the amplification of tobacco mosaic virus (TMV) RNA was increased to 62°C.

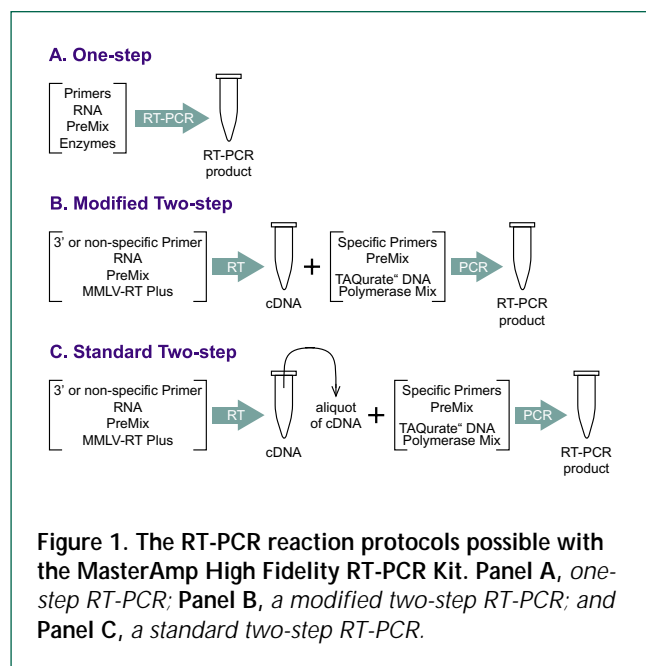
The modified two-step amplifications included 0.5 µg random hexamer oligonucleotides or 25 pmoles of oligo (dT)₁₈ during the reverse transcription reaction and were incubated for 30 minutes at 37°C. After first strand synthesis, 12.5 pmoles of each specific primer were added and the reactions were cycled as described previously.

Results and Discussion

Efficient amplification of a variety of RNA templates

RNA templates used for RT-PCR vary in complexity, structure, and length. Figure 2A demonstrates the amplification of messages from sources including total cellular human, bacterial, and viral RNA preparations.

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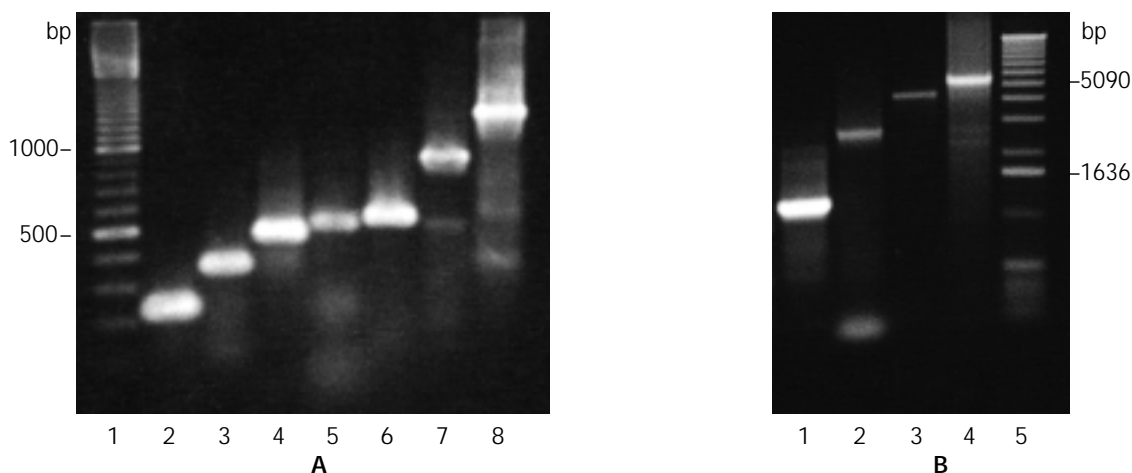


Figure 2. Efficient amplification of a variety of RNA templates using the MasterAmp High Fidelity RT-PCR Kit. Panel A: Lane 1, 100 bp ladder; Lane 2, 250 bp human lung β -actin; Lane 3, 350 bp human lung GAPDH; Lane 4, 463 bp region of TMV; Lane 5, 479 bp *E. coli* 16s rRNA; Lane 6, 589 bp human placental CG α ; Lane 7, 850 bp human heart β -actin; and Lane 8, 1248 bp region of TMV. **Panel B:** Long amplifications of TMV RNA. Lane 1, 1,248 bp; Lane 2, 2,795 bp; Lane 3, 4,517 bp; Lane 4, 5,503 bp; and Lane 5, kb ladder.

Figure 2B demonstrates the ability of the MasterAmp High Fidelity RT-PCR Kit to amplify full-length RT-PCR product from specific regions of TMV RNA. RT-PCR amplifications were all performed using the convenient one-step protocol (Figure 1A) with template-specific primers for 30 cycles. No reaction optimization was required to obtain substantial yields from each of these targets.

The simultaneous amplification of more than one RNA target or multiplex amplifications can also be performed in a single tube using the standard one-step protocol. Figure 3 shows the co-amplification of chorionic gonadotropin (CG) α and β -actin from human placental RNA.

Specific amplification using the modified two-step protocol

A modified two-step procedure is demonstrated in Figure 4. Reverse transcription was performed on cellular RNA from human lung with either random hexamers or oligo d(T)₁₈ primers. β -Actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequences were then amplified by the addition of TAQurate DNA polymerase mix and gene specific primers. As a comparison, the specific rather than random primers, were used for first-strand synthesis. All three reactions produced a high yield of specific product with this modified two-step protocol.

Full-length amplification products from long RNA templates

Standard RT-PCR kits can not be used to adequately produce full-length cDNAs from targets longer than 3kb. Figure 5 (lane 2) demonstrates that a 5.5kb segment of TMV RNA, containing complex secondary structure, was only amplified with high specificity using the MasterAmp High Fidelity

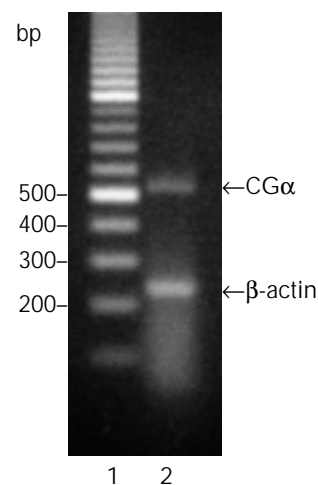


Figure 3. Multiplex RT-PCR using the MasterAmp High Fidelity RT-PCR Kit. Lane 1, 100 bp ladder; Lane 2, co-amplification of 589 bp CG α and 250 bp β -actin from human placental RNA.

RT-PCR Kit. Although the one-step reaction procedure was used, a two-step amplification also produced specific, full-length product (not shown). The same thermal cycling parameters were used in a standard two-step amplification with MMLV-RT and a standard Taq DNA polymerase. Not using the MasterAmp blend of thermostable DNA polymerases resulted in a small amount of full-length product, but also produced numerous, undesirable, smaller amplification products (Figure 5, Lane 3). A one-step,

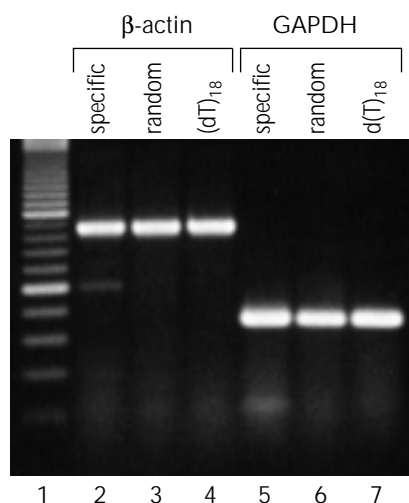


Figure 4. The modified two-step protocol using the MasterAmp High Fidelity RT-PCR Kit to amplify RNA reverse transcribed with non-specific primers. Total cellular RNA purified from human lung tissue was reverse transcribed with either message-specific (Lanes 2 & 5), random hexamer (Lanes 3 & 6), or oligo (dT)₁₈ (Lanes 4 & 7) primers prior to PCR with message-specific primers for β -actin or GAPDH.

high-temperature reverse transcription with MasterAmp Tth DNA polymerase performed at 60°C for 60 minutes with identical PCR cycling conditions, resulted in no detectable full-length product (Figure 5, Lane 4). These results show that the MasterAmp High Fidelity RT-PCR Kit is a more reliable and specific approach for the accurate amplification of long regions of RNA.

Summary

The MasterAmp High Fidelity RT-PCR Kit accurately and conveniently reverse transcribes and amplifies cDNA from RNA templates. The amplification of sequences from a variety of RNA templates was demonstrated, including the ability to amplify long templates with better efficiency and specificity than standard methods. One-step or two-step amplifications with random, oligo dT, or specific primers can all be performed with the MasterAmp High Fidelity RT-PCR Kit. This protocol flexibility, along with the MasterAmp 2X RT-PCR PreMix and the use of the TAQurate DNA Polymerase Mix, makes the MasterAmp High Fidelity RT-PCR Kit the most versatile and convenient high-fidelity RT-PCR Kit for cloning and expression of cDNA.

References

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2. Cline, J. *et al.* (1996) *Nucl. Acids Res.* **24** (18), 3546.
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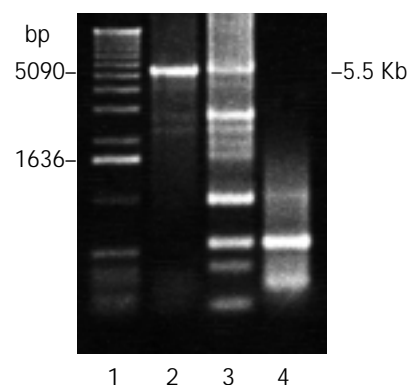


Figure 5. The MasterAmp High Fidelity RT-PCR Kit more efficiently amplifies full-length products from long RNA templates. RT-PCR products from TMV RNA with primers that amplify a 5.5 kb region of the viral RNA. Lane 1, kb ladder; Lane 2, amplification using the convenient one-step protocol; Lane 3, amplification with a standard two-step procedure using MMLV-RT and Taq DNA polymerase; Lane 4, amplification with Tth DNA polymerase.

MasterAmp™ High Fidelity RT-PCR Kit

25 Reactions	RF91025
100 Reaction	RF910100

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