Throw Away the Script!

Promega

Using ImProm-II™ Reverse Transcription System for Coupled RT-PCR

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Abstract

Promega's new ImProm-II™ Reverse Transcription System (Cat.# A3800) is suitable for coupled RT-PCR. This article demonstrates the high degree of detection sensitivity and proportional response exhibited using the ImProm-II™ Reverse Transcriptase in combination with Promega Taq *DNA Polymerase (Cat.# M1661) for coupled RT-PCR.*

The ImProm-II™ System is designed to be used for quantitative synthesis of high-quality cDNA in preparation for gene-specific analysis such as by PCR.

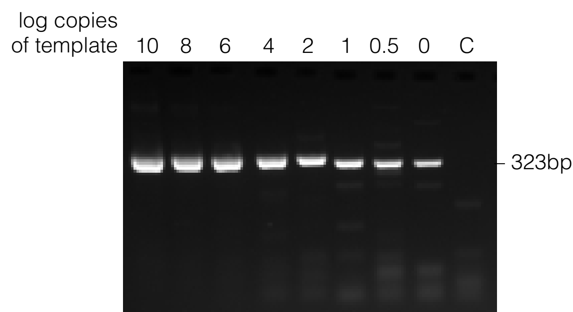
Introduction

Promega's new ImProm-II™ Reverse Transcription System (Cat.# A3800) combines a newly formulated reverse transcriptase, an optimized reaction buffer and the associated reagents qualified for efficient synthesis of first-strand cDNA. The ImProm-II™ System is designed to be used for quantitative synthesis of high-quality cDNA in preparation for gene-specific analysis such as by PCR[a]. The optimized ImProm-II™ Reaction Buffer enables robust activity by both ImProm-II™ Reverse Transcriptase and Promega *Taq* DNA Polymerase[a] (Cat.# M1661). The buffer also combats the inhibiting effect of reverse transcriptases on thermophilic DNA polymerases, which is often encountered in RT-PCR (1).

The ImProm-II™ Reverse Transcription System can be used for coupled or uncoupled RT-PCR; therefore, the system is suitable for the approach dictated by your experimental questions. The components of the ImProm-II™ System are provided separately, giving the greatest flexibility in performing first-strand cDNA synthesis, coupled or uncoupled to PCR amplification. The individual packaging also allows for better optimization in the cDNA synthesis step (2).

In this article the ImProm-II™ System is used in combination with Promega *Taq* DNA Polymerase for single-tube, coupled RT-PCR. Detailed methods for RNA isolation, first-strand cDNA synthesis and gene-specific amplification and analysis are available in the enhanced online article at: www.promega.com/enotes/applications/ap0036_tabs.htm.

A. Kanamycin Primers Alone



B. Kanamycin Primers plus Oligo(dT)

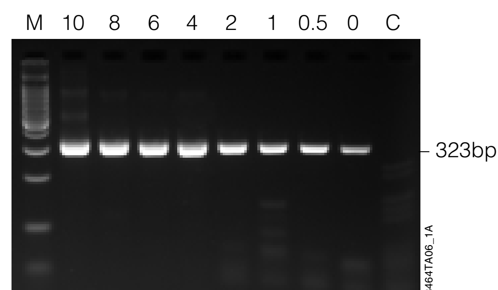


Figure 1. Fewer than five copies of a transcript were detected using the ImProm-II™ Reverse Transcription System supplemented with *Taq* DNA Polymerase in coupled RT-PCR. Kanamycin Positive Control RNA (Cat.# C1381) over a range of 1 x 10^{10} (0.1µg) to approximately a single copy. We combined aliquots of this diluted RNA template with gene-specific primers for kanamycin-specific RT-PCR (Tables 1 and 2). In parallel, we combined aliquots of the diluted RNA template with gene-specific primers plus oligo(dT) to demonstrate that oligo(dT) can be used for cDNA priming with no deleterious effect on the quality or the RT-PCR. RNA/primer combinations were denatured and then reactions were assembled and incubated for coupled RT-PCR as follows. First-strand cDNA synthesis (RT): Annealing 25°C for 5 minutes; extension 42°C for 60 minutes, inactivation 95°C for 5 minutes. PCR: 94°C for 1 minute; 60°C for 1 minute; 72°C for 2 minutes, 38 cycles, followed by final extension at 72°C for 5 minutes. Samples (2µl) of each 20µl RT-PCR were analyzed for the presence of the 323bp product by electrophoresis on a 4% NuSieve®:GTG® agarose gel. Lane M, 100bp DNA Ladder (Cat.# G2101); lane C, no RNA control.

Published first in eNotes!

This article was first published on the Promega web site at: www.promega.com/enotes/applications/ap0036_tabs.htm. Additional information on methods are available in the online version.

Table 1. Target RNA and Gene-Specific Oligonucleotide Primers Used.

Target	Primers	Product Size
1.2kb Kanamycin Positive Control RNA	Kanamycin Sense (GCCATTCTCACCGGATTCAGTCGTC) & Antisense (AGCCGCCGTCCCGTCAAGTCAG) **-or-** Kanamycin Sense & Antisense plus Oligo$(dT)_{15}$	323bp
Human γ-Actin mRNA	γ-Actin Sense (AAGTACCCCATTGAGCATGGC) & Antisense (CACAGCTTCTCCTTGATGTCGC) **-or-** γ-Actin Sense & Antisense plus Oligo$(dT)_{15}$	449bp

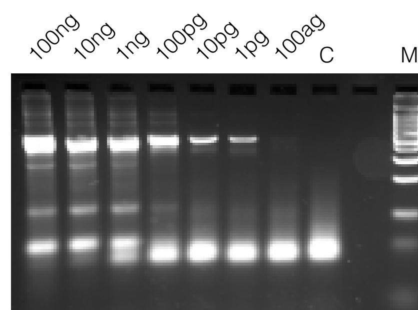
Notes: Kanamycin Positive Control RNA: 0.1µg, 2.5fmol (~10^{10} copies) to 2.5ymol (~1 copy). Kanamycin Control Primers: 20pmol each. Oligo$(dT)_{15}$ Primer (Cat.# C1101): 0.5mg. Human γ-actin mRNA, medium abundance (0.4%) (3). Human Jurkat cell total RNA: 100ng to 100ag. Human γ-actin primers: 20pmol each.

Table 2. RT-PCR Setup.

Component	Final Conc.	Volume (µl)
Nuclease-Free Water		5.9
ImProm-II™ Reaction 5X Buffer	1X	4.0
$MgCl_2$, 25mM	2mM	1.6
PCR Nucleotide Mix, 10mM	0.5mM	1.0
rRNasin® Ribonuclease Inhibitor[b,c]	1u/µl	0.5
ImProm-II™ Reverse Transcriptase		1.0
Taq DNA Polymerase	0.25u/µl	1.0
	Subtotal	**15.0**
RNA + primers		5.0
	Total	**20.0**

Notes: RNA, maximum 1µg per reaction; primers, 0.4–1µM.

A. γ-Actin Primers Alone



B. γ-Actin Primers plus Oligo(dT)

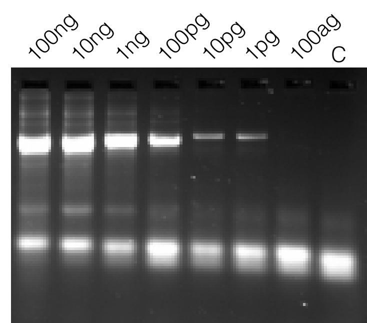


Figure 2. Detection of medium-abundance message for γ-actin starting with as little as 1pg of total RNA using components of ImProm-II™ System supplemented with *Taq* DNA Polymerase in coupled RT-PCR. Human Jurkat total RNA, which had been prepared using SV Total RNA Isolation System (Cat.# Z3100), was titrated over a range of approximately 100ng to 100ag. Aliquots of the diluted RNA template were combined with γ-actin-specific primers. In parallel, aliquots of the RNA were combined with the γ-actin primers plus oligo(dT). We denatured the RNA/primer combinations, assembled the reactions and incubated for coupled RT-PCR. Reactions were performed as in Figure 1 except PCR consisted of 40 cycles and 5µl of product were analyzed for the presence of the 449bp product. Lane M, 100bp DNA Ladder (Cat.# G2101); lane C, no RNA control.

Results

As evident in Figure 1, fewer than five copies of a transcript were detected using the ImProm-II™ Reverse Transcription System supplemented with *Taq* DNA Polymerase in coupled RT-PCR. Kanamycin Positive Control RNA[b] (1.2kb) was titrated over 11 orders of magnitude (from approximately 10^{10} to a single copy). We combined aliquots of the diluted RNA template with gene-specific primers for kanamycin-specific RT-PCR. In parallel, we combined aliquots of the diluted RNA template with gene-specific primers plus oligo(dT) to demonstrate that oligo(dT) can be used for cDNA priming with no deleterious effect on the quality or the RT-PCR.

Figure 2 shows that the ImProm-II™ System can be used to detect a medium-abundance message (γ-actin; 3) in 1pg of total RNA when used with *Taq* DNA Polymerase for coupled RT-PCR. Human Jurkat total RNA, which had been prepared using SV Total RNA Isolation System (4) (Cat.# Z3100), was titrated over a range of approximately 100ng to 0.1pg.

Conclusions

We demonstrated the extremely sensitive detection and proportional response using the ImProm-II™ Reverse Transcription System for coupled RT-PCR. The single-species, polyadenylated kanamycin transcript could be amplified from fewer than five copies of starting material, and medium-abundance γ-actin message could be detected from as little as 1pg of human Jurkat total RNA. This optimized application, a modification of the uncoupled RT-PCR protocol provided in the ImProm-II™ System Technical Manual (#TM236), offers a simple method for sensitive analysis of RNA sequences.

References

1. Chandler, D.P., Wagnon, C.A. and Bolton, H. (1998) *Appl. Environ. Microbiol.* **64**, 669–677.
2. *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation.
3. Adams, M.D. *et al.* (1995) *Nature* **377(Suppl)**, 3–174.
4. *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation.

Protocols

- *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation. (www.promega.com/tbs/tm236/tm236.html)
- *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation. (www.promega.com/tbs/tm048/tm048.html)

Table 3. Observations About Coupled RT-PCR Using the ImProm-II™ System.

- The volume of the RT-PCR may be changed as long as the concentration of ImProm-II™ Reverse Transcriptase and *Taq* DNA Polymerase remain proportionally constant.
- Do not substitute any other reaction buffer for ImProm-II™ Reaction Buffer.
- Optimal dNTP concentration for the first-strand synthesis is 0.5mM.
- Though the magnesium concentration range for first-strand cDNA synthesis is between 1.5–8mM, the acceptable concentration for *Taq* DNA Polymerase activity is more limited and should be optimized between 1.5–2.5mM for coupled RT-PCR.
- Addition of rRNasin® Ribonuclease Inhibitor is optional but highly recommended for efficient coupled RT-PCR.
- The primer concentraions may be optimized based on knowledge of the sequences.
- For first-strand synthesis, the primer annealing temperatures may be altered based on T_m of the sequence.
- Extension temperature for first-strand cDNA synthesis is optimally between 37–42°C. For templates with troublesome secondary structure, try a temperature >42°C.
- It is critical to thermally inactivate the reverse transcriptase (at 95°C for 5 minutes) prior to initiating amplification.

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Ordering Information

Product	Size	Cat.#
ImProm-II™ Reverse Transcription System*	100 reactions	A3800
ImProm-II™ Reverse Transcriptase*	500 reactions	A3803
	100 reactions	A3802
	10 reactions	A3801
Taq DNA Polymerase in Storage Buffer B[(a)]*	500 units	M1665
	100 units	M1661

**For Laboratory Use.*

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GTG is a registered trademark of FMC Corporation. NuSieve is a registered trademark of BMA, Inc.

[(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. Nos. 4,966,954 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

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

Technical Questions?

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