

Promega RT-PCR Systems Explained

By Hanan Abramovici, M.S., Promega Corporation

Abstract

PCR has proven to be one of the most flexible and powerful techniques in modern molecular biology. Reverse transcription-PCR (RT-PCR) adds a new dimension, coupling the conversion of RNA into complementary DNA (cDNA) by viral reverse transcriptases with the amplification of the cDNA by thermostable polymerases. (More information on this topic is available online at: www.promega.com/amplification/.) Promega offers two systems for RT-PCR: The Access RT-PCR System and the Reverse Transcription System.

Editor's Note: Promega has introduced a new reverse transcription system—ImProm-II™ Reverse Transcription System* (Cat.# A3800). Reverse transcribe RNA templates starting with either total RNA, poly(A)+ mRNA or synthetic transcript RNA. The optimized reaction buffer and powerful enzyme provided in the ImProm-II™ System enable robust, full-length cDNA synthesis for the reproducible analysis of rare or long messages. See page 26 for more information.

What is the difference between the Access RT-PCR System and the Reverse Transcription System?

The Access RT-PCR System^(a,b) (Cat.# A1260, A1250, A1280) is a one-tube, one-step, two-enzyme system that allows the user to perform RT-PCR with minimum hands-on time and labor as well as decreased possibility of sample contamination. The system begins with total RNA, mRNA or in vitro-transcribed RNA as templates, and uses gene-specific primers to produce a single cDNA product.

The Reverse Transcription System (Cat.# A3500) is a two-tube, two-step, two-enzyme system that allows the user to produce multiple cDNAs by using oligo(dT)₁₅ or random hexamer primers in the initial first-strand cDNA synthesis step from either total RNA, mRNA or an in vitro transcript. This first reaction can then be divided into multiple second-strand cDNA syntheses and PCR amplifications using different gene-specific primers to

generate pools of unique cDNAs. Other differences include the ability to vary the magnesium concentrations in both the RT and the PCR steps of the Reverse Transcription System and the use of *Thermus flavus* (*Tfl*) DNA Polymerase^(a) in the Access RT-PCR System.

Why does the Access RT-PCR System use *Tfl* instead of *Taq* DNA Polymerase?

While both *Taq* and *Tfl* DNA Polymerases are similar in many respects, experiments performed at Promega have shown that the *Tfl* DNA Polymerase amplifies larger (3–6kb) fragments with greater success than *Taq* DNA Polymerase^(a).

Why do Promega's RT-PCR Systems use AMV RT instead of M-MLV RT?

AMV RT (Avian Myeloblastosis Virus Reverse Transcriptase) displays optimal activity at a higher temperature than M-MLV RT (Moloney Murine Leukemia Virus Reverse Transcriptase) (48 versus 37°C, respectively), making it particularly useful for reverse transcription of RNA templates that exhibit significant secondary structure. However, AMV RT also has a stronger intrinsic RNase H activity, making generation of longer (>5kb) cDNAs more difficult. For long cDNA, it may be preferable to use Promega's M-MLV RT (Cat.# M1701, M1705).

Is it possible to use oligo(dT) or random hexamers with the Access RT-PCR System?

Yes, however users will have to make slight modifications to the standard protocol, as it is designed for gene-specific oligonucleotide primers. Random Hexamers (Cat.# C1181) or Oligo(dT)₁₅ Primer (Cat.# C1101) at concentrations of 1µM may be included, *in addition to the gene-specific primers*, for first-strand synthesis. Incubate the reaction for 10 minutes at room temperature to ensure that the primers anneal and are extended adequately to remain bound to the template. Then proceed with first-strand synthesis (42°C for 35 minutes), second-strand synthesis and PCR amplification.

*For Laboratory Use.

What are the important parameters to keep in mind when performing RT-PCR?

Template integrity and purity, primer design and concentration, magnesium concentration, dNTP concentration and cycling parameters can all significantly impact on the success and quality of RT-PCR (1). Therefore, a thorough understanding of how these parameters can affect the successful amplification of a desired transcript is highly recommended. For assistance troubleshooting such parameters, we refer readers to Promega's *Amplification Assistant*SM PCR Troubleshooting Program online. This web tool assists with the selection of many parameters and helps users troubleshoot aspects of amplification based on their data input.

The *Amplification Assistant*SM web tool is available at: www.promega.com/amplification/assistant/.

How can I easily clone my RT-PCR-generated cDNAs?

PCR produces cDNA fragments that contain 3'-A overhangs added nonspecifically by certain thermostable DNA polymerases, such as *Taq* or *Tfl* DNA Polymerases, that lack "proofreading" 3'→5' exonuclease activity. This feature permits the easy cloning of PCR products into linearized vectors that contain complementary T overhangs in the multiple cloning site. Promega offers four different T vectors for such purposes: pGEM[®]-T (Cat.# A3600, A3610) and pGEM[®]-T Easy (Cat.# A1360, A1380) Vector Systems^(c,d) for routine cloning, PinPoint[™] Xa-1 T-Vector System^(c,d,e) (Cat.# V2610, V2850) for bacterial expression and purification of fusion proteins, and pTARGET[™] Mammalian Expression Vector System^(d,f) (Cat.# A1410). Following completion of RT-PCR, the product can either be resolved on an agarose gel and purified using Promega's Wizard[®] PCR Preps DNA Purification System^(g) (Cat.# A7170) or it can be excised from an agarose gel and released from agarose using AgarACE[®] Agarose-Digesting Enzyme^(h) (Cat.# M1741, M1743). Alternatively, RT-PCR products may be used directly in T-vector ligation without prior purification. However, it will then be necessary to screen a greater number of clones to identify those of interest; primer-dimers or non-specific amplification products may be cloned into the T-vector as well.

How sensitive is RT-PCR?

The minimum amount of RNA that can be amplified by RT-PCR depends on the purity and complexity of the RNA template and on the primer concentration. In general, starting with 10pg to 1µg of total RNA will provide excellent amplification although successful amplification has been achieved with as little as 1pg of total RNA using the Access RT-PCR System (2). For poly(A)⁺ mRNA, we generally recommend between 1pg and 100ng. When using highly homogeneous samples such as in vitro generated transcripts, amplification can be achieved using as little as 100 molecules of starting template (2). Using the Access RT-PCR System, scientists at Promega and elsewhere have successfully amplified a message from a single cell without RNA isolation and purification (3,4) and from as few as 100 cells in a mixed cell population (unpublished observations, Promega).

References

1. Kephart, D. (1999) *Promega Notes* **73**, 14–16.
2. Miller, K. and Storts, D.R. (1995) *Promega Notes* **53**, 2–5.
3. Benoehr, P. *et al.* (2000) Single-cell RT-PCR of laser capture-micro-dissected Purkinje cells, enotes online:
◆ www.promega.com/enotes/applications/ap0013_tabs.htm
4. Betz, N. (2000) Single-cell RT-PCR using RNasin[®] Ribonuclease Inhibitor, enotes online:
◆ www.promega.com/enotes/applications/ap0026_tabs.htm

AgarACE, *pGEM*, and *Wizard* are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. *PinPoint* and *pTARGET* are trademarks of Promega Corporation. *Amplification Assistant* is a service mark of Promega Corporation.

^(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. 4,966,964, 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. 4,766,072.

^(d)Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

^(e)For research purposes only. Not for diagnostic or therapeutic use. For nonresearch uses of the portion of the vector encoding the biotinylation sequence, please contact Promega Corporation for licensing information. For bulk purchases of the SoftLink[™] Resin, contact TosoHaas, 156 Keystone Drive, Montgomeryville, PA 18936, 1-800-366-4875 or 215-283-5000.

^(f)The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

^(g)Licensed under U.S. Pat. No. 5,075,430.

^(h)U.S. Pat. No. 5,814,487, 5,869,310 and 6,001,636.

Technical Questions?

E-mail Promega Technical Services at:

techserv@promega.com