TP enhancer for sensitive RT-PCR

Reverse transcription is an enzymatic reaction that transcribes genetic information from RNA to DNA. This reaction is catalyzed by enzyme reverse transcriptase, which occurs in many types of retroviruses. Currently, reverse transcription is routinely used in a number of applications in research and diagnostic assays based on molecular biology approaches, including isolation of spliced gene variants and evaluation of transcription levels of various genes in different cell types. For amplification of difficult-to-amplify DNA fragments with polymerase chain reaction (PCR), we prepared a new reaction buffer, called TPP buffer, which improves PCR amplification of GC-rich and long DNA fragments. In this project we examined whether this buffer also improves reverse transcription with Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase. We found that when reverse transcription of difficult-to-transcribe genes is performed in TPP buffer, better results are obtained than with a standard M-MLV buffer (Figure 1).

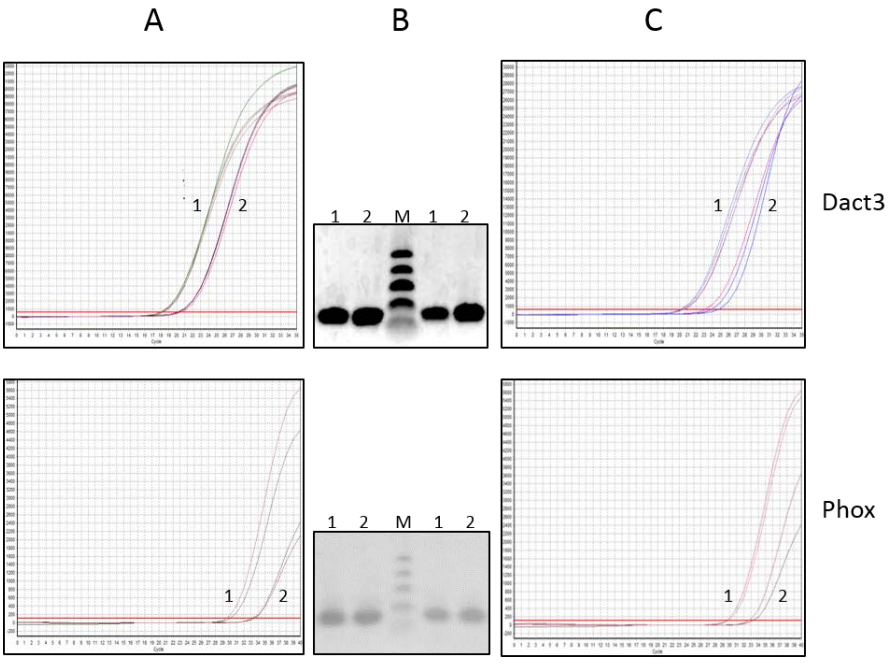

Figure 1: Comparison of reverse transcription performance using TPP buffer or regular buffer for reverse transcription with M-MLV reverse transcriptase. Two gene transcripts were examined, Dact3 (upper panel) and Phox (lower panel). M-MLV-mediated reverse transcription was performed in TPP buffer (1) or regular M-MLV reverse transcriptase buffer (2), followed by cDNA amplification in PCR using the TPP buffer supplemented with nucleotides, Taq DNA polymerase, and SYBR green I intercalating fluorescence dye. The results are presented as fluorescence versus PCR cycle (panel A and C) and agarose gel electrophoresis (panel B). In these experiments, two PCR oligonucleotide primer sets for each gene were used. The results with one primer set are shown in the left panel (A) and the left part of panel B, and the results with the second primer set are shown in the right panel (C) and the right part of panel B. DNA markers in the B panel are also shown (M).

Quantification of mRNA using PCR with routinely used Taq DNA polymerase is usually a two-step procedure performed in two tubes. In the first step/tube, mRNA is reverse transcribed to cDNA, and in the second step/tube, an aliquot from the first step is amplified in PCR. Our experiments showed that using TPP buffer it is possible to perform both steps (reverse transcription and PCR) in the same reaction tube and with smaller amounts of both RNA and M-MLV reverse transcriptase. Direct comparison of the results of the two-tube assay and one-tube assay is shown in Figure 2. The data show that one-tube assays with a reduced amount of RNA and M-MLV reverse transcriptase in the TPP buffer give comparable results to those using a higher amount of the RNA and reverse transcriptase and performed in two tubes. The combined data indicate that TPP buffer improves reverse transcription of difficult-to-transcribe genes and allows simplified quantification of specific mRNAs in one tube.

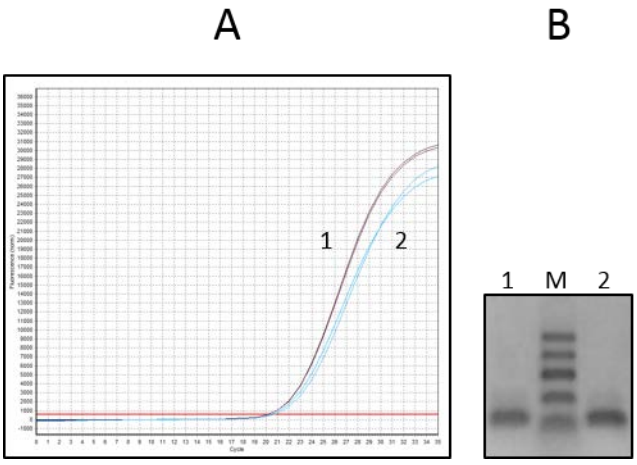

Figure 2: Comparison of the results of reverse transcription combined with PCR in two-tube and one-tube assays. In the two-tube experiment (1), M-MLV-mediated reverse transcription was performed in a regular M-MLV reverse transcription buffer using isolated RNA (50 ng/µl) and reverse transcriptase (20 U/µl) in a total volume of 10 µl in one tube. One µl (10%) of the reaction mixture after reverse transcription was then used for amplification in complete TPP buffer-based PCR master mix. For one-tube experiment (2), reverse transcription was performed in TPP buffer and with reduced concentration of both RNA (5 ng/µl) and reverse transcriptase (2.5 U/µl). PCR was then performed in the same tube after adding nucleotides, Taq DNA polymerase, oligonucleotide primers, and SYBR green I. The results are presented as fluorescence versus PCR cycles (panel A) and photography of agarose gel electrophoresis of the PCR amplicons stained with ethidium bromide (panel B). Positions of DNA markers in the B panel are also shown (M).

Conclusion: The results show that TPP buffer improves efficiency of reverse transcription and allows simplified quantification of specific mRNAs using reduced amounts of both RNA and M-MLV reverse transcriptase.

To get more information on this project and/or to purchase a nonexclusive license for the prototype of simplified, economical and accelerated analysis of RNA from low cell numbers, contact the **Centre for Technology Transfer,** IMG AS CR, Vídeňská 1083, 14220 Prague 4, Czech Republic; Tel. 420-241 063 227 or 420-602 892 876, e-mail: ctt@img.cas.cz