



TargetAmp™ Aminoallyl-aRNA is a Suitable Template for cDNA Synthesis

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T7-based, linear RNA amplification methods are used to increase the small amount of RNA obtained from limited samples so that the RNA can be used for microarray analysis. When sufficient RNA is available, a portion of the cDNA produced early in the RNA amplification procedure can be saved and used later in real-time RT-PCR to validate gene expression differences. However, with rare or precious RNA samples, using the abundant antisense RNA (aRNA) product of the amplification process is preferable. Frequently aminoallyl-UTP (AA-UTP) is incorporated into the aRNA during the amplification procedure to produce aminoallyl-aRNA (AA-aRNA), which can be labeled and used as target for microarray studies. To verify that AA-aRNA is a suitable template for real-time RT-PCR, we tested AA-aRNA samples produced using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 (See page 6). Here we report that AA-aRNA can be used effectively as a template for real-time RT-PCR to identify differences in gene expression or to validate microarray results.

Methods

MonsterScript™ 1st-Strand cDNA Synthesis

Total cellular rat RNA from heart and kidney was amplified using the TargetAmp Aminoallyl-aRNA Amplification Kit 101. For comparison, heart and kidney RNA samples were also amplified without the incorporation of AA-UTP to produce unmodified aRNA. First-strand cDNA was synthesized from 200 ng of AA-aRNA (rat heart and kidney) and aRNA (rat heart and kidney) using random nonamer primers and EPICENTRE's new MonsterScript 1st-Strand cDNA Synthesis Kit (See page 22).

TAQurate™ GREEN Real-Time PCR

One microliter from each 20- μ l cDNA synthesis reaction was used in a TAQurate GREEN Real-Time PCR reaction containing 1X TAQurate™ GREEN Real-Time PCR MasterMix, and 20 pmoles of each message-specific primer.¹ Rat messages detected were: β -2-microglobulin (B2M), a ubiquitously expressed housekeeping gene; protease serine 15 (Prss15), a mitochondrial ATP-dependent protease; myomegalin, a phosphodiesterase D4

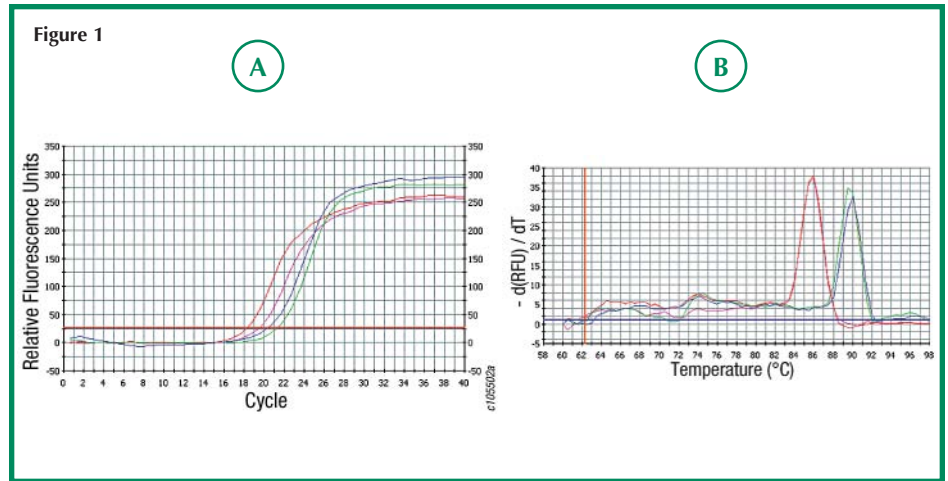


Figure 1. MonsterScript™ 1st-Strand cDNA, made from both AA-aRNA and unmodified aRNA, amplifies efficiently by real-time PCR using TAQurate™ GREEN Real-Time PCR Master Mix. Real-time PCR was performed, as described in the text, using AA-aRNA and aRNA. **A.** PCR amplification plot of B2M (AA-aRNA, pink; aRNA, red) and Prss 15 (AA-aRNA, green; aRNA, blue). Primers used for B2M were: 5' CGT GCT TGC CAT TCA GAA AAC 3' and 5'-TCT GAG GTG GGT GGA ACT GAG 3', and for Prss15 were: 5' GTC ACA TCC CAC ATC CAC CTG C 3' and 5' GTG ATG AAG GGA GCC AAG TCT GA 3'. **B.** Melt curve analysis of the PCR products indicated a specific product for each primer pair tested.

interacting protein found to be highly expressed in rat heart and skeletal muscle,² with lower expression in other cell types; and kallikrein, a serine protease found to be highly expressed in rat kidney, with lower expression in other cell types.³ Real-time PCR was performed and results were analyzed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad) using the following cycling profile: 95°C (2 minutes), followed by 45 cycles of 92°C (20 seconds), 64°C (30 seconds), and 72°C (30 seconds).

Results

B2M and Prss15

The AA-aRNA and aRNA samples produced similar results by real-time PCR analysis for the expression of B2M and Prss15. The threshold cycles (C_T) for the AA-aRNA samples demonstrated a slight delay in amplification, as indicated by an increase in C_T of about one cycle, compared to the aRNA samples, for both heart and kidney (Figure 1A and data not shown). The slight increase in C_T appears to be consistent among AA-aRNA PCR amplified samples and is probably due to less efficient reverse transcription of the AA-aRNA template. Therefore, for accu-

rate and meaningful comparisons, relative (comparative) real-time RT-PCR should be performed using either AA-aRNA samples or aRNA samples, but not a combination of both. No differences between PCR products were observed by melt curve analysis (Figure 1B).

Myomegalin and kallikrein

The expression levels of both rat myomegalin and kallikrein in heart and kidney were compared by relative real-time PCR using both AA-aRNA and aRNA templates. Real-time RT-PCR results confirm that expression of myomegalin is high in rat heart tissue and low in rat kidney as demonstrated by a C_T difference of approximately 5 cycles, for both AA-aRNA ($\Delta C_T=5.0$) and aRNA ($\Delta C_T=5.4$) samples (Figure 2A). Kallikrein expression is very low in heart and high in kidney as shown by a C_T difference of approximately 9 cycles, for AA-aRNA ($\Delta C_T=9.1$) and aRNA ($\Delta C_T=9.7$) samples (Figure 3A).

For myomegalin and kallikrein, the C_T values from the AA-aRNA samples again showed a slight delay in amplification, as demonstrated by an increase of up to one cycle, when compared to the aRNA samples (Figures 2A, 3A). Melt curve analysis

of the PCR products showed only the expected, specific products with the myomegalin primers (Figure 2B). In heart samples, where the kallikrein message was extremely limited, some primer-dimer products were synthesized in addition to the specific products, for both the AA-aRNA and the aRNA. The negative control, which contained no cDNA produced only primer-dimers.

Conclusions

Aminoallyl-aRNA, produced using the TargetAmp Aminoallyl-aRNA Amplification Kits, is a suitable substrate for real-time RT-PCR to assay gene expression and validate microarray results. For relative real-time PCR, we recommend that all samples, including target and reference genes, are PCR amplified from the same type of starting RNA; either unamplified,

amplified without aminoallyl-labeling, or amplified with aminoallyl-labeling, to obtain the most accurate expression comparisons.

References

1. Grunenwald, H. *et al.* (2004) *EPICENTRE Forum* **11**(5), 9.
2. Verde, I. *et al.* (2001) *J. Biol. Chem.* **276**(14), 11189.
3. Saed, G.M. *et al.* (1990) *Circ. Res.* **67**, 510.
4. Heil, S.G. *et al.* (2003) *BioTechniques* **35**, 502.

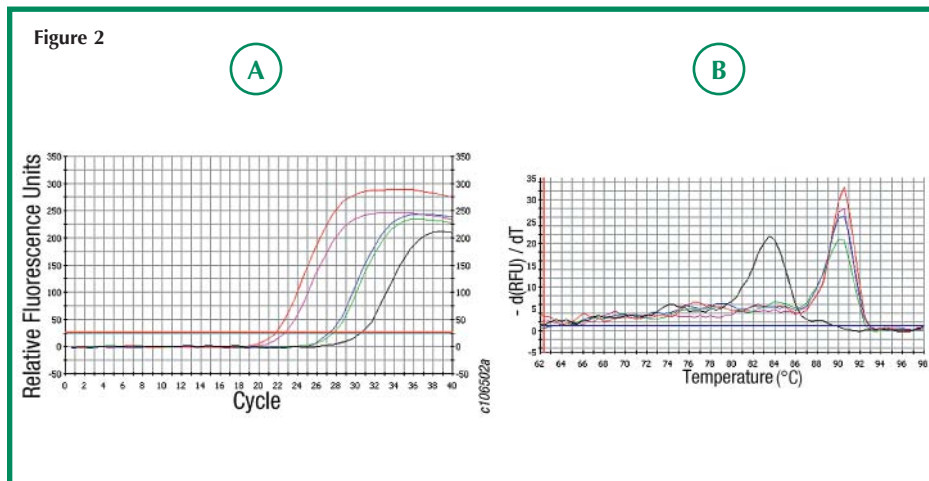


Figure 2. Myomegalin expression level differences in rat heart and kidney RNA were determined by real-time RT-PCR using both AA-aRNA and aRNA. Real-time RT-PCR was performed, as described in the text, from rat heart and kidney RNA. **A.** PCR amplification plot for myomegalin reactions (heart AA-aRNA, pink; heart aRNA, red; kidney AA-aRNA, green; kidney aRNA, blue; no cDNA, black). Myomegalin primers were 5' ACA GCA GCA GAT TGG GGA AGG G 3' and 5' GGG ACA CTT GGG CTC GCA GGT 3'. **B.** Melt curve analysis shows only specific myomegalin products for all reactions containing cDNA and only a primer-dimer peak in the control sample containing no cDNA.

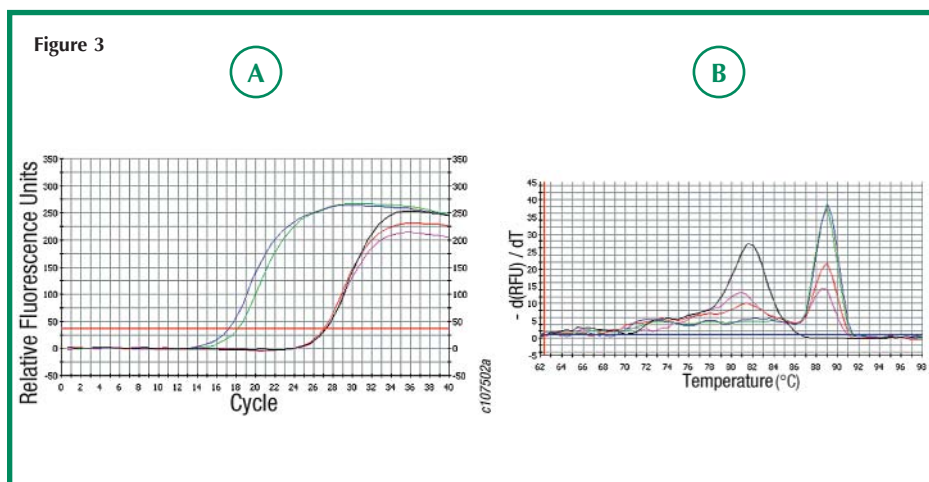


Figure 3. Kallikrein expression level differences in rat heart and kidney RNA were determined by real-time RT-PCR using both AA-aRNA and aRNA. Real-time RT-PCR was performed, as described in the text, from rat heart and kidney RNA. **A.** PCR amplification plot for kallikrein reactions (heart AA-aRNA, pink; heart aRNA, red; kidney AA-aRNA, green; kidney aRNA, blue; no cDNA, black). Kallikrein primers were 5' CTG CCC ACT GAG GAG CCC AA 3' and 5' TAA GTT TGG TGT AGA TGG CTG GC 3'. **B.** Melt curve analysis shows specific kallikrein PCR products in the kidney samples, where kallikrein expression is high. In the heart, where kallikrein expression is very low, both specific products and primer-dimers were made. Only a primer-dimer peak is seen in the control sample containing no cDNA.

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TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

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MonsterScript™ Reverse Transcriptase

MSTA5110	10 Reactions
MSTA5124	24 Reactions

Includes MonsterScript™ 5X Reaction buffer

MonsterScript™ 1st-Strand cDNA Synthesis Kit

MS040910	10 Reactions
MS041050	50 Reactions

Contents:
 MonsterScript™ Reverse Transcriptase (with RNase Inhibitor)
 MonsterScript™ PreMix Solution (with Mg²⁺, dNTPs and Betaine*)
 V₃-Oligo(dT)₂₁ Primer
 Random Nonamer Primer
 Sterile RNase-Free Water

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TAQurate™ GREEN Real-Time PCR MasterMix

TM049096	96 25-µl Reactions
TM046400	400 25-µl Reactions

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 TAQurate™ Real-Time PCR Enzyme Blend
 TAQurate™ GREEN Real-Time 2X PCR MasterMix
 Passive Reference Dye
 Stabilizer

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