ABSTRACT

The future of research into human disease will require a better understanding of the regulation of gene expression. In addition, RNA viruses are common pathogens and virome sequencing is increasingly important in understanding a variety of viral diseases. Next generation sequencing platforms provide a powerful tool to study the human transcriptome and RNA virome; however, the value of this information depends directly on the accuracy of the libraries created for these analyses. Currently, used library construction protocols are compromised by low fidelity enzymes for cDNA synthesis and amplification, and technical limitations in library construction. These limitations lead to frequent errors and rearrangements during cDNA synthesis, bias due to secondary structure and nucleotide composition, and the loss of information on the direction of transcription. This is due to complicated library construction methods and their reliance on retroviral RTs, particularly those of Moloney Murine Leukemia Virus (M-MLV) and Avian Myeloblastosis Virus (AMV), to synthesize cDNA. Deficiencies inherent to retroviral RTs include: 1) Low thermostability. 2) Low fidelity. 3) Frequent rearrangements of the products. 4) Secondary activities. 5) High bias enzymes. Novel thermostable DNA polymerases (Pols), discovered in natural thermal environments are being developed as improved reagents for detecting and analyzing nucleic acids. PyroScript Reverse Transcriptase (RT) is the first thermostable viral DNA polymerase and the first Pol from any source with RT activity for efficient single-enzyme RT-PCR. In addition, this enzyme has an inherently high fidelity PCR capability, significantly higher than Taq Pol. These attributes improve the detection and quantification of transcript RNAs and RNA viruses and should facilitate the more accurate, efficient construction of transcriptome and RNA virome libraries for sequence analysis.

Comparison Data

PyroPhage RT vs. MMLV RT

2-step RT-PCR human mRNA

Total human liver RNA (1 µg) was reverse transcribed by Moloney Murine Leukemia Virus or by PyroPhage RT. Then PCR amplified using Lucigen EconoTaq® PLUS Master Mix. Shown are targets of 144, 246 and 298 bp.

CONCLUSIONS

- Thermostable PyroPhage RT directly detects and RT-PCR amplifies viral RNA and human transcripts.
- Effective for quantitative real-time and conventional RT-PCR analyses.
- Enhanced signal with magnetic beads.
- PyroPhage RT is effective in RT-LAMP.
- PyroScript RT-PCR 2X Master Mix is a robust and convenient RT-PCR solution.

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