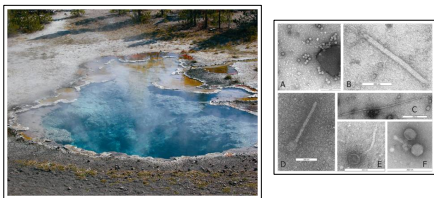


## ABSTRACT

Known limitations in reverse transcription enzymes (RTs) compromise current RT PCR and RNA sequence analyses. Most common RT protocols use retroviral RTs that impair analysis due to low accuracy, strand switching and bias. Since none of these RT enzymes are truly thermostable, a second DNA polymerase is needed to perform PCR amplification, which creates additional handling steps and necessitate a compromise in optimal conditions between the two enzymes. Recent screens of metagenomic libraries from hot spring viruses have uncovered novel DNA polymerases including PyroPhage™ RT. Thermostable PyroPhage RT is the first viral reverse transcriptase that is also capable of PCR. This allows single-enzyme, one-step RT PCR with sensitivity, specificity and accuracy equivalent to or better than multi-enzyme RT PCR systems, with the added benefit of reduced hands-on time and downstream error. Extreme thermostability expedites sample preparation by allowing complete denaturation to 94°C prior to reverse transcription and cDNA synthesis at 70°C, which improves analysis of highly structured RNA templates. PyroPhage RT has extremely high PCR fidelity, equivalent to the most accurate PCR enzymes, raising the prospect of highly accurate transcriptome analysis with reduced amplification bias and strand switching as compared to the alternatives. Additionally, PyroPhage RT was identified as part of a viral replication complex that includes thermostable helicase and primase subunits. These accessory proteins are now being expressed and studied as adjuncts to further improve PyroPhage RT performance. Details on usage of the PyroPhage RT enzyme in both RT PCR and transcriptome analysis are provided.

## Thermophilic Phage Metagenomics

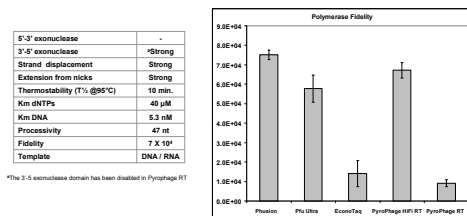


### Viral Metagenomics

Thermophilic phage (electron micrographs, right) were isolated from the Hot Springs of Yellowstone National Park (left) and used to construct metagenomic libraries that were screened to identify novel DNA polymerases.

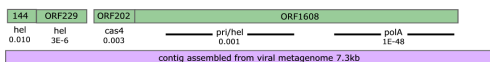
*Applied Environmental Microbiology* 74(13):4164-4174, 2008.

## High Fidelity PCR with PyroPhage RT



## Accessory Proteins

### The PyroPhage RT Replication Operon



The PyroPhage RT gene is part of a 7.3 kb contig assembled from the viral metagenome. This contig contains an apparent operon that includes the polA-like replicase (PyroPhage RT) and four apparent accessory proteins that are being developed to improve RT PCR and transcriptome and RNA virome library preparation.

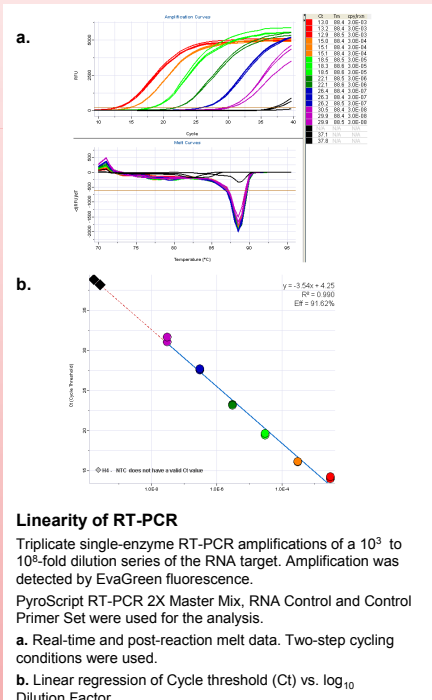
## PyroScript™ RT-PCR Master Mix Kit

- PyroScript RT-PCR 2X Master Mix
  - Buffer
  - dNTPs
  - PyroPhage RT Polymerase
- RNA control
  - Direct RT-PCR control
  - Extractable Control
- Control Primer Set
- 100 mM Magnesium Sulfate
- Nuclease-free Water

Just add template, primers and cycle

Compatible with end point (gel-based) or real-time analysis

## RT-PCR with PyroPhage RT

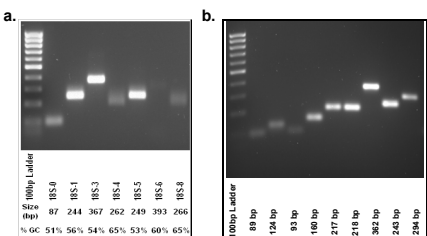


### Linearity of RT-PCR

Triplicate single-enzyme RT-PCR amplifications of a 10<sup>3</sup> to 10<sup>8</sup>-fold dilution series of the RNA target. Amplification was detected by EvaGreen fluorescence.

PyroScript RT-PCR 2X Master Mix, RNA Control and Control Primer Set were used for the analysis.

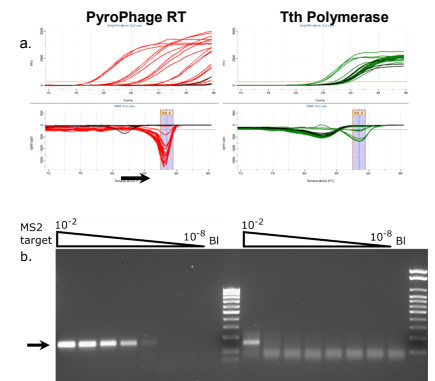
- Real-time and post-reaction melt data. Two-step cycling conditions were used.
- Linear regression of Cycle threshold (Ct) vs. log<sub>10</sub> Dilution Factor.



### Single Enzyme RT-PCR

- Human 18S rRNA sequences were amplified from 100 pg total A549 cell line RNA. Seven primer sets targeting amplicons from 51 to 65% GC content and from 87 to 393 bp in length were tested.
  - Viral RNA (Enterobacteriophage MS2, ATCC 15597-B1) was amplified by 40 cycles of RT-PCR without background. Products from 89 to 362 bp in length were amplified.
- One-step single-enzyme RT-PCR cycling conditions:  
15 sec @ 94°C, (10s @ 94°C, 30s @ 72°C)\*40

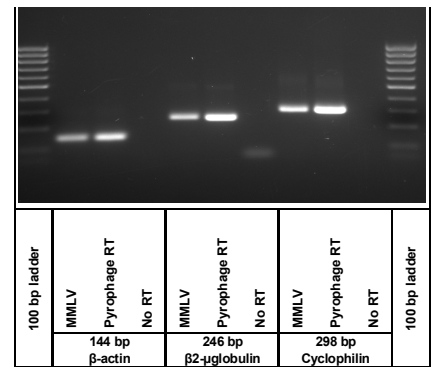
## Comparison Data



### PyroPhage RT vs. Tth Polymerase

Single-enzyme, one-step RT-PCR of a 160 bp amplicon using a 10<sup>2</sup> to 10<sup>8</sup>-fold dilution series of MS2 RNA.

- Real-time and post-reaction melt data
- Corresponding Agarose gel data. Tth polymerase used with Mn<sup>2+</sup> as directed (Epicentre). Arrows show correct melt T<sub>m</sub> (top) and amplicon (bottom).



### 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.
- PyroPhage RT is effective in RT-LAMP.
- PyroScript RT-PCR 2X Master Mix is a robust and convenient RT-PCR solution.

### Acknowledgements

This work was supported by NSF and NIH NIAID grants. Sequencing of viral metagenomes was performed by JGI.

