UltraMarathonRT

RNAConnect

# UltraMarathonRT: enabling greater visibility and performance to power the future of multiomics

## ABSTRACT

Sensitive, quantitative detection of full-length RNA transcripts is essential for accurately capturing information on gene expression and transcriptomic diversity. However, the reverse transcriptase (RT) enzymes used to detect RNA molecules during processes such as RNA-seq and RT-PCR are notoriously sensitive to variations in RNA sequence, transcript length, RNA structure and reaction conditions. MarathonRT, a novel group II intron-encoded RT, was developed to address these limitations. It efficiently copies highly structured and modified long RNAs end-to-end without stopping, even at low temperatures. This contrasts with conventional RTs which are inherently nonprocessive and which are blocked by RNA structures in the template. To overcome these issues, conventional RTs have been engineered to function at high reaction temperatures that melt RNA template structures. However, high temperature conditions cause RNA degradation that fractures long RNA molecules. This prevents the capture of full-length transcripts, eliminates positional information on RNA processing variants, and reduces the amount of RNA available for analysis.

However, despite its many positive attributes, the original MarathonRT enzyme had suboptimal sensitivity for detection of low abundance RNA transcripts, which limited its full potential as a tool for building sequencing libraries. To address this issue, RNAConnect developed an optimized version of MarathonRT called UltraMarathonRT (uMRT). A diversity of experimental metrics, described below, establish that uMRT maintains the ultra-high processivity of MarathonRT while exhibiting superior sensitivity to low abundance RNA transcripts, uniform transcript coverage, and high enzyme efficiency when compared to conventional reverse transcriptases. In addition, our new proprietary Boost, a uMRT reaction enhancer, greatly improves uMRT sensitivity under conditions of low RNA input making it applicable for routine cDNA synthesis with limited RNA input, single-cell and spatial transcriptomics investigations. These improvements, along with a carefully formulated reaction buffer and lower reaction temperatures that preserves RNA integrity, establishes uMRT as the leading enzyme for routine RNA amplification, sequencing, and cutting-edge transcriptomics applications.

## INTRODUCTION

MarathonRT is a group II intron-encoded RT that was discovered and developed by Dr. Anna Marie Pyle and Dr. Chen Zhao at Yale University during structural studies of proteins involved in group II intron splicing[1]. The structure has been solved to high resolution in multiple states[2-4] resulting in unprecedented knowledge about the architecture and function of this enzyme. MarathonRT was shown to rapidly copy highly structured long RNA molecules in a single pass[1,5], even in cases where the template contained highly stable RNA structural motifs[6].MarathonRT displayed extraordinary levels of processivity, fidelity, synchronicity, and unwinding power (Table 1). Upon systematic characterization, the enzyme showed remarkable performance in a variety of applications including single-cycle reverse transcription of long RNAs[1,5,6], mutational profiling with DMS and acylation reagents (DMS-MaP and SHAPE-MaP[7-9]), and the detection of natural RNA base modifications[5,10].

**Table 1: MarathonRT is an ultra-processive, high performance enzyme.**

| Attribute | MarathonRT | SSIV |
|---|---|---|
| Processivity | 30,000 | 124 |
| Fidelity | $9.9 \times 10^{-5}$ | $1.8 \times 10^{-4}$ |
| Synchrony | High | None |
| Velocity | 25 nt/s | N/A |
| Single Cycle | Yes | Weak |
| Unwinding Power | 2°, 3° structures | None |

One drawback of the natural MarathonRT enzyme is the presence of secondary RNA binding sites that nonspecifically associate with nucleic acid, thereby reducing productive recognition of primertemplate initiation sites. To address this issue, we engineered a highly optimized variant called UltraMarathonRT. UltraMarathonRT (uMRT) generates >5-fold more cDNA than MarathonRT when analyzing low abundance long RNA by qPCR. In addition to protein engineering, we have designed a new reaction buffer enhancer called Boost that further optimizes uMRT's sensitivity to RNA input as low as 1 pg.

Here we provide metrics showing how uMRT combines the ultra-high processivity of MarathonRT with superior sensitivity to low abundance RNA transcripts, enabling detailed profiling of complex RNA libraries under real-world conditions that preserve the integrity of RNA samples.

## MATERIALS AND METHODS

**Commercial RNA Samples:** Commercial RNA samples used included Universal Human Reference RNA (Invitrogen™, Cat. No. QS0639) and SIRV-Set 4 (Lexogen, SKU 141.01).

**RNA sample preparation:** Total cellular RNA from SARS-CoV-2-infected VeroAT cells was extracted using TRIzol reagent (Invitrogen™, Cat. No. 15596026) and diluted to a starting concentration of 100 ng/µL followed by 5x ten-fold dilutions (100 ng – 1 pg/µL). The HCV small genome RNA was prepared by in vitro transcription.

**cDNA preparation:** For enzyme efficiency analysis, cDNA synthesis was performed using the UltraMarathonRT™ Reverse Transcription Kit (RNAConnect™ Cat. No. R1002) using the First-Strand cDNA synthesis protocol with the MarathonRT and UltraMarathonRT enzyme. cDNA was synthesized by SuperScript™ IV (Invitrogen™, Cat. No. 18091300), Maxima H Minus (ThermoScientific™, Cat. No.K1652), Induro® (NEB™, Cat. No. M0681), ProtoScript® II (NEB™, Cat. No. M0368), and SMARTScribe™ (Takara™, Cat. No. 639536) using their standard firststrand cDNA synthesis protocol.

For sensitivity to low RNA input analysis, cDNA was synthesized using UltraMarathonRT™ (RNAConnect™ Cat. No. R1002) with Boost, SuperScript™ III (Invitrogen™, Cat. No. 18080093), Maxima H Minus (ThermoScientific™, Cat. No. K1652), and Induro® (NEB™, Cat. No. M0681).

**PCR amplification of cDNA:** cDNA was amplified using LongAmp® Taq 2X Master Mix (NEB™, Cat. No. M0287) using their standard PCR protocol.

**Analysis of RNA degradation:** HCV small genome RNA samples were incubated in UltraMarathonRT (RNAConnect™ Cat. No. R1002), Maxima H Minus (ThermoScientific™, Cat. No. K1652), SuperScript™ IV (Invitrogen™, Cat. No. 18091300), and Induro® (NEB™, Cat. No. M0681) reaction buffer at their recommended reaction temperatures for 0, 10, 20, and 30 min respectively. Samples were then analyzed using the Agilent™ RNA 6000 Pico Kit (PN# 5067-1513) on a 2100 Bioanalyzer™.

**RNA-seq:** Universal human reference RNA was used as the templates. The cDNA libraries were first prepared using the template switching activities of uMRT and SuperScript II (Invitrogen™, Cat. No. 18064022) respectively and then amplified using KAPA HiFi DNA polymerase (Roche, Cat. No. 07958897001) followed by DNA purification by AMPure XP beads (Beckman, Cat. No. A63881). The sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, Cat. No. FC-131-1024)

**Bioinformatic analysis:** The sequencing reads were first trimmed with Trimmomatic (v0.40) to remove the adapter sequences, and the trimmed reads were then mapped to human reference GRCh38 by HISAT2 (v2.2.1). The resulting SAM files were converted to BAM files and then sorted by template-coordinate using samtools (v1.17). The resulting BAM files were used to count the mapped reads for each gene using software featureCounts (v2.0.6), and the raw counts were then converted to TPM using program language R. The gtf annotation file (basic gene annotation, release 45) used was downloaded from GENCODE website.

## RESULTS

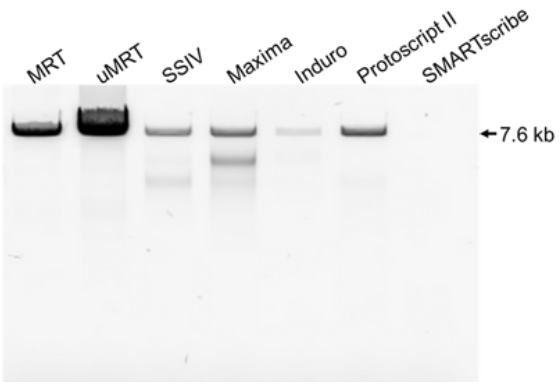


**Figure 1.** RT-PCR detection of a 7.6kb HCV small genome by UtraMarathonRT and other leading RTs.

### UltraMarathonRT enzyme efficiency with long, highly structured RNA templates.

Using first-strand cDNA synthesis followed by the same number of cycles of PCR amplification, we compared the performance of uMRT with MRT and other leading RTs for detection of a 7.6 kb HCV genomic RNA fragment using standard RT-PCR (Figure 1). UltraMarathonRT provided significantly higher cDNA yields for PCR amplification.

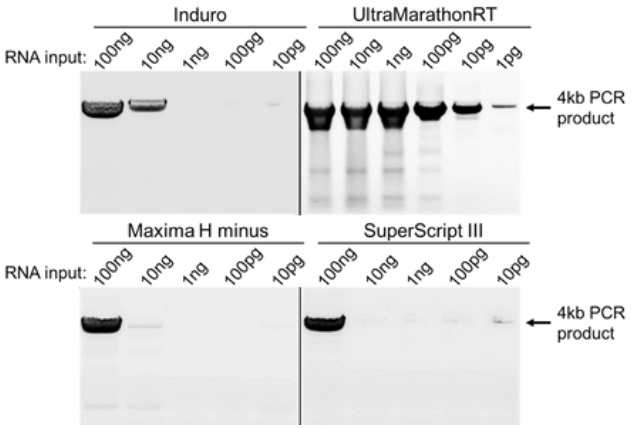


**Figure 2.** UltraMarathonRT sensitivity to low RNA input compared to other RTs.

## UltraMarathonRT sensitivity to low RNA input using new buffer enhancer Boost.

The concentration of total RNA greatly impacts the efficiency of reverse transcriptases and low RNA input significantly decreases cDNA synthesis levels. We developed a new reaction buffer enhancer called Boost that enables uMRT to copy full-length long RNA efficiently regardless of RNA input concentration. Using RT-PCR at varying total RNA input concentrations ranging from 100 ng to 10 pg, we compared UltraMarathonRT with Induro, Maxima H minus and SuperScript III RTs for long RNA amplification (Figure 2). The results show that UltraMarathonRT synthesizes long cDNAs more efficiently and from lower RNA input than other RTs. Additionally, UltraMarathonRT synthesized long cDNAs at every level of RNA input tested, including 1 pg.

## Preserving RNA template integrity.

Unlike other RTs, UltraMarathonRT has intrinsic helicase activity for RNA structure unwinding, making the high temperature reaction conditions utilized by other RTs totally unnecessary. It is important to graphically illustrate the extensive RNA damage that is incurred by incubation at elevated temperatures. To demonstrate this point, we analyzed the stability of an HCV genome fragment (7.6 kb) using manufacturer recommended buffers and incubation temperatures for a broad range of commercial RTs over a time course of 30 minutes (Figure 3).

As expected, the higher the temperature and duration of incubation, the more the RNA template degraded. These results demonstrate the advantage of using an RT that functions efficiently at ambient temperatures.

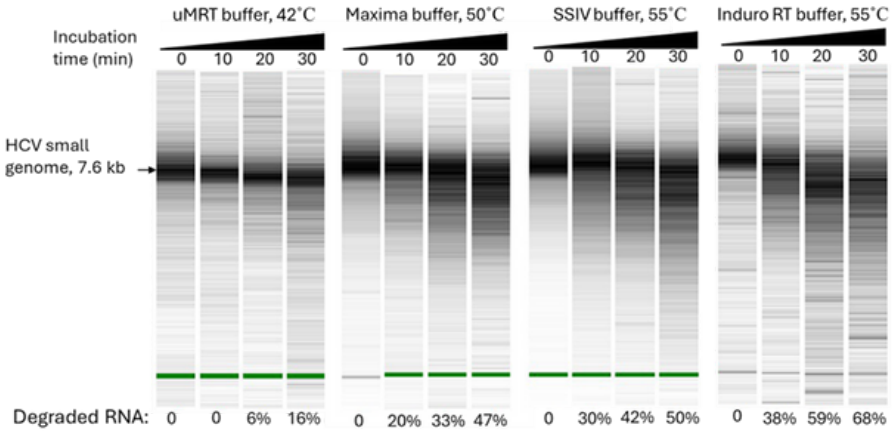


**Figure 3**. UltraMarathonRT reaction conditions promote RNA integrity.

## Comparison of gene coverage by UltraMarathonRT and SuperScript II in RNA-seq.

One of the fundamental reasons why UltraMarathonRT was developed was to generate accurate and complete sequencing libraries through experiments such as RNAseq. We therefore compared UltraMarathonRT and SuperScript II in RNA-seq experiments using human total cellular RNA (Figure 4). The resulting reads show that UltraMarathonRT detects 25% more genes than SuperScript II. In every class of RNA examined (Figure 4), UltraMarathonRT shows superior performance relative to SuperScript II. Notably, 65% more long non-coding RNA molecules (lncRNAs) were detected with UltraMarathonRT relative to SuperScript II. This suggests that UltraMarathonRT performs well on a wide variety of templates, accommodating those with unusual structures, modifications, and more diverse characteristics than mRNA molecules. By incorporating an ultra-processive RT enzyme into RNA-seq pipelines, we have unleashed power of RNA-seq for profiling complex RNA libraries that contain mixtures of long, structured RNA transcripts.

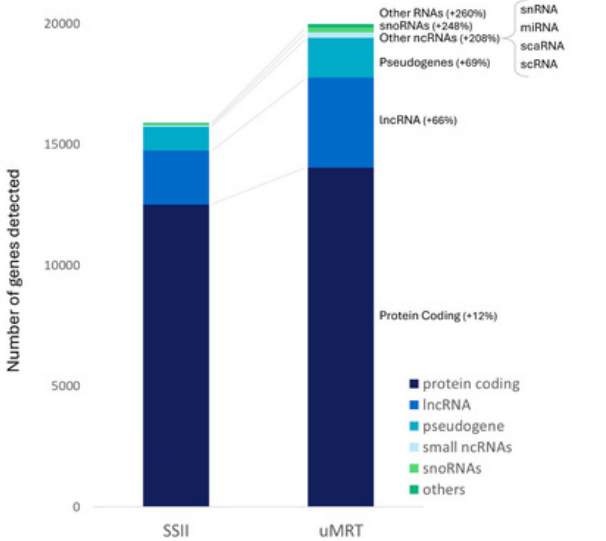


**Figure 4.** UltraMarathonRT shows superior gene coverage using RNA-seq.

## CONCLUSIONS

As biotechnology and molecular medicine advance, more powerful tools will be needed to accurately monitor and manipulate the complex RNA landscape of living systems. UltraMarathonRT unlocks this potential by reporting on the full transcriptomic complexity of RNA in cells and tissues under a broad set of real-world reaction conditions. Given its unmatched processivity, unwinding efficiency, low abundance RNA sensitivity, and low operating temperature, UltraMarathonRT is an essential component of the genomics toolkit.

## PRODUCTS LIST

| Product | Quantity | Catalog No. |
|---|---|---|
| UltraMarathonRT Reverse Transcription Kit | 20 reactions | R1002S |
| | 50 reactions | R1002M |
| | 100 reactions | R1002L |
| UltraMarathonRT Template Switching Kit | 20 reactions | R1004S |
| | 50 reactions | R1004M |
| | 100 reactions | R1004L |



## REFERENCES

1. Zhao, C., Liu, F. & Pyle, A. M. An ultraprocessive, accurate reverse transcriptase encoded by a metazoan group II intron. RNA **24**, (2018).

2. Zhao, C. & Pyle, A. M. Crystal structures of a group II intron maturase reveal a missing link in spliceosome evolution. Nat Struct Mol Biol **23**, (2016).

3. Chung, K. et al. Structures of a mobile intron retroelement poised to attack its structured DNA target. Science (1979) **378,** (2022).

4. Xu, L., Liu, T., Chung, K. & Pyle, A. M. Structural insights into intron catalysis and dynamics during splicing. Nature **624**, (2023).

5. Guo, L. T. et al. Sequencing and Structure Probing of Long RNAs Using MarathonRT: A Next-Generation Reverse Transcriptase. J Mol Biol **432**, (2020).

6. Guo, L. T., Olson, S., Patel, S., Graveley, B. R. & Pyle, A. M. Direct tracking of reverse-Transcriptase speed and template sensitivity: Implications for sequencing and analysis of long RNA molecules. Nucleic Acids Res **50**, (2022)

7. Huston, N. C. et al. Comprehensive in vivo secondary structure of the SARS-CoV-2 genome reveals novel regulatory motifs and mechanisms. Mol Cell **81**, (2021).

8. Tavares, R. de C. A., Mahadeshwar, G., Wan, H., Huston, N. C. & Pyle, A. M. The Global and Local Distribution of RNA Structure throughout the SARS-CoV-2 Genome. J Virol **95**, (2021).

9. Wan, H., Adams, R. L., Lindenbach, B. D. & Pyle, A. M. The In Vivo and In Vitro Architecture of the Hepatitis C Virus RNA Genome Uncovers Functional RNA Secondary and Tertiary Structures. J Virol **96**, (2022).

10. Araujo Tavares, R. de C., Mahadeshwar, G., Wan, H. & Pyle, A. M. MRT-ModSeq – Rapid Detection of RNA Modifications with MarathonRT. J Mol Biol **435**, (2023).

# CliniSciences Group

**Austria**
Company: CliniSciences GmbH
Address: Sternwartestrasse 76, A-1180 Wien - Austria
Telephone: +43 720 115 580
Fax: +43 720 115 577
Email: **oesterreich@clinisciences.com**
Web: **https://www.clinisciences.com**

**Belgium**
Company: CliniSciences S.R.L
Address: Avenue Stalingrad 52, 1000 Brussels - Belgium
Telephone: +32 2 31 50 800
Fax: +32 2 31 50 801
Email: **belgium@clinisciences.com**
Web: **https://www.clinisciences.com**

**Denmark**
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: **danmark@clinisciences.com**
Web: **https://www.clinisciences.com**

**Finland**
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: **suomi@clinisciences.com**
Web: **https://www.clinisciences.com**

**France**
Company: CliniSciences S.A.S
Address: 74 Rue des Suisses, 92000 Nanterre- France
Telephone: +33 9 77 40 09 09
Fax: +33 9 77 40 10 11
Email: **info@clinisciences.com**
Web: **https://www.clinisciences.com**

**Germany**
Company: Biotrend Chemikalien GmbH
Address: Wilhelm-Mauser-Str. 41-43, 50827 Köln - Germany
Telephone: +49 221 9498 320
Fax: +49 221 9498 325
Email: **info@biotrend.com**
Web: **https://www.biotrend.com**

**Iceland**
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: **island@clinisciences.com**
Web: **https://www.clinisciences.com**

**Ireland**
Company: CliniSciences Limited
Address: Ground Floor, 71 lower Baggot street Dublin D02 P593 - Ireland
Telephone: +353 1 6971 146
Fax: +353 1 6971 147
Email: **ireland@clinisciences.com**
Web: **https://www.clinisciences.com**

**Italy**
Company: CliniSciences S.r.l
Address: Via Maremmana inferiore 378 Roma 00012 Guidonia Montecelio - Italy
Telephone: +39 06 94 80 56 71
Fax: +39 06 94 80 00 21
Email: **italia@clinisciences.com**
Web: **https://www.clinisciences.com**

**Netherlands**
Company: CliniSciences B.V.
Address: Kraijenhoffstraat 137A, 1018RG Amsterdam, Netherlands
Telephone: +31 85 2082 351
Fax: +31 85 2082 353
Email: **nederland@clinisciences.com**
Web: **https://www.clinisciences.com**

**Norway**
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: **norge@clinisciences.com**
Web: **https://www.clinisciences.com**

**Poland**
Company: CliniSciences sp.Z.o.o.
Address: ul. Rotmistrza Witolda Pileckiego 67 lok. 200 - 02-781 Warszawa -Poland
Telephone: +48 22 307 0535
Fax: +48 22 307 0532
Email: **polska@clinisciences.com**
Web: **https://www.clinisciences.com**

**Portugal**
Company: Quimigen Unipessoal LDA
Address: Rua Almada Negreiros, Lote 5, Loja 14, 2615-275 Alverca Do Ribatejo - Portugal
Telephone: +351 30 8808 050
Fax: +351 30 8808 052
Email: **info@quimigen.com**
Web: **https://www.quimigen.pt**

**Spain**
Company: CliniSciences Lab Solutions
Address: C/ Hermanos del Moral 13 (Bajo E), 28019, Madrid - Spain
Telephone: +34 91 269 40 65
Fax: +34 91 269 40 74
Email: **espana@clinisciences.com**
Web: **https://www.clinisciences.com**

**Sweden**
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: **sverige@clinisciences.com**
Web: **https://www.clinisciences.com**

**Switzerland**
Company: CliniSciences Limited
Address: Marktgasse 18 8302 Kloten - Switzerland
Telephone: +41 (044) 805 76 81
Fax: +41 (044) 805 76 75
Email: **switzerland@clinisciences.com**
Web: **https://www.clinisciences.com**

**UK**
Company: CliniSciences Limited
Address: 11 Progress Business center, Whittle Parkway, SL1 6DQ Slough- United Kingdom
Telephone: +44 (0)1753 866 511
or +44 (0) 330 684 0982
Fax: +44 (0)1753 208 899
Email: **uk@clinisciences.com**
IWeb: **https://www.clinisciences.com**

**USA**
Company: Biotrend Chemicals LLC
Address: c/o Carr Riggs Ingram, 500 Grand Boulevard, Suite 210 Miramar Beach, FL 32550- USA
Telephone: +1 850 650 7790
Fax: +1 850 650 4383
Email: **info@biotrend-usa.com**
Web: **https://www.biotrend-usa.com**