UltraMarathonRT

RNAConnect

Enabling Complete and Accurate cDNA Synthesis for Targeted RNA or Whole Transcriptome Sequencing with UltraMarathonRT®

INTRODUCTION

Reverse transcriptase (RT) enzymes are indispensable tools for synthesizing complementary DNA (cDNA) from RNA templates. This process is crucial for various applications, including gene expression analysis, target RNA sequence analysis, and RNA-seq library preparation. UltraMarathonRT (uMRT) has emerged as a next-generation enzyme to provide precise RNA sequence and abundance analysis for these applications due to its exceptional processivity, high-fidelity, efficient template switching activity, and ability to synthesize full-length cDNA from challenging RNA templates. This application note highlights the performance advantages of UltraMarathonRT in cDNA synthesis and compares it with other commercially available reverse transcriptase enzymes.

UltraMarathonRT: A SUPERIOR CHOICE FOR cDNA SYNTHESIS

UltraMarathonRT is derived from a group II intron maturase found in *Eubacterium rectale*. The enzyme evolved to be highly processive, enabling it to easily transcribe long and structurally complex RNA templates into full-length cDNA. UltraMarathonRT's robust performance is attributed to its unique structural features, including an α-loop in the finger subdomain, which maintains the enzyme's engagement with the RNA template throughout the synthesis process resulting in intrinsic helicase activity. In addition to its exceptional processivity, uMRT is also sensitive to RNA inputs as low as 0.1 pg.

KEY FEATURES OF UltraMarathonRT

High Yield of Targeted Full-Length cDNA

Importantly, while other RTs often require more than 1 ng of RNA input, uMRT will make full length cDNAs even from input levels below 10 pg. uMRT will efficiently reverse transcribe templates >12 kb (Figure 1).

Figure 1. RT reactions for all reverse transcriptase enzymes were performed using 1 ng of total cellular RNA containing 10 pg of SIRV-Set 4 spike-in RNA and an incubation time of 20 minutes. The target RNA templates are SIRV-Set4 transcripts of different length.

High Processivity and Sensitivity

UltraMarathonRT is capable of synthesizing cDNA from RNA templates with length ranging from less than 0.1 kb to greater than 30 kb for end-to-end coverage at single-cell levels of RNA input (Figure 2).

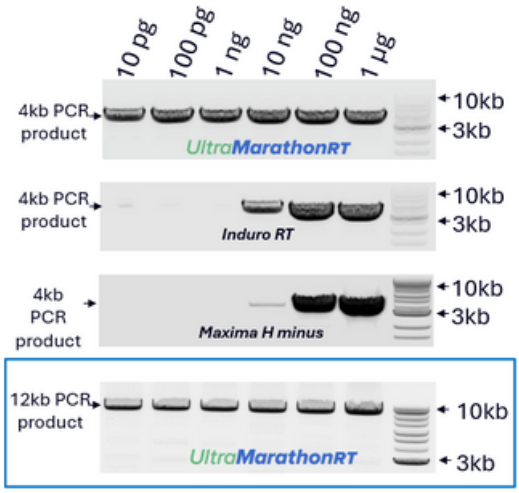
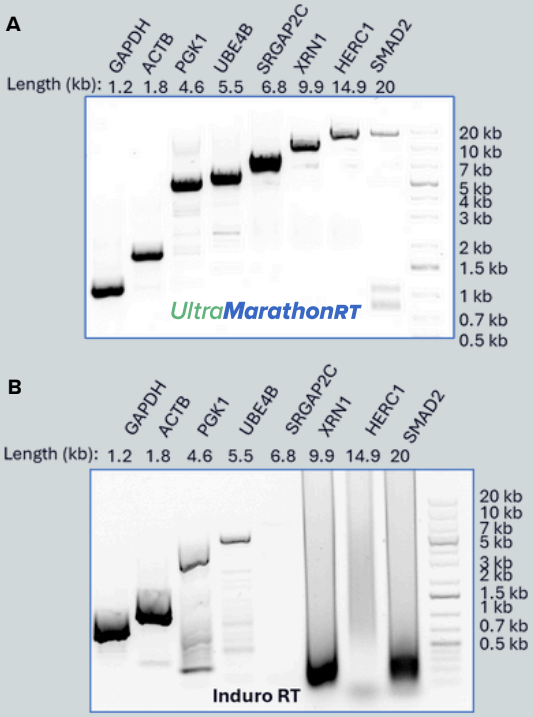


Figure 2. UltraMarathonRT sensitivity to low RNA input transcribing a 4 kb and 12 kb template compared to other RTs.



Easily Amplifies Complex Targets

Even long (20 kb) target RNAs with complex secondary structures do not present a problem for UltraMarathonRT (Figure 3). uMRT has an intrinsic helicase activity derived from its unique protein structure that enables it to naturally unwind all secondary structures including those with high GC-content.

Figure 3. A) Amplification of transcripts by UltraMarathonRT from 1.2 kb to 20 kb using 100 ng Hela total RNA. **B)** The cDNAs of the same target transcripts generated by Induro RT were amplified by LongAmp Taq. Reverse transcription was performed using Oligo(dT)18 primer.

Optimal Operating Temperature

UltraMarathonRT functions optimally at 30°C. Higher reaction temperatures (>50°C) are used by other RTs to melt secondary structures. However, these high temperatures cause degradation of target RNA (which is not observed at 30°C) (Figure 4).

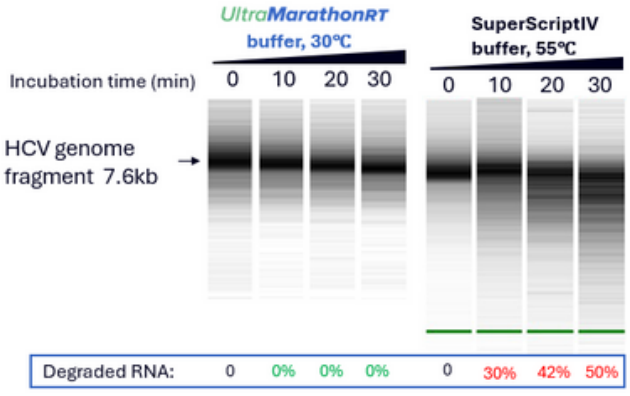


Figure 4. Monitoring the degradation of a 7.6 kb HCV RNA in the denoted RT buffer and temperature over time.

Consistent, Highly Efficient Template Switching

UltraMarathonRT has efficient terminal transferase activity. It consistently adds three non-templated adenosines to the end of each cDNA. Utilizing uMRT's Template Switching Oligonucleotide allows for adapters to be added to both ends of the transcript. This enables end-to-end RNA sequencing methods that capture 5'-sequence content without cumbersome library manipulations.

COMPARATIVE PERFORMANCE ANALYSIS

End-to-End Synthesis

Traditional reverse transcriptases, known for their frequent premature stops during cDNA synthesis, struggle with long or structured RNAs. These RTs frequently pause and terminate prematurely, leading to incomplete cDNA synthesis. When libraries are made from these cDNAs, a distinct 3' bias can be observed. uMRT reduces this bias by creating full length cDNAs (Figure 5).

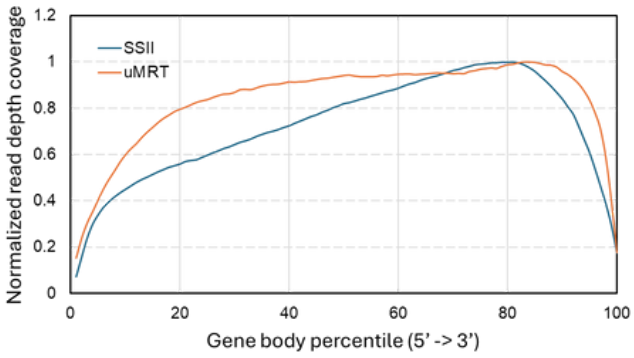


Figure 5. Gene body coverage by SMART-seq reads from the uMRT and SSII libraries prepared using universal human reference RNA. Each line represents the coverage of mapped reads from every library along with transcripts from 5' to 3' ends.

APPLICATION BENEFITS OF UltraMarathonRT

cDNA Library Construction

Combining UltraMarathonRT's ability to generate high yields of full-length cDNA with its efficient template-switching activity significantly simplifies the construction of cDNA libraries. This approach ensures accurate capture of RNA transcript diversity, streamlines library preparation workflows, and minimizes the risk of sequence artifacts.

Long RNA Templates

UltraMarathonRT excels in synthesizing cDNA from long RNA templates, including viral genomes, long noncoding RNAs (lncRNAs), and full-length messenger RNAs (mRNAs).

Sensitive, Quantitative and Reliable

UltraMarathonRT has ultra-high sensitivity to RNA input as low as 0.1 pg. Combined with its high fidelity and ability to create full length cDNA from long RNAs, including those with high GC content, uMRT ensures an accurate representation of any RNA template. Having consistent, full-length cDNAs improves the reliability of gene expression analysis produced from RNA-seq and RT-qPCR data. Regardless of the type of RT primer or location of qPCR primer pairs, the uMRT RT-qPCR kit will deliver consistent C_t values (Figure 6). This greatly simplifies primer pair selection and improves data reliability.

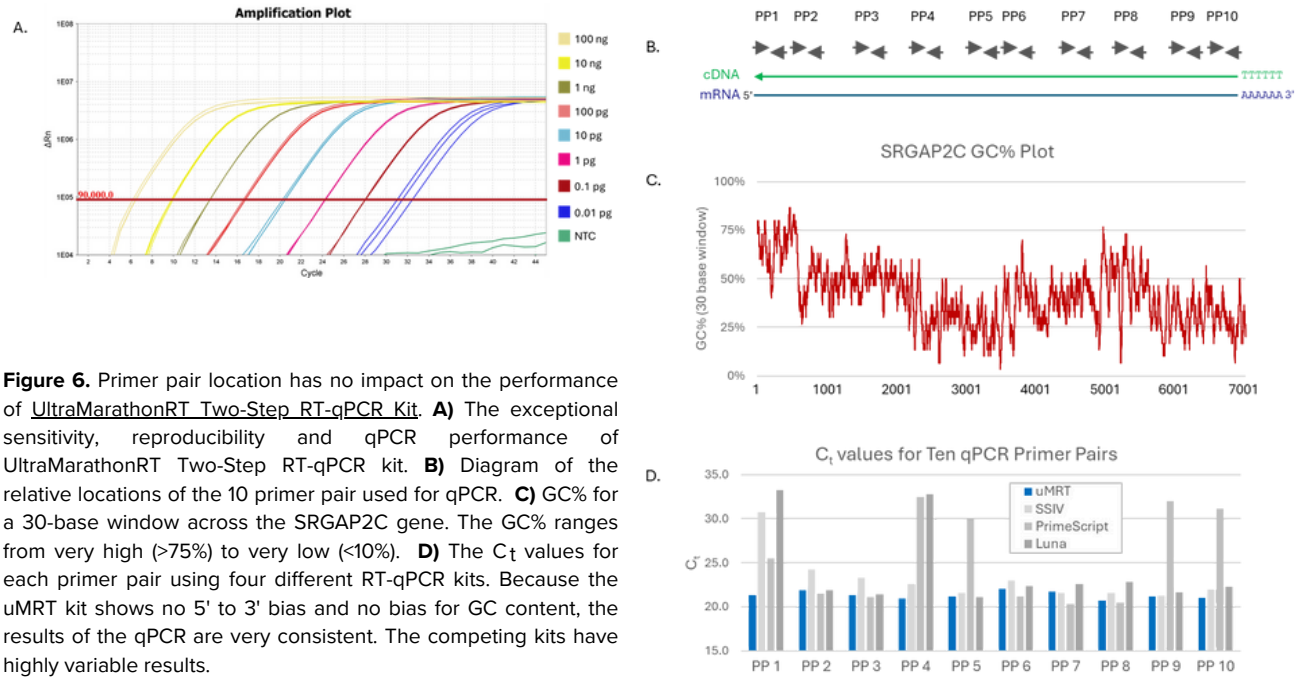


Figure 6. Primer pair location has no impact on the performance of UltraMarathonRT Two-Step RT-qPCR Kit. **A)** The exceptional sensitivity, reproducibility and qPCR performance of UltraMarathonRT Two-Step RT-qPCR kit. **B)** Diagram of the relative locations of the 10 primer pair used for qPCR. **C)** GC% for a 30-base window across the SRGAP2C gene. The GC% ranges from very high (>75%) to very low (<10%). **D)** The C_t values for each primer pair using four different RT-qPCR kits. Because the uMRT kit shows no 5' to 3' bias and no bias for GC content, the results of the qPCR are very consistent. The competing kits have highly variable results.

CONCLUSIONS

UltraMarathonRT offers a powerful solution for researchers and laboratories requiring reliable and efficient cDNA synthesis from complex RNA templates. Superior processivity, high-fidelity, and operational flexibility make it a valuable tool for constructing complete and accurate cDNA libraries. UltraMarathonRT provides consistent high performance, particularly in applications involving long and structured RNA molecules.

For laboratories seeking to optimize their cDNA synthesis workflows and achieve the highest quality results, UltraMarathonRT is the enzyme of choice. Its unique capabilities position it as a leader in the field of reverse transcription, supporting the ongoing advancement of RNA research and biotechnology.

ORDERING INFORMATION

For more information on UltraMarathonRT, including pricing and availability, please contact our technical support team or visit our website at www.rnaconnect.com.

This product and its labels and packaging may be covered by one or more patents, trademarks or copyrights owned or licensed by RNAConnect, Inc. The use of this product may require additional third-party intellectual property rights for certain applications.

This product is for research use only and is not intended for use in humans.