Improved applications for RNA analysis

qScript Ultra, a novel, engineered, inhibitor tolerant reverse transcriptase enables rapid, full-length synthesis of cDNA at elevated temperatures

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Abstract

First-strand cDNA synthesis by a reverse transcriptase (RT) is an indispensable step that underpins many methods for RNA analysis including monitoring changes in gene expression levels, profiling noncoding RNA, RNA pathogen quantification and sequencing of full transcripts or RNA viruses. Despite the pivotal role of RTs, most commercially available enzymes have limited speed, processivity, are adversely affected by sample matrix, or lack activity at temperatures sufficient to rival RNA secondary structure. These deficiencies typically result in incomplete and biased cDNA synthesis. Through a program of directed mutagenesis and enzyme variant screening, we have engineered a novel, inhibitor tolerant RT (iRT) called qScript Ultra that displays superior velocity and processivity with elevated activity over a broad range of temperatures.

Introduction

First-strand cDNA synthesis. For two-step RT-PCR approaches we developed the qScript Ultra RT mix, which provides qScript Ultra RT in an optimized and stablized master mix format and delivers efficient full-length cDNA synthesis with maximal yield at temperatures as high as 60°C. The enhanced velocity and processivity enables rapid and efficient conversion of long transcripts at elevated temperatures, critical for capturing RNA with high GC-content on regions of secondary structure. For RT-PCR applications that demand the highest consistency, reproducibility, and unbiased representation of the transcriptome in cDNA product we developed qScript Ultra cDNA SuperMix. Incorporating both anchored oligo(dT) and randomers, it contains all required components for cDNA synthesis except RNA template. The improved speed, processivity, and expanded thermal activity profile of qScript Ultra provides efficient linear conversion of RNA to cDNA in a 10-minute reaction at 55-60°C with exceptionally broad linear dynamic range.

One-step reverse transcription qPCR. For one-step, single-tube RT-qPCR applications we developed optimized master mixes containing qScript Ultra RT, DNA polymerase, and a thermoleable virul RNA glycosylase for PCR competitor contamination control. In addition, a nucleic acid aptamer is included, effectively blocking RT activity at temperatures up to 35°C, yet allowing full RT activity at temperatures of 55-60°C. This highly stringent warm start feature is particularly important for allowing room temperature reaction setup and for maintaining full specificity in complex multiplexed reactions. The robust, inhibitor tolerant characteristics of qScript Ultra allow for linear and consistent cDNA synthesis over a broad range of RNA input quantity and quality, including picogram levels of RNA and samples containing inhibitors derived from extraction chemicals, blood, plant, and animal tissues. Finally, qScript Ultra RT is highly tolerant of ligation, displaying full functional activity after drying and rehydration, enabling formulations with extended stability at room temperature and allowing shipping without a requirement for dry ice and foam packaging.

Methods

Total human RNA samples used in this study was Agilent Universal Human Reference (UHR) RNA. First-strand cDNA synthesis reactions were performed using the qScript Ultra Flex or the qScript Ultra SuperMix. First-strand cDNA synthesis reactions were analyzed by qPCR using the PerfeCTa SYBR Green FastMix. 1-step RT-qPCR master mixes were compared using the CDC Influenza SARS-CoV-2 Multiplex assay. Ligation of 1-step RT-qPCR master mix was done in 3 ml glass vials using a Microfuge Strata SBSVE freeze dryer. Dahlia Latent Virioid ssRNA was synthesized in reactions using 5 ng total human RNA and a stable LDHA gene expression levels, profiling noncoding RNA, RNA pathogen quantification and sequencing of full transcripts or RNA viruses. Despite the pivotal role of RTs, most commercially available enzymes have limited speed, processivity, are adversely affected by sample matrix, or lack activity at temperatures sufficient to rival RNA secondary structure. These deficiencies typically result in incomplete and biased cDNA synthesis. Through a program of directed mutagenesis and enzyme variant screening, we have engineered a novel, inhibitor tolerant RT (iRT) called qScript Ultra that displays superior velocity and processivity with elevated activity over a broad range of temperatures.

Conclusions

• The improved properties of the engineered qScript Ultra RT allow for increased reaction temperatures with high yields of long >17 kb cDNA products in only 10-minute reaction times.
• Optimized master mix formulations display broad RNA input compatibility with either flexibility in choice of priming method or the consistency and reproducibility of a single tube SuperMix format.
• 1-step RT-qPCR formulations with qScript Ultra and warm-start aptamer are compatible with ligation and display high sensitivity and specificity and improved inhibitor tolerance. High RT temperatures enable sensitivity with difficult templates.

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