

cDNA synthesis

Fast cDNA synthesis protocol using
SuperScript V VILO Master Mix

Generate cDNA with reverse transcription incubation times as low as 1 min

Introduction

Reverse transcription is a critical first step in gene expression analysis workflows and often determines the overall time required to generate RT-qPCR results. Traditional cDNA synthesis workflows require multiple steps, including DNase treatment, inactivation, primer annealing, reverse transcription, and termination, resulting in extended processing time. In contrast, Invitrogen™ SuperScript™ V VILO™ Master Mix reduces the total workflow time by shortening incubation times. Additionally, Invitrogen™ ezDNase™ Master Mix, supplied in single-tube format, allows rapid genomic DNA removal without an inactivation step. The primer annealing step is also eliminated, enabling a standard RNA-to-cDNA workflow in approximately 13 minutes.

By further omitting the DNase treatment step, reverse transcription can be completed in 11 minutes or less followed by qPCR in under 40 minutes (Figure 1).

This application note demonstrates that efficient target detection by RT-qPCR can be achieved with reverse transcription times as short as 1 minute across a broad reaction temperature range. We further show that cDNA can be generated in as few as 30 seconds for shorter transcripts, highlighting the exceptional catalytic efficiency of Invitrogen™ SuperScript™ V products and the potential to further reduce workflow time for suitable applications.

Traditional reverse transcriptase workflow with DNase I (105 min)



SuperScript IV VILO Master Mix workflow with ezDNase Enzyme (27 min)



SuperScript V VILO Master Mix workflow with ezDNase Master Mix (13 min)



SuperScript V VILO Master mix, simplified workflow (~11 min)



Complete qPCR results in 40 minutes or less when combined with TaqMan Fast Advanced Master Mix



Streamlining the RT-qPCR workflow

- Omit DNase treatment by using primers that span exon-exon junctions (or other gDNA-discriminating designs)
- Shorten the incubation time to as little as 1 minute for short-target detection in rapid workflows, such as RT-qPCR
- Use TaqMan Fast Advanced Master Mix to obtain qPCR results in 40 minutes or less

■ DNase treatment ■ DNase inactivation ■ Primer annealing
■ Reverse transcription reaction ■ Reverse transcription inactivation

Figure 1. Streamlined workflow reduces reverse transcription time and enables rapid RT-qPCR results. For optimal performance across a broad range of RNA sample types, inputs, and transcript lengths, 10-minute reverse transcription is recommended.

Materials and methods

Reagents

- SuperScript V VILO Master Mix (Cat. No. 11800001)
- SuperScript V VILO Master Mix, with gDNA removal (Cat. No. 11800111)
- Invitrogen™ Cervical Adenocarcinoma (HeLa-S3) Total RNA (Cat. No. AM7852)
- Invitrogen™ Millennium™ RNA Markers (Cat. No. AM7150)
- Applied Biosystems™ TaqMan™ Fast Advanced Master Mix (Cat. No. 4444556)
- Thermo Scientific™ Phusion™ Plus PCR Master Mix (Cat. No. F632S)
- ARTIC SARS-CoV-2 primer panel
- Invitrogen™ Colibri™ ES DNA Library Prep Kit for Illumina Systems (Cat. No. A38605024)

cDNA synthesis for two-step RT-qPCR

To evaluate the impact of short reverse transcription times on target detection by qPCR, reverse transcription reactions were prepared using SuperScript V VILO Master Mix according to the user manual with 10 ng of HeLa total RNA. cDNA synthesis was performed under varying conditions, including reaction temperatures from 37°C to 65°C and incubation times ranging from 1 to 10 minutes. The resulting cDNA was analyzed by qPCR using TaqMan Fast Advanced Master Mix and fast run mode.

cDNA synthesis using Millennium RNA Markers

To evaluate the impact of reaction time on cDNA length, reverse transcription reactions were set up according to the recommended protocols using SuperScript V VILO Master Mix, Primer-Free, oligo(dT)₁₈, and 1 µg of Millennium RNA Markers per 20 µL reaction. cDNA synthesis reactions were carried out for 0.5 to 10 minutes. The resulting cDNA was analyzed by gel electrophoresis using 2 µL of sample per well.

Next-generation sequencing (NGS) workflow compatibility

To evaluate the performance of cDNA generated using short reaction times in NGS workflows, 5 ng of HeLa total RNA was mixed with 100–10,000 copies of synthetic SARS-CoV-2 control RNA (nonoverlapping ~5 kb fragments) to simulate the RNA background and target abundance encountered in clinical samples. The RNA mixture was then reverse transcribed using SuperScript V VILO Master Mix stored at –20°C (control) or at 25°C for 4 weeks. Reverse transcription was performed using either 5- or 10-minute incubation protocols. cDNA was amplified using the ARTIC SARS-CoV-2 primer panel and Phusion Plus PCR Master Mix. Sequencing libraries were prepared using the Colibri ES DNA Library Prep Kit for Illumina Systems and sequenced on an Illumina™ MiSeq™ platform. Sequencing performance was evaluated using target genome coverage and amplicon recovery (47,000 reads per sample).

Results

Consistent target detection across temperatures and times

RT-qPCR analysis of *ACTB* (high-abundance) and *FZD6* (low-abundance) targets using 10 ng total RNA demonstrated consistent cDNA synthesis across a broad temperature range (37–65°C) and reduced reverse transcription incubation times (1–10 minutes) (Figure 2). At 50°C, reducing the incubation time from 10 to 5 minutes resulted in an approximately 0.5-cycle C_t delay, indicating only a modest reduction in cDNA yield. Further reducing the incubation time to 2 or even 1 minute increased the C_t delay but still provided reliable target detection, suggesting that these shorter incubation times may be suitable for rapid workflows where turnaround time is prioritized over maximum cDNA yield (Figure 3).

Efficient cDNA synthesis across time points

Agarose gel analysis was performed with cDNA synthesized from Millennium RNA Markers (0.5–9 kb) at 60°C using reaction times ranging from 30 seconds to 10 minutes (Figure 4). Efficient cDNA synthesis from short RNA fragments was observed across all time points, with clear detection of a 0.5 kb fragment even after just 30 seconds of reverse transcription. Increasing incubation time enhanced overall yield and synthesis of longer fragments. These results demonstrate the ability to synthesize a 9 kb fragment in just 5–10 minutes and the possibility to reduce reaction time even further for shorter transcripts.

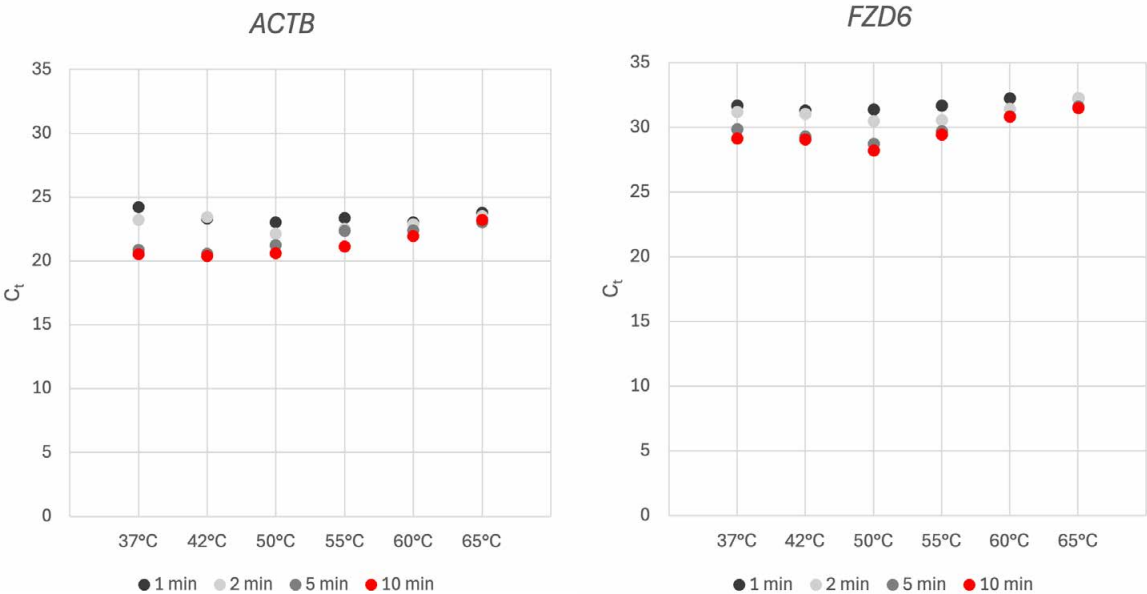


Figure 2. Reliable target detection with reduced reaction times.

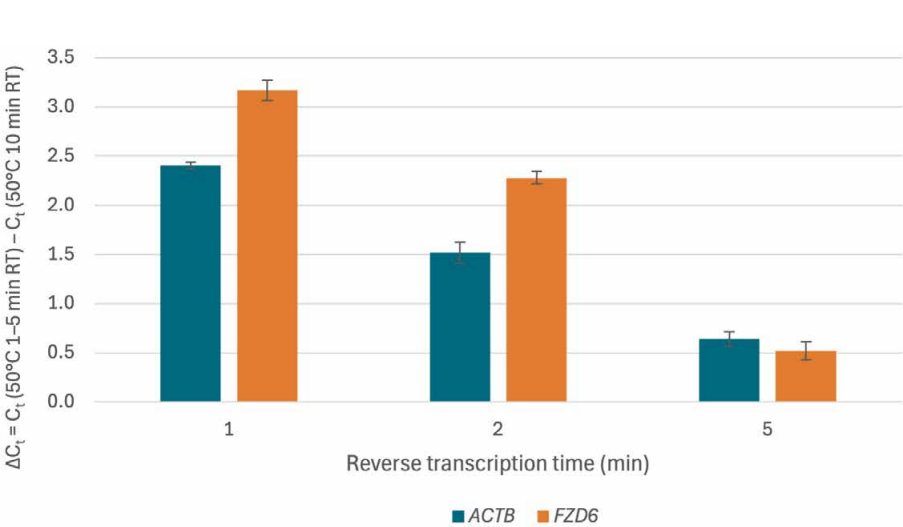


Figure 3. C_t delay compared to the standard protocol (50°C, 10 minutes).

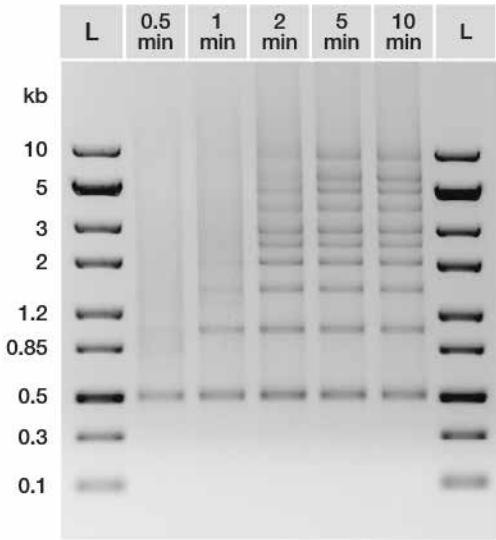


Figure 4. Rapid cDNA synthesis enables detection in as little as 30 seconds.

Short reaction times are compatible with NGS workflows

Shortening the reverse transcription incubation from 10 to 5 minutes maintained comparable sequencing performance across the evaluated SARS-CoV-2 RNA input levels. Mean sequencing coverage across the target genome and amplicon-level sequencing profiles were similar between the 5- and 10-minute reactions. Comparable results were also observed with SuperScript V VILO Master Mix stored at room temperature (25°C) for 4 weeks relative to the frozen control (Table 1).

Sequencing signal was evaluated by calculating the mean number of sequencing reads covering each position across the target genome. Amplicon-level sequencing profiles remained similar across the 5- and 10-minute reaction times and between storage conditions.

Table 1. Average sequencing signal by SARS-CoV-2 RNA copy level.

RNA copies	Average signal by RNA copy level			
	Control sample stored at -20°C		Sample stored for 4 weeks at 25°C	
	5 min reaction	10 min reaction	5 min reaction	10 min reaction
10,000	549	544	558	556
1,000	547	539	558	552
100	574	562	581	582

Amplicon coverage across the ~30 kb SARS-CoV-2 target sequence was assessed using the sequencing depth of each ARTIC amplicon. For visualization, each amplicon was plotted at its genomic midpoint, calculated as (amplicon start position + amplicon end position)/2, and its sequencing depth was classified into one of four coverage ranges: <10x, 10–99x, 100–999x, or ≥1,000x. Amplicon-level coverage profiles were comparable between the 5- and 10-minute reverse transcription protocols (Figure 5).

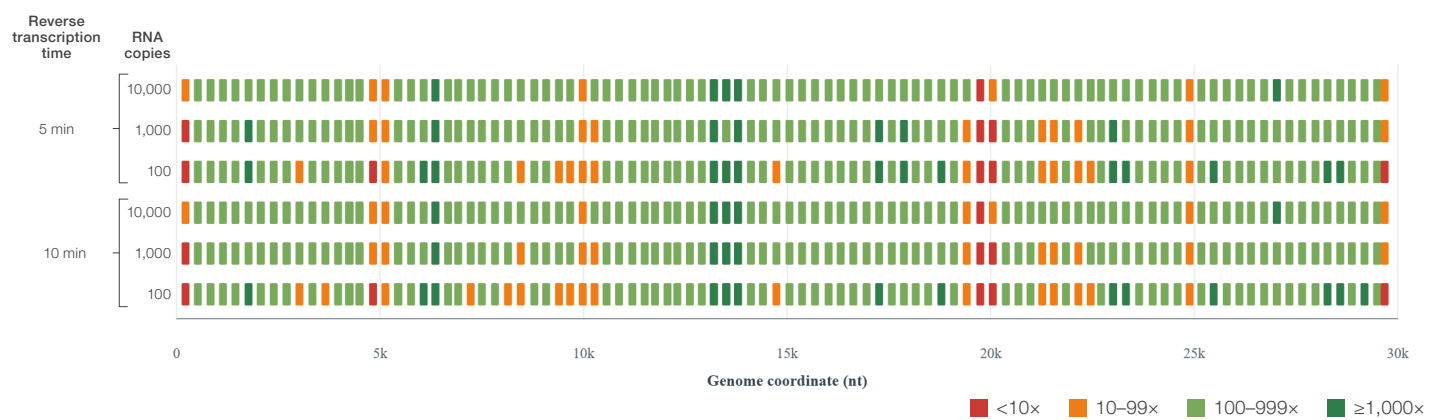


Figure 5. Shortened protocol with SuperScript V VILO Master Mix supports NGS workflows. Each row represents an individual sample; values were not averaged across samples. Coverage gaps may occur where amplicons span boundaries between the nonoverlapping ~5 kb template RNA fragments or where alternative primers that target the same region produce little or no signal.

Summary

SuperScript V products are designed for high-performance cDNA synthesis with the flexibility to reduce reverse transcription times and perform efficiently across a broad reaction temperature range. This study demonstrates detectable cDNA synthesis in 0.5–1 min for shorter transcripts. Combined with rapid genomic DNA removal and a simplified workflow, SuperScript V products enable substantial reductions in RNA-to-cDNA workflow time while maintaining robust RT-PCR and RT-qPCR performance.

Ordering information

Product	Quantity	Cat. No.
SuperScript V Reverse Transcriptase	10 reactions	19090010
	50 reactions	19090050
	200 reactions	19090200
SuperScript V VILO Master Mix	50 reactions	11800001
	500 reactions	11800002
SuperScript V VILO Master Mix, with gDNA removal	50 reactions	11800111
	500 reactions	11800112
SuperScript V VILO Master Mix, Primer-Free	50 reactions	11900001
	500 reactions	11900002
SuperScript V VILO Master Mix, Primer-Free, with gDNA removal	50 reactions	11900111
	500 reactions	11900112

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