Advanced analysis with the SuperScript IV One-Step RT-PCR System

Introduction

In one-step RT-PCR, reverse transcription (RT) of RNA and amplification of the resulting synthesized cDNA are performed in the same reaction tube. A one-step approach is often preferred over the separate two steps because it enables a faster and simpler workflow, requires fewer pipetting steps, and therefore minimizes possible contamination and improves data reproducibility.

Most one-step RT-PCR systems are based on conventional RT and PCR enzymes that offer good performance with standard RNA templates, but that often fail in more challenging applications. We developed the Invitrogen™ SuperScript™ IV One-Step RT-PCR System, which pairs the thermostable Invitrogen™ SuperScript™ IV Reverse Transcriptase with the high-fidelity Invitrogen™ Platinum™ SuperFi™ DNA Polymerase—enzymes featuring exceptionally high processivity and robustness. The combination of these two enzymes and an optimized reaction buffer offers high performance in one-step RT-PCR with standard as well as challenging RNA templates. In this study, we compare the sensitivity, specificity, inhibitor tolerance, and accuracy of the SuperScript IV One-Step RT-PCR System and the older-generation one-step RT-PCR systems based on SuperScript II and SuperScript III Reverse Transcriptases.

Materials and methods

The products used in the study included:

- SuperScript IV One-Step RT-PCR System (Cat. No. 12594025), containing SuperScript IV Reverse Transcriptase
- SuperScript One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Cat. No. 10928034), containing SuperScript II Reverse Transcriptase
- SuperScript One-Step RT-PCR System for Long Templates (Cat. No. 11922010), containing SuperScript II Reverse Transcriptase
- SuperScript III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Cat. No. 12574018), containing SuperScript III Reverse Transcriptase
- SuperScript III One-Step RT-PCR System with Platinum *Taq* High Fidelity DNA Polymerase (Cat. No. 12574030), containing SuperScript III Reverse Transcriptase

RNA samples

RT-PCR reactions were performed using total RNA from HeLa cells (commercial component of Ion Total RNA-Seq Kit v2, Cat. No. 4475936). Total RNA from human blood was isolated with the Thermo Scientific™ MagJET™ Whole Blood RNA Kit (Cat. No. K2751) and used in experiments to compare the fidelity of RT-PCR kits.

One-step RT-PCR

RT-PCR reactions were performed following the standard protocols recommended for each one-step RT-PCR system, unless stated otherwise. The final reaction volume was always 50 µL. For all experiments, gene-specific primers were used that generated amplicons of different lengths: β-actin, 353 bp; GNE, 528 bp; HTF, 1,006 bp; GNE, 1,492 bp; BF, 2,441 bp; VIN, 4,497 bp; DNCH, 7,818 bp. The temperature for the PCR annealing step was determined using a T_m calculator (**thermofisher.com/tmcalculator**). Amplified fragments were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The ladders in the Thermo Scientific™ ZipRuler™ Express DNA Ladder Set (Cat. No. SM1373) were used as size standards.

Fidelity measurements

The fidelity of different RT-PCR systems was measured by next-generation sequencing (NGS). A 1,006-base target was copied and amplified from 1 µg of total human RNA following the recommended protocols of the respective kits. The PCR fragments obtained by amplification were used to generate libraries and sequenced using the Illumina™ MySeq™ system. Collected sequences were aligned to the template, amplification errors were identified, and error rates were determined.

Results and discussion

Sensitivity

The high sensitivity of the one-step RT-PCR system enables detection of low-abundance RNA targets and allows for one-step RT-PCR experiments even when RNA input is limited. We compared the ability of the SuperScript IV One-Step RT-PCR System and other one-step RT-PCR kits to amplify targets from as little as 0.01 pg of total RNA. The highest sensitivity was demonstrated by the SuperScript IV One-Step RT-PCR System, as it produced a detectable product with 1/10 to 1/1,000 of the amount of input RNA required by other RT-PCR systems (Figure 1).

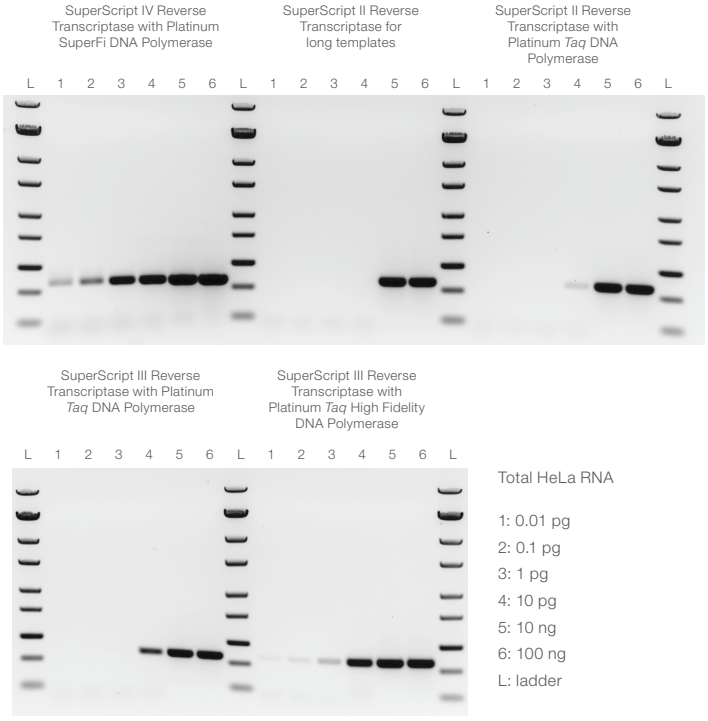

Figure 1. Detection in low amounts of input RNA. A 0.35 kb target in β-actin RNA was detected in a 0.01 pg to 100 ng range of total HeLa RNA input, using different one-step RT-PCR systems.

Specificity

To obtain accurate results, high specificity of one-step RT-PCR is critical. We compared amplification of various targets ranging from 0.3 to 7.8 kb in length using different one-step RT-PCR kits. While amplification of longer targets was challenging for some older one-step RT-PCR kits, we obtained the highest specificity, demonstrated by clean and strong bands for up to 7.8 kb in length, with the SuperScript IV One-Step RT-PCR System (Figure 2).

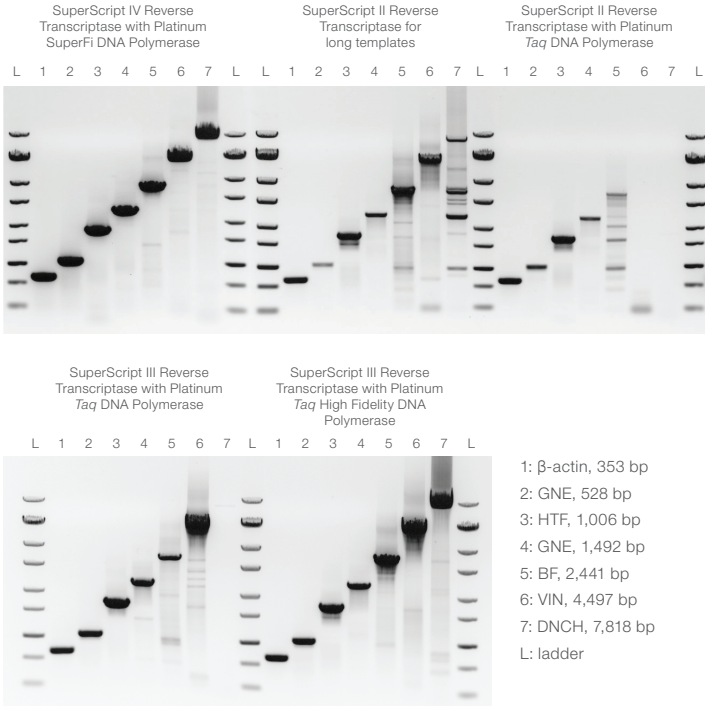

Figure 2. Versatility across a broad range of target lengths. Targets ranging from 0.3 to 7.8 kb were detected in 100 ng of total HeLa RNA using different one-step RT-PCR systems.

Inhibitor tolerance

Numerous compounds that have inhibitory effects on RT and PCR enzymes are commonly found in RNA samples, even after employing thorough purification methods. These inhibitors include compounds co-purified from biological samples or reagents used for RNA purification. The SuperScript IV One-Step RT-PCR System leverages the ability of SuperScript IV Reverse Transcriptase and Platinum SuperFi DNA Polymerase to work in the presence of impurities. In experiments to test inhibition, the SuperScript IV One-Step RT-PCR System withstands the effects of various tested inhibitors, in contrast to other one-step RT-PCR kits (Figure 3). This robustness makes the SuperScript IV One-Step RT-PCR System less dependent on RNA sample quality to achieve reliable results.

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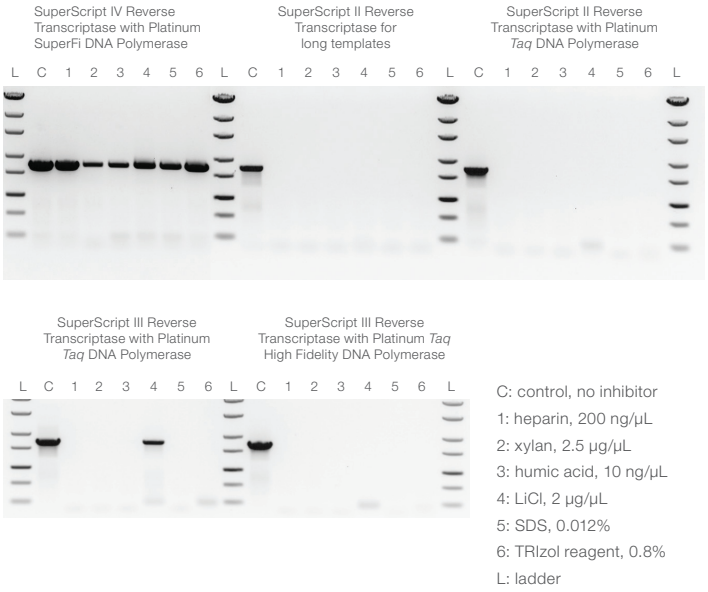

Figure 3. Resistance to inhibitors. Detection of a 1 kb RNA target from 100 ng of total HeLa RNA using the SuperScript IV One-Step RT-PCR System or other one-step RT-PCR kits in reaction mixtures containing the indicated inhibitors.

Sequence accuracy of RT-PCR product

Platinum SuperFi DNA Polymerase, used in the SuperScript IV One-Step RT-PCR System, is one of the most accurate enzymes available for DNA amplification. However, the target in RT-PCR is RNA, which has to be reverse-transcribed into cDNA prior to PCR. As reverse transcriptase enzymes possess no proofreading activity, they may introduce errors during cDNA synthesis. The high-fidelity DNA polymerase ensures that no additional errors are introduced during the subsequent PCR step.

The comparison of results confirmed that the SuperScript IV One-Step RT-PCR System provides the lowest error rate and generates the most accurate products among the tested RT-PCR kits (Figure 4).

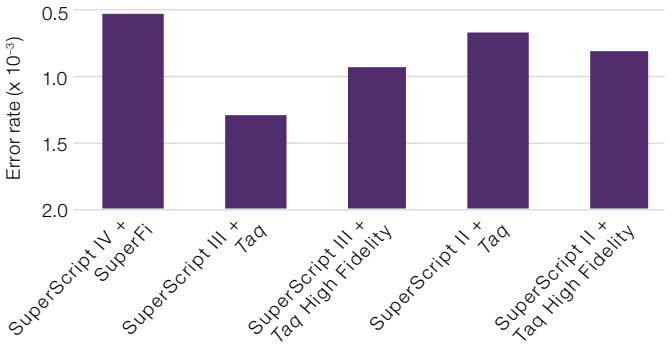

Figure 4. Fidelity in RT-PCR amplification. Target amplification was performed with different one-step RT-PCR kits. The errors in the final product were determined by NGS, and error rates (number of misincorporated nucleotides per total number of nucleotides polymerized) for the kits were determined. The error rate scale is presented in reverse order on the y-axis, so that the heights of the bars represent accuracy/fidelity.

Conclusions

As RNA detection and analysis pushes the boundaries for high sensitivity and increase in throughput, the older-generation one-step RT-PCR systems may not perform adequately to these requirements. In cases where RNA quantity is low or sample purity is compromised, the newly developed, superior SuperScript IV One-Step RT-PCR System provides the most robust and specific synthesis of RT-PCR products. The combination of SuperScript IV Reverse Transcriptase and high-fidelity Platinum SuperFi DNA Polymerase enables amplification of targets that are longer and with the least errors introduced. Researchers can confidently replace the older one-step RT-PCR kits in their current workflows with the SuperScript IV One-Step RT-PCR System.

Find out more at **thermofisher.com/ssiv-onestep**

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