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Score winning cDNA yields with SuperScript™ III RT

SuperScript™ III offers:

- Higher cDNA yields
- Higher thermal stability
- Longer half-life

… than any other RT you could use

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Announcing SuperScript™ III Reverse Transcriptase

How could we possibly improve on SuperScript™ II RT? After all, more researchers all over the world depend on SuperScript™ than any other RT. Well, we did it. Higher thermostability. Longer half-life. The one RT you trust is now even more reliable. Get full-length cDNA and higher sensitivity. Perform RT at higher temperatures and get even higher yields. Whether you're cloning, analyzing a single gene, or monitoring expression of every gene, SuperScript™ III RT will move your research forward even faster.

SuperScript™ improved

SuperScript™ III reverse transcriptase (RT)[138], a point mutant of SuperScript™ II RT[5], provides increased thermal stability and higher yields. Like M-MLV RT, it's a DNA polymerase that synthesizes a complementary DNA strand from single-stranded DNA, RNA, or an RNA:DNA hybrid. And like SuperScript™ II RT, it's engineered to be RNase H^-, delivering high yields and full-length cDNA. But compared to all other RTs, SuperScript™ III has full activity at 50°C and an incredibly long half-life of 220 minutes. You'll get even higher cDNA yields and you can work at higher temperatures for superior performance in all your RT experiments (Table 1).

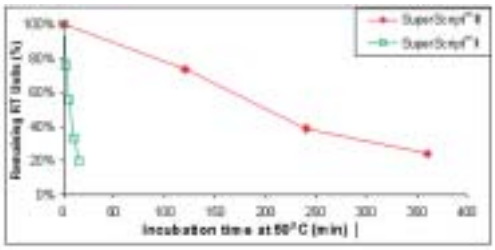
Table 1 – SuperScript™ III features and benefits

Feature	Benefit
Half-life of 220 minutes at 50°C	High cDNA yields
Thermal stability at 50°C	Reduced background with gene-specific primer Results with RNA secondary structure
RNase H negative	High cDNA yields Full-length cDNA to 12 kb Routine detection from as low as 1.0 pg total RNA
Increased units	Can increase RT units without inhibiting subsequent PCR (1). Use 1-10 µl of 20 µl RT reaction for PCR.

Increased half-life for higher yields

The longer half-life of SuperScript™ III RT gives you greater cDNA yields. At its optimal temperature of 50˚C, SuperScript™ III RT has a half-life of 220 minutes—that's 35 times longer than SuperScript™ II and 70 times longer than M-MLV RT. The longer half-life makes SuperScript™ III more robust (Figure 1) and your experiments more dynamic.

Figure 1 - Half-life of SuperScript™ III RT and SuperScript™ II RT at 50˚C

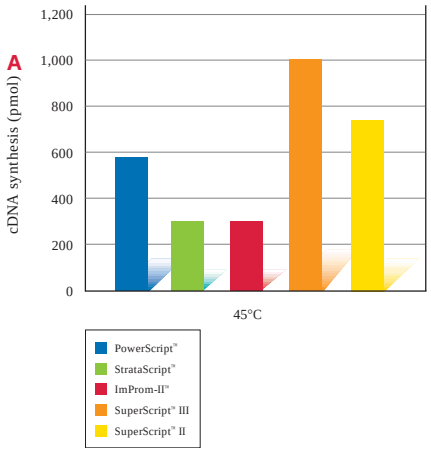


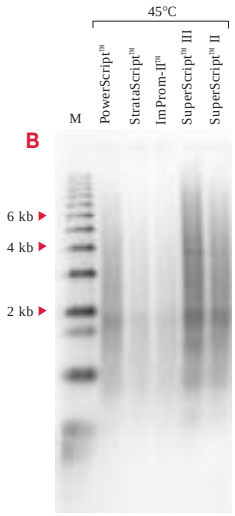
RT was incubated in 1X first-strand buffer at 50˚C. Samples were taken at time points indicated and activity measured by unit assay.

More cDNA, period

SuperScript™ III RT generates more cDNA than any other RT. This includes cDNA from total RNA or a specific gene. To demonstrate, we compared cDNA yield from various RTs (Figure 2). The results speak for themselves. It's simple: For maximized cDNA yield in library construction, array applications, or RT-PCR, use SuperScript™ III RT and make every message count.

Figure 2 – SuperScript™ III RT generates the highest cDNA yields from total RNA





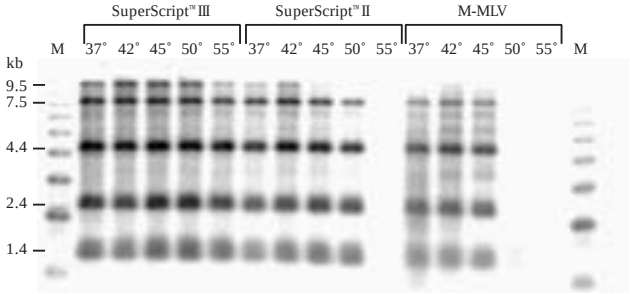
The cDNA synthesis reactions were performed in 20 µl with 20 µg HeLa total RNA, 0.5 µg of oligo(dT)$_{25}$ and 0.1 µl of α-^{32}P-dCTP (10 µCi/µl). Competitive products followed the manufacturer's recommended protocols. After the reaction mixtures were pre-incubated at 45°C for 2 min, 1 µl of each RT (400 U for SuperScript™ III) was added and incubated at 45°C for another 50 min. The reactions were stopped by adding 10 µl of 0.5 M EDTA. 5 µl of each reaction was TCA-precipitated and dried on GF/C filters.
A. The total cDNA synthesized was calculated by ^{32}P counts.
B. The rest of the samples were ethanol precipitated and electrophoresed on 1.2% agarose gel. The dried gel was exposed on the film for 1 hour.

Active at higher temperatures

SuperScript™ III is active in a wide temperature range, making it more useful than any other RT you could select (Figure 3). It's fully active at 50˚C but can be used from 45-55˚C (Figure 4). Incubation at higher temperature is important when using gene-specific primers (Figure 5) or tackling RNA with secondary structure (Figure 6).

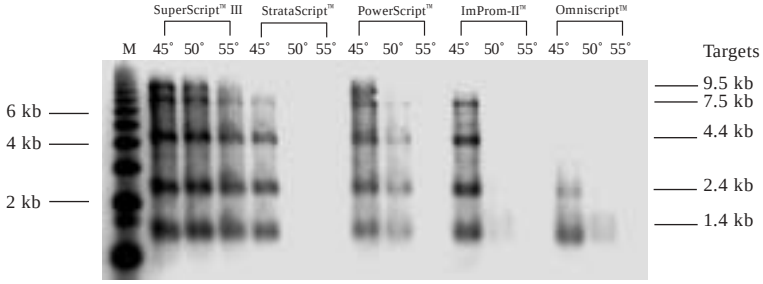
Figure 3 – More full-length, first-strand cDNA with SuperScript™ III RT

Figure 3A – SuperScript™ III compared to SuperScript™ II and M-MLV



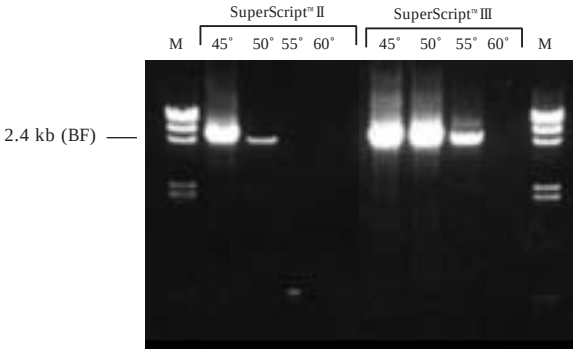
An autoradiograph is shown of ^{32}P-labeled cDNA synthesized from a mixture of 0.25 µg each of 1.35 kb, 2.4 kb, 4.4 kb, 7.5 kb, and 9.5 kb with 200 units of each RT at various temperatures. Lane M is ^{32}P-labeled 1 kb DNA ladder.

Figure 3B - SuperScript™ III outperforms other RT's



^{32}P-labeled cDNA was synthesized in 20 µl at temperatures indicated (˚C) for 50-60 min. from a mixture of 0.2 µg each of 1.4 kb, 2.4 kb, 4.4 kb, 7.5 kb, and 9.5 kb poly(A)-tailed RNA and 0.5 µg oligo(dT)$_{25}$ using buffers and components supplied with each kit. 1 µl of each RT or 400 U of SuperScript™ III was added to pre-warmed reaction mixtures (hot start). The reactions were stopped by adding 10 µl 0.5 M EDTA. After ethanol precipitation, the ^{32}P-labeled cDNA products were loaded on 1.2% alkaline agarose gel. The dried gel was exposed on film for 2 hours.

Figure 4 - Activity of SuperScript™ III RT at various temperatures

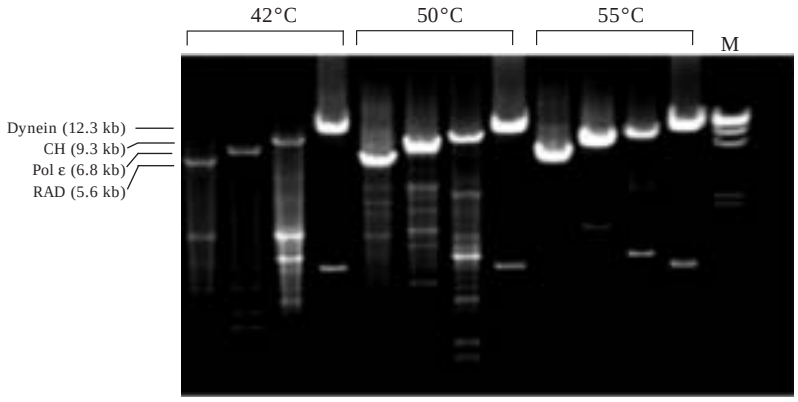


cDNA was synthesized in 20 µl for 50 minutes at temperatures indicated from 500 ng HeLa total RNA with oligo(dT) primer. 400 U SuperScript™ II or SuperScript™ III RT was added to prewarmed reaction mixtures (hot start). 2 µl of cDNA was added to 50 µl PCR reaction containing primers for 2.4 kb Human B-Factor properdin (BF) gene.

Increased priming specificity with gene specific primer

SuperScript™ III RT's activity at higher temperatures allows you to use gene-specific primers with a high T_m, increasing specificity, reducing background, and generating higher yield of your specific product. Use SuperScript™ III RT at 55˚C to achieve maximum yield with GSPs (Figure 5).

Figure 5 - Increased specificity with various genes

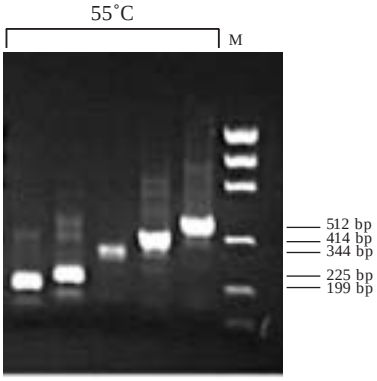


cDNA was synthesized in 20 µl from 2 µg total rat brain RNA for the Dynein 12.3 kb gene (Oligo dT_{20}) or from 1 µg total HeLa RNA for the other targets (GSP) using 200 units of SuperScript™ III, 50 min at 42°C, 50°C, or 55°C. One tenth of each reaction was amplified with Platinum® *Taq* DNA Polymerase High Fidelity.

Relax: GC-rich RNA is not a problem

High temperatures relax RNA secondary structure—temperatures where SuperScript™ III is active when other RTs aren't. Increasing the reaction temperature to 55˚C melts hairpin structures, allowing synthesis to proceed. To demonstrate, we performed RT-PCR on a region of 28S rRNA that is 84% GC-rich. SuperScript™ III RT performed brilliantly, processing through all regions with high yield and low background (Figure 6).

Figure 6 - High yields with GC-rich RNA and gene specific primer

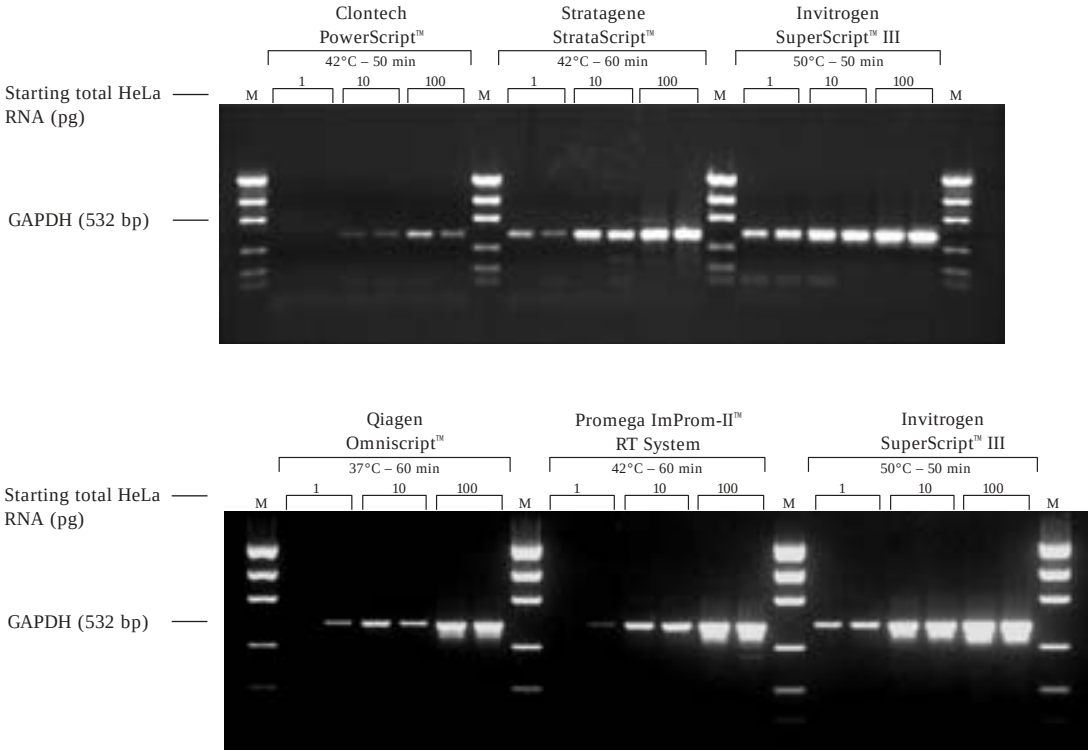


cDNA was synthesized from 100 ng HeLa total RNA with gene-specific primer (GSP) and 200 U SuperScript™ III RT in 20 µl for 50 minutes at 55˚C. 2 µl of cDNA was added to 50 µl PCR reaction containing primers for regions on 28S rRNA indicated and 1 U of Platinum® *Taq* DNA Polymerase High Fidelity, at 97˚C for 2 min., then 40 cycles, at 97˚C for 30 sec., 60˚C for 30 sec., and 68˚C for 1 min.

Routine detection in 10 pg total RNA

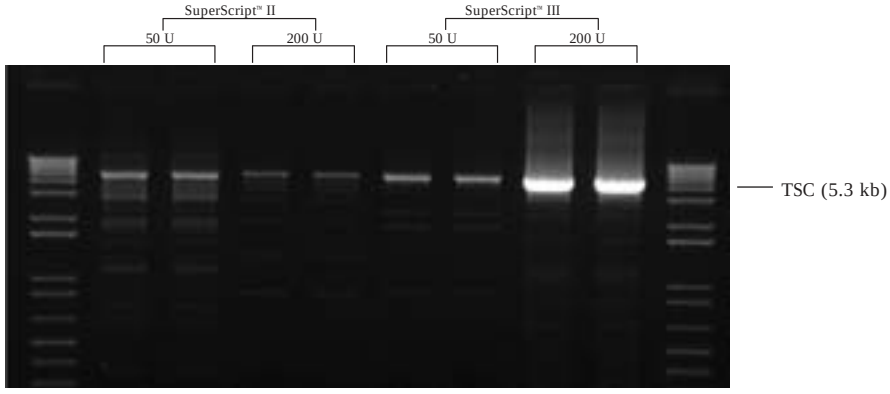
SuperScript™ III RT has high sensitivity to work with small sample sizes. You can routinely detect genes from low inputs of total RNA down to 1.0 pg. Compared to other RTs, SuperScript™ III RT not only detects genes from low input RNA, it also produces the highest yield (Figure 7). And increased units or cDNA volume is not inhibiting to PCR (Figure 8).

Figure 7 - SuperScript™ III RT generates highest yield with low input RNA



cDNA was synthesized from HeLa total RNA with oligo(dT) primer using buffer supplied with each RT and recommended conditions (400 U of SuperScript™ III RT). 10% of cDNA reaction was added to 50 µl PCR reaction containing primers for 532 bp GAPDH gene and 2 U of Platinum® *Taq* DNA Polymerase, 40 cycles, 1 min/kb.

Figure 8 - Effect of RT units and cDNA volume on PCR

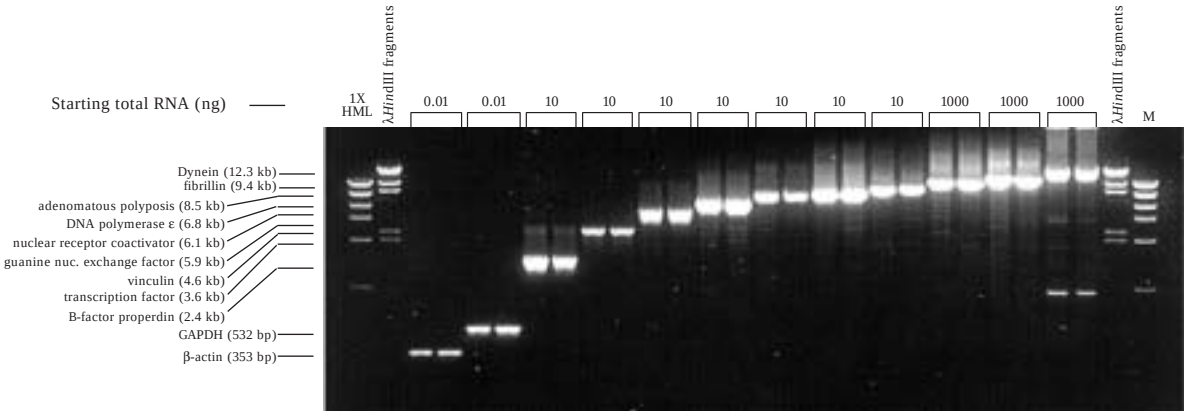


cDNA was synthesized from 100 ng total HeLa RNA with oligo(dT) primers using SuperScript™ II at 45˚C or SuperScript™ III at 50˚C. One third (7 µl) of the 20 µl reaction was amplified in 50 µl with 1 unit of Platinum® *Taq* DNA Polymerase High Fidelity at 94˚C for 2 min, then 35 cycles at 94˚C for 15 sec, 55˚C for 30 sec, and 68˚C for 6 min. PCR was performed with primers for human tuberous sclerosis (TSC 5.3 kb).

Full-length cDNA, high yields

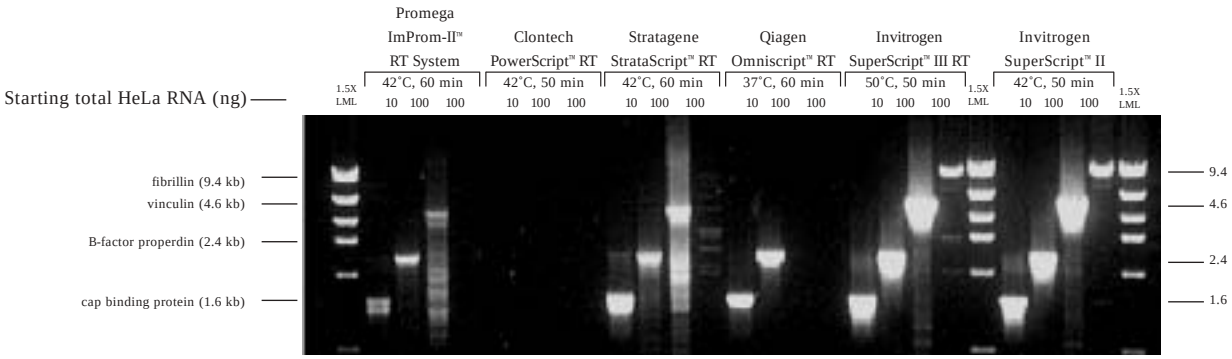
Generate full-length cDNA with high yields from mimimal starting material for gene analysis or cloning with SuperScript™ III (Figure 9). At 200 U/µl, you can set up reactions for high-throughput or increase units to increase yield. Compared to other RTs, SuperScript™ III RT not only generates the highest yields, but it was also able to generate the 9.4 kb fibrillin target (Figure 10).

Figure 9 - SuperScript™ III RT generates highest yield with various-sized targets



cDNA was synthesized from HeLa total RNA or rat total RNA for Dynein with oligo(dT) primer using 400 U of SuperScript™ III RT at 50°C. 10% of cDNA reaction was added to 50 µl PCR reaction containing primers for each gene and 2 U of Platinum® *Taq* DNA Polymerase or 1 U of Platinum® *Taq* DNA Polymerase High Fidelity, 35 or 40 cycles, 1 min/kb.

Figure 10 - SuperScript™ III RT outperforms the competition



cDNA was synthesized from 10 ng HeLa total RNA for cap binding protein (1.6 kb) or 100 ng HeLa total RNA for B-factor properdin (2.4 kb), vinculin (4.6 kb), and fibrillin (9.4 kb) with oligo(dT) primer using buffer and recommended conditions supplied with each RT (400 U of SuperScript™ III RT). 10% of the cDNA reaction was added to a 50 µl PCR reaction containing primers for each gene and 2 U Platinum® *Taq* DNA Polymerase or 1 U Platinum® *Taq* DNA Polymerase High Fidelity, 35 cycles, 1 min/kb.

Easily optimize reaction conditions

You can optimize SuperScript™ III RT for specific applications to ensure the best results. We provide SuperScript™ III at 200 U/µl to allow flexibility in experimental design (Table 2).

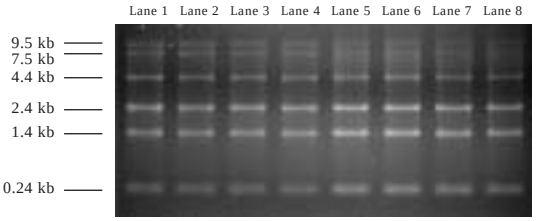
Table 2 – SuperScript™ III RT specifications

Units	To increase yields, use 2 µl, or 400 U. Increasing units does not inhibit subsequent PCR as with other RNase H minus RTs. Use 400 U when synthesizing long targets (≥5 kb) at high temperature (≥ 55˚C).
cDNA Volume	1-10 µl of cDNA reaction can be used in 50 µl PCR reaction.
Temperature	Even though SuperScript™ III RT is fully active at 50˚C, you can obtain good results at 45˚C. You should increase the temperature to 55˚C when using a gene-specific primer or working with highly structured RNA.
Buffer	SuperScript™ III RT uses the same 5X first-strand buffer as SuperScript™ II RT, making it easy to switch. Buffer formulation: [250 mM Tris-HCl (pH 8.3), 375 mM KCl,15 mM $MgCl_2$]
Storage	As a single polypeptide, SuperScript™ III is very stable. It is shipped on dry ice to ensure full activity upon arrival. While SuperScript™ III RT is stable at –80˚C and can be kept at 4°C overnight without activity loss, we recommend storage at –20˚C.

Stringent quality control to ensure your results

Each lot of SuperScript™ III RT is stringently quality controlled. Tests include SDS-PAGE to analyze purity; contamination assays to confirm absence of endodeoxyribonuclease, 3´ and 5´ exodeoxyribonuclease, and ribonuclease activities (Figure 11); and cDNA synthesis of 7.5-kb target to meet yield and length criteria.

Figure 11 - SuperScript™ III RT is RNase-free



5 µg of 0.24-9.5 kb RNA ladder was incubated with 1000 units of SuperScript™ II and III at 37˚C for 30 min followed by phenol extraction, then analyzed on a 1.25% formaldehyde/agarose/EtBr gel. Lane 1 and 2: RNA Ladder with RNase-free water kept on ice for 30 min. Lane 3 and 4: RNA ladder with SuperScript™ storage buffer incubated at 37˚C for 30 min. Lane 5 and 6: RNA ladder with 1000 units SuperScript™ II incubated at 37˚C for 30 min followed by phenol extraction. Lane 7 and 8: RNA ladder with 1000 units SuperScript™ III incubated at 37˚C for 30 min followed by phenol extraction.

Try SuperScript™ III today

Maximize cDNA yield, increase specificity, and enhance the results in all your RT experiments. Call Invitrogen and order SuperScript™ III RT today.

Product	Quantity	Cat. no.
SuperScript™ III Reverse Transcriptase	10,000 U	18080-044

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Reference: 1. Huang, L. *et al.* (2000) Focus® **22**:6.

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