



High performance RT for reliability in every experiment

To achieve the best results in your reverse transcriptase applications, you need the best reverse transcriptase. SuperScript™ II reverse transcriptase delivers the highest cDNA yields, the most full-length, and the greatest sensitivity of any RT enzyme. Combine SuperScript™ II RT with your choice of optimized buffers, protocols, and kits to get high performance and consistent reliability in a range of applications.

High yields of full-length cDNA

SuperScript™ II reverse transcriptase⁵ is a point mutant of Moloney murine leukemia virus RT (M-MLV RT) engineered to be RNase H⁻. Like M-MLV, it is a DNA polymerase that synthesizes a complementary DNA strand from single-

stranded DNA, RNA, or a RNA:DNA hybrid. But compared with wild type RTs, SuperScript™ II delivers higher yields of full-length cDNA—superior performance that enhances results in all your experiments.



Eliminating RNase H activity is the key

In wild type RTs such as M-MLV and AMV, RNase H activity competes with 5'→3' DNA polymerase activity (Table 1) for the hybrid formed between the RNA template and the DNA primer. This competition inhibits the elongation of template-primer complex, resulting in lower yields of cDNA (Figure 1). RNase H activity also acts to diminish the yield and reduce the size of the cDNA by hydrolyzing

the RNA template as chain growth occurs. The elimination of RNase H activity allows higher yields and significantly greater full-length cDNA (1). You'll typically achieve > 50% more full-length cDNA synthesis and substantially greater yields of first-strand synthesis than obtained with AMV RT or M-MLV RT.

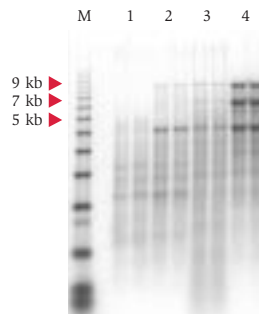
Table 1 - Reverse transcriptase activities

Reverse Transcriptase Activities		
Enzyme	DNA Polymerase	RNase H*
AMV RT	46,000	494
M-MLV RT	350,000	30
SuperScript™ II RT	320,000	not detected

Relative activities of RTs. Numbers represent units/mg. A unit is a nmole substrate incorporated or nmole substrate solubilized in 10 min. Both polymerase and RNase H activities listed were determined with MgCl₂ (as used in first-strand cDNA synthesis).

* For RNase H activity, the substrate used was [³H](A)n•(dT)n. For AMV RT, optimal unit reaction conditions use MgCl₂. For M-MLV RT, optimal unit reaction conditions use MnCl₂ (not MgCl₂). With MnCl₂, M-MLV RT has an RNase H activity of 2,670 and SuperScript™ II RT has an activity of 0.0018.

Figure 1 - More full-length, first-strand cDNA with SuperScript™ II



cDNA was synthesized in duplicate according to manufacturers' instructions from a mixture of 0.5 µg each of MAP-4 mRNA (5.2 kb), MAP-4/APC hybrid mRNA (9.5 kb), and 7.5 kb mRNA. Reactions were incubated at recommended temperatures.

Lanes 1: Supplier Q1
Lanes 2: Supplier Q2
Lanes 3: Supplier I
Lanes 4: SuperScript™ II

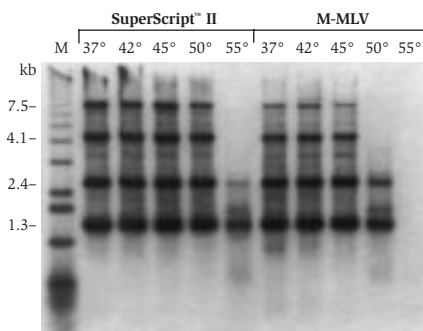
Higher thermostability for enhanced synthesis

The point mutations leading to inactivity of the RNase H domain in SuperScript™ II RT also improve the temperature stability, allowing first-strand synthesis at temperatures as high as 50°C (Figure 2).

Reverse transcription at higher temperatures improves

cDNA synthesis from RNA that has high GC content or secondary structure, or both. With higher thermostability than wild type RTs, SuperScript™ II lets you meet these challenges—while giving you the high yield and full length you need.

Figure 2 - Effect of incubation temperature on RT performance

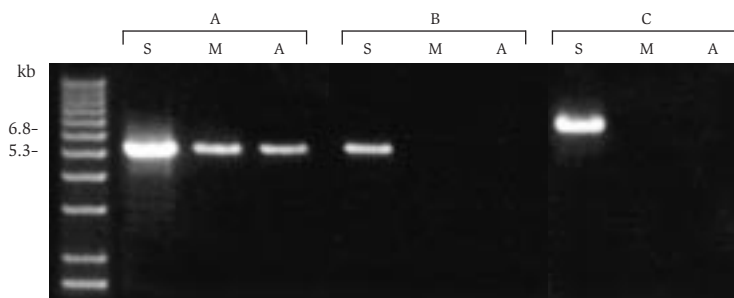


An autoradiograph is shown of ³²P-labeled cDNA synthesized from a mixture of 0.25 µg each of 1.3-kb, 2.4-kb, 4.1-kb, and 7.5-kb RNA with 200 units of each RT at various temperatures.

Superior performance, even in long RT-PCR

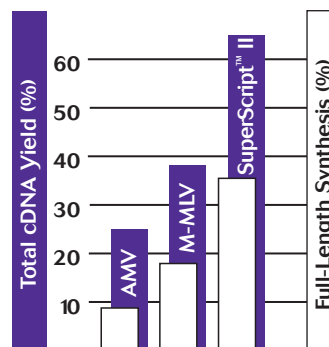
The superior performance of SuperScript™ II extends to long RT-PCR. Although other RTs are able to synthesize full-length cDNA from long transcripts, SuperScript™ II RT outperforms them. First, more RNA is converted to cDNA, and second, significantly more of the cDNA synthesized is full length (Figure 3). The increase in the amount of full-length transcript generated can have a marked effect on the yield of long (> 5 kb) RT-PCR products (2,3). As Figure 4 shows, only SuperScript™ II RT-derived cDNA yielded a signal for the 6.8-kb transcript.

Figure 4 – Comparison of RTs for long RT-PCR



A comparison of RTs for long RT-PCR. Synthesis of cDNA was catalyzed by SuperScript™ II RT (S), M-MLV RT (M), or AMV RT (A). All samples were treated with RNase H. An amount equivalent to 0.5% of the starting RNA was amplified with Elongase® Enzyme Mix. (A) 5 µg total HeLa RNA for TSC 5.3 kb; (B) 1 µg total HeLa RNA for TSC 5.3 kb; (C) 5 µg total HeLa RNA for Pol ε 6.8 kb.

Figure 3 - Comparison of RTs for long RT-PCR



Comparison of first-strand cDNA synthesis. Each RT was used in the amount (10 units AMV RT and 200 units other RTs) and under conditions determined optimal for each enzyme using 1 µg of a 7.5-kb RNA template. Total yield of first-strand cDNA (■) was calculated from TCA precipitations analysis. Full-length synthesis (□) was determined following electrophoresis on an alkaline agarose gel and counting and the full-length band and the rest of the lane. The counts of the full-length band were divided by total counts from the lane and multiplied by 100%.

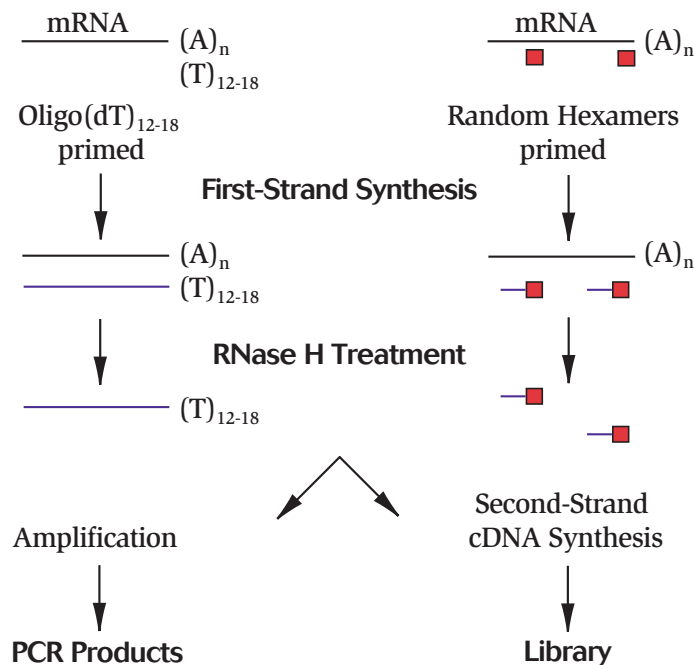
Multiple kit formats, greater options

With SuperScript™ II RT, you have the ideal tool to prepare cDNA copies of mRNA for both polymerase chain reaction (PCR) or cDNA library construction (Figure 5). SuperScript™ II RT is available in the following formats:

- *Stand-alone enzyme* – reliable first-strand synthesis with flexibility for any protocol
- *First-strand synthesis kit* – pre-qualified, optimized reagents for the best first-strand synthesis, combined with flexibility in choosing a PCR enzyme
- *One-step RT-PCR kits* – convenient, single-tube RT-PCR for the highest sensitivity, suitable for higher throughput applications
- *Double-strand synthesis kit* – high-quality cDNA with flexibility for any protocol or cloning vector
- *Library construction kits* – complete kits for high-quality libraries

Whatever format you choose, you'll get the high yield and full length you need for all your downstream experiments.

Figure 5 – Process of first-strand synthesis to PCR or double-strand synthesis

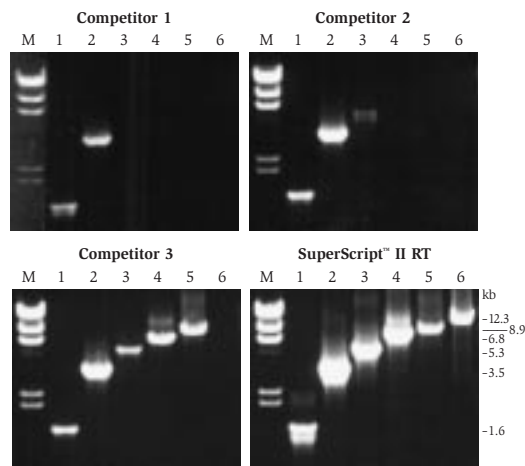


Complete kit for high-quality cDNA, every time

The SuperScript™ First-Strand Synthesis System for RT-PCR^{4,5} is a complete kit optimized to generate high-quality first-strand cDNA from total or poly(A)⁺ RNA. Use the kit in conjunction with PCR to detect the presence of rare messages, quantify specific mRNA from small samples, or clone specific cDNA without cDNA library construction. With this format,

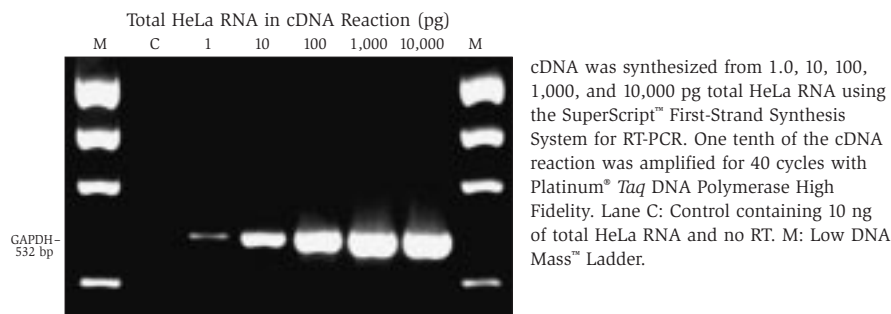
you can choose the PCR enzyme best suited for your application. You'll get consistently high first-strand yields and full-length cDNA up to 12 kb (Figure 6) as well as exceptional sensitivity. Detect as little as 50 to 100 molecules of RNA template from samples as small as 1.0 pg total RNA (Figure 7).

Figure 6 - Comparison of yield and length



cDNA was synthesized from 10 ng total HeLa RNA for CBP 1.6 kb, 100 ng total HeLa RNA for Pol ϵ 3.5 kb and 6.8 kb and TSC 5.3 kb, 0.5 μ g total HeLa RNA for APC 8.9 kb, and 2 μ g total rat brain RNA for Dynein 12.3 kb. One-tenth of the cDNA reaction was used for 35 cycles of PCR with Platinum® *Taq* DNA Polymerase High Fidelity. Lane M: λ DNA/*Hind* III Fragments.

Figure 7 - Sensitivity



cDNA was synthesized from 1.0, 10, 100, 1,000, and 10,000 pg total HeLa RNA using the SuperScript™ First-Strand Synthesis System for RT-PCR. One-tenth of the cDNA reaction was amplified for 40 cycles with Platinum® *Taq* DNA Polymerase High Fidelity. Lane C: Control containing 10 ng of total HeLa RNA and no RT. M: Low DNA Mass™ Ladder.

One-step kits for unrivaled sensitivity

High performance. Convenient set-up. With SuperScript™ One-Step RT-PCR Systems ^{1,2,5,14}, you get both. These easy-to-use kits combine SuperScript™ II RT with Platinum® automatic hot-start PCR technology to deliver optimal RT-PCR results. Add total or poly(A)⁺ RNA to a pre-mixture of RT and PCR enzymes in a single tube, and you'll get the high yield, sensitivity, and specificity required for successful gene expression analysis. Two SuperScript™ One-Step RT-PCR Systems are available, both with streamlined protocols:

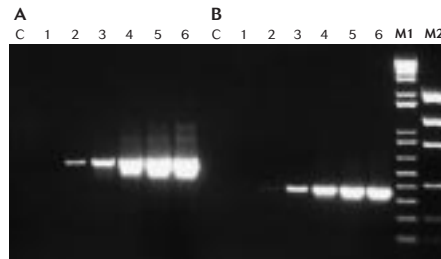
- *SuperScript™ One-Step RT-PCR System with Platinum® Taq DNA Polymerase.* For fast and easy screening of gene expression, detection of rare RNA and viral RNA, and

quantification of specific messages. You'll detect as little as 5 to 10 molecules of RNA template from as little as 0.1 pg of total RNA (Figure 8).

- *SuperScript™ One-Step RT-PCR System for Long Templates.* Made with Platinum® Taq DNA Polymerase High Fidelity, allowing you to amplify RNA targets up to 9 kb (Figure 9). It's the ideal kit for cloning and expression of your RT-PCR products.

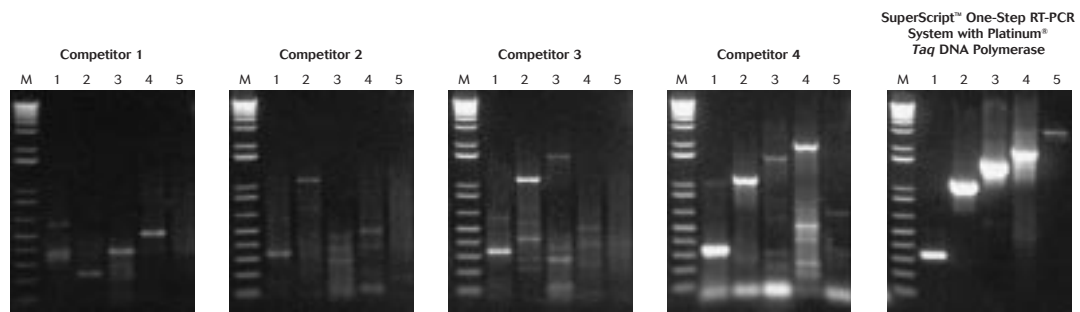
With SuperScript™ One-Step Systems, you get a single-tube format to reduce reaction variability and lower risk of contamination. The improved reproducibility and rapid protocol facilitate higher throughput applications.

Figure 8 - Increased sensitivity with SuperScript™ One-Step RT-PCR System with Platinum® Taq DNA Polymerase.



RT-PCR reactions contained 0.01, 0.1, 1.0, 10, 100, and 1,000 pg total HeLa RNA (lanes 1–6, respectively) and gene specific primers to detect GAPDH 532 bp (Panel A) or β -actin 353 bp (Panel B). Lane C: Control. Lane M1: 1 Kb Plus DNA Ladder™. Lane M2: Low DNA Mass™ Ladder.

Figure 9 - Comparison of RT-PCR products, ranging in fragment size, from various RT-PCR systems.



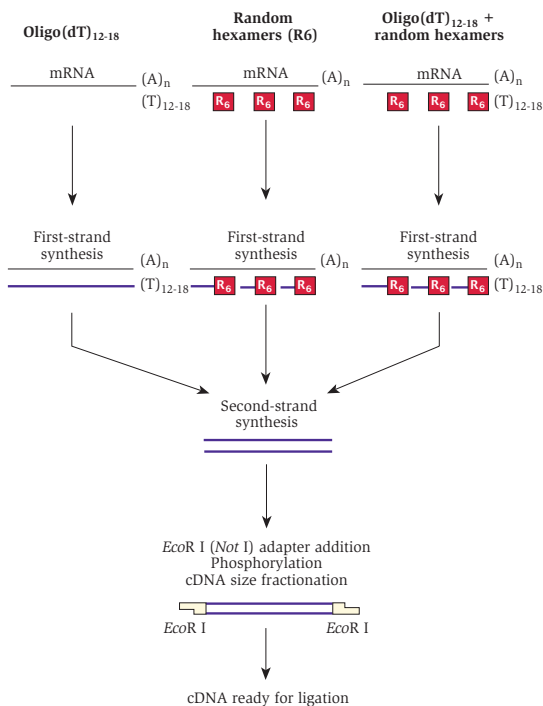
Five different targets were amplified from 100 ng total HeLa RNA: Pol ϵ 338 bp, PP2A 1,093 bp, CBP 1,590 bp, RPA 2,057 bp, and Pol ϵ 2,980 bp (lanes 1–5 respectively). All reactions were run using the manufacturer's recommended reaction and cycling conditions. Lane M: 1 Kb Plus DNA Ladder™.

High-quality library construction

A winning strategy depends on a solid foundation. SuperScript™ II delivers the superior performance you need to construct high-quality cDNA libraries. With a choice of three formats, you're sure to find the optimal system for your needs:

- *SuperScript™ Choice System*⁵. Generate ready-to-clone double-stranded cDNA from a purified mRNA population (Figure 10). This complete kit contains everything you need, including a detailed protocol, to ensure you construct a high-quality library the first time.
- *SuperScript™ Plasmid Systems with Gateway™ Technology*^{5,19,45}. These systems include specialized vectors that enable you to construct Gateway™-compatible cDNA libraries with large numbers of full-length clones. For more information about the power of Gateway™ Technology, please visit our web site at www.invitrogen.com.
- *SuperScript™ Double-Stranded cDNA Synthesis Kit*⁵. Synthesize high-quality double-stranded cDNA from total or poly(A)⁺ RNA. Supply your RNA primer, adaptors, purification columns, cloning vector, and competent cells, for maximum flexibility in library design.

Figure 10 - Outline of procedure of SuperScript™ Choice System



Advanced RACE kit for full-length genes

The GeneRacer™ RLM RACE kit uses SuperScript™ II RT and an RNA ligase-mediated method to capture the full-length 5' end from long and complex mRNA. For more information, please visit our web site at www.invitrogen.com.

Use only the best RT

Why take needless risks with your samples? Capture the high performance and consistent reliability that only SuperScript™ II reverse transcriptase can deliver—and set the stage for success in all your downstream experiments.

Product	Quantity	Cat. no.	
SuperScript™ II RT	2,000 U	18064-022	
	10,000 U	18064-014	
	4 x 10,000 U	18064-071	
SuperScript™ First-Strand Synthesis System	50 rxns	11904-018	
SuperScript™ One-Step RT-PCR System <i>with Platinum® Taq DNA Polymerase</i>	25 rxns	10928-034	
	100 rxns	10928-042	
SuperScript™ One-Step RT-PCR System for Long Templates <i>with Platinum® Taq DNA Polymerase High Fidelity</i>	25 rxns	11922-010	
	100 rxns	11922-028	
SuperScript™ Double-Stranded cDNA Synthesis Kit	10 rxns	11917-010	
SuperScript™ Choice System for cDNA Synthesis	3 rxns	18090-019	
SuperScript™ Plasmid System for cDNA Synthesis and Plasmid Cloning with Gateway™ Technology			
	<i>with UltraMAX™ DH5α-FT™ Competent Cells</i>	3 rxns	18248-013
	<i>with MAX Efficiency® DH10B™ Competent Cells</i>	1 kit	18248-047
	<i>with MAX Efficiency® DH5α™ Competent Cells</i>	1 kit	19613-017
	<i>with ElectroMAX™ DH10B™ Competent Cells</i>	1 kit	19617-018
	1 kit	19625-011	
GeneRacer™ Kit <i>with SuperScript™ II RT and TOPO TA Cloning® Kit for Sequencing</i>	1 kit	L1502-01	

References:

1. Gerard, G. *et al.* (1989) *Focus*® 11:66.
2. Westfall, B. *et al.* (1995) *Focus*® 17:62.
3. Nathan, M. *et al.* (1995) *Focus*® 17:78.

^{1,2,4,5,14,19,45} Products mentioned above are subject to the Limited Use Label License indicated by the superscript numbers. Please refer to the Invitrogen web site or catalog for the Limited Use Label License corresponding to the numbers indicated.



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