# Be SMART™ About First-Strand cDNA Synthesis

SMARTScribe™ Reverse Transcriptase—the RT of choice for all of our SMART Kits

- **Synthesize long, full-length cDNA**
- **Amplify rare transcripts**
- **Maintain the complexity of the original RNA**
- **Robust system with consistent performance**

Reliable reverse transcription requires a quality reverse transcriptase (RT) that can efficiently generate full-length, first-strand cDNA from a variety of RNA transcripts. **SMARTScribe Reverse Transcriptase** is a high-performance enzyme that allows unbiased cDNA synthesis and library construction from any RNA transcript. SMARTScribe RT is a modified Moloney Murine Leukemia Virus Reverse Transcriptase that generates long, full-length cDNA and amplifies rare transcripts, while preserving the relative transcript proportions of the original RNA sample. It is the ideal RT to use with all of our SMART Kits (1).

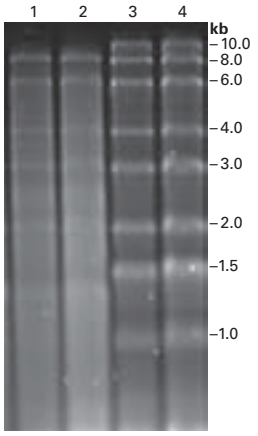


**Figure 1. SMARTScribe RT generates long, first-strand cDNA.** SMART MMLV RT (Lanes 1 & 2) and SMARTScribe RT (Lanes 3 & 4) were each used to synthesize first-strand cDNA using a polyA⁺ RNA ladder as template. The samples were analyzed by denaturing agarose gel electrophoresis. SMARTScribe was able to generate large amounts of single-stranded cDNA from RNA of all sizes, including the 10 kb transcripts.

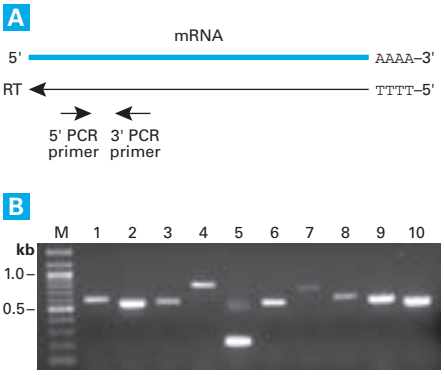


**Figure 2. SMARTScribe RT synthesizes long, full-length cDNA.** SMARTScribe was used to reverse transcribe first-strand cDNA from Human Universal Reference Total RNA using oligo(dT)$_{18}$ primers (**Panel A**). The resulting single-stranded cDNA was then analyzed by PCR using primers that generated 200–800 bp amplicons from the 5' ends of 10 genes (**Panel B**, Lanes 1–10). See Table I for information on the genes analyzed and the size of the amplicon generated from each; the lane numbers correspond to the sample number in the table. The successful generation of each amplicon indicates that SMARTScribe RT was able to synthesize single-stranded cDNA transcripts as long as 14.7 kb (from LRP2 mRNA; Lane 10). Lane M: 100 bp DNA size marker.

**Table I: Genes Analyzed by RT-PCR and the Minimum Lengths of the cDNA Transcripts Generated**

| Sample | Accession No. | Gene Name | Amplicon Size (bp) | Minimum Length of cDNA Transcript (bp) |
|---|---|---|---|---|
| 1 | NM_002223 | Human inositol 1,4,5-triphosphate receptor, type 2 (ITPR2) | 599 | 11,607 |
| 2 | NM_000267 | Human neurofibromin 1 (NF1) | 554 | 11,739 |
| 3 | NM_001408 | Human cadherin, EGF LAG seven-pass G-type receptor 3 (CELSR3) | 572 | 10,057 |
| 4 | NM_057164 | Human collagen, type VI, alpha 3 (COL6A3) | 811 | 9,122 |
| 5 | NM_015092 | Human PI-3-kinase-related kinase SMG-1 (SMG1) | 224 | 13,007 |
| 6 | NM_000426 | Human laminin, alpha 2 (LAMA2) | 597 | 8,598 |
| 7 | NM_000059 | Human breast cancer 2, early onset (BRCA2) | 792 | 10,158 |
| 8 | NM_004010 | Human dystrophin (DMD) | 644 | 13,166 |
| 9 | NM_002332 | Human low density lipoprotein-related protein 1 (LRP1) | 598 | 13,432 |
| 10 | NM_004525 | Human low density lipoprotein-related protein 2 (LRP2) | 563 | 14,652 |

# Be SMART™ About First-Strand cDNA Synthesis...continued

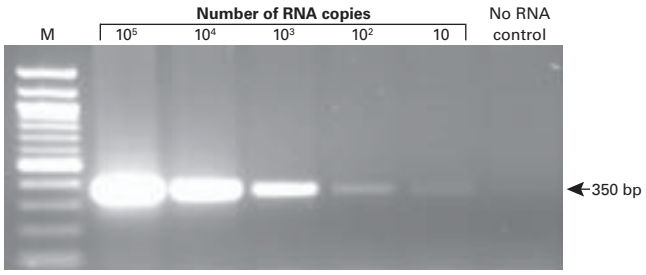


**Figure 3. SMARTScribe RT exhibits exceptional sensitivity.** RT-PCR assays were performed using SMARTScribe RT and synthetic RNA. The synthetic RNA was serially diluted 10X to obtain $10^5$–10 copies per sample; diluted template was then spiked into 50 ng of total RNA from HeLa cells to increase complexity. First-strand cDNA was synthesized with SMARTScribe RT, then amplified with **Advantage® 2 DNA Polymerase Mix** in 36 cycles of PCR using primers specific for a 350 bp amplicon. The PCR reactions were then analyzed by agarose gel electrophoresis. SMARTScribe was able to generate single-stranded cDNA from as few as 10 copies of synthetic RNA. Lane M: 100 bp DNA size marker.

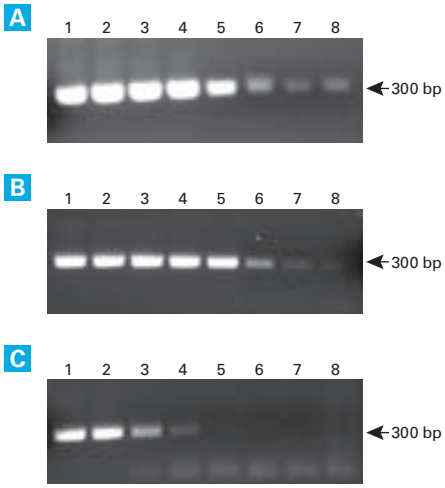


**Figure 4. SMARTScribe RT is more sensitive than the competition.** Human Universal Total RNA was serially diluted 10X (from 1 µg to 0.1 pg), then reverse transcribed using either SMARTScribe RT (**Panel A**), Competitor P (**Panel B**) or Competitor Q (**Panel C**). Each RT reaction was performed according to the enzyme manufacturer's specifications. One microliter of each RT reaction was then used in PCR reactions with primers specific for a 300 bp portion of the 5' end of the β-actin gene. The samples were analyzed by agarose gel electrophoresis. The amount of template RNA used in each reaction is as follows: Lane 1: 1 µg. Lane 2: 100 ng. Lane 3: 10 ng. Lane 4: 1 ng. Lane 5: 100 pg. Lane 6: 10 pg. Lane 7: 1 pg. Lane 8: 0.1 pg. SMARTScribe RT exhibited superior sensitivity at all of the template concentrations tested.

## Generate Long, Full-Length cDNA

SMARTScribe RT contains modifications that allow optimal reverse transcription of virtually any RNA, including rare and long transcripts (Figure 1). In addition, the enzyme is subject to the same proprietary purification process as **SMART MMLV RT.** This ensures that SMARTScribe preparations are exceptionally pure, with all contaminating nucleases removed.

SMARTScribe's ability to synthesize long, full-length cDNA was demonstrated in reverse transcription PCR assays (RT-PCR; Figure 2) in which SMARTScribe RT was used to generate first-strand cDNA from **Human Universal Reference Total RNA.** The resulting single-stranded cDNA was then analyzed via PCR reactions that generated short (200–800 bp) amplicons from the 5' region of each of the genes listed in Table I (Figure 2, Panels A and B). The successful generation of these amplicons demonstrates that SMARTScribe RT was able to synthesize single-stranded cDNA transcripts of up to 14.7 kb (Table I; Figure 2, Panel B, Lane 10). The ability to produce long, high-quality cDNA makes SMARTScribe RT the enzyme of choice for all applications requiring long, full-length, first-strand cDNA.

## Amplify Rare or Low Copy Transcripts

SMARTScribe RT exhibits exceptional sensitivity, which results in maximum amounts of first-strand cDNA regardless of template size or abundance. As a result, rare or precious RNA samples can be preserved. This was demonstrated in RT-PCR assays, where SMARTScribe RT was used to generate first-strand cDNA from either synthetic RNA template (Figure 3) or Human Universal Reference Total RNA (Figure 4, Panel A). The resulting single-stranded transcripts were amplified by PCR and visualized on agarose gels. SMARTScribe was able to synthesize single-stranded cDNA from as few as 10 copies of synthetic RNA (Figure 3), and as little as 0.1 pg of total RNA (Figure 4, Panel A, Lane 8).

## More Sensitive than the Competition

We compared the sensitivity of SMARTScribe RT with that of two competitor enzymes in the RT-PCR assay in Figure 4. In this assay, each enzyme was used to reverse transcribe Human Universal Reference Total RNA that had been serially diluted 10X from 1 µg to 0.1 pg. The resulting single-stranded cDNA was then used in PCR assays to generate a 300 bp amplicon from the 5' end of β-actin cDNA. The samples were analyzed by agarose gel electrophoresis and compared. SMARTScribe RT (Figure 4, Panel A) exhibited superior sensitivity down to 0.1 pg total RNA—the lowest RNA concentration used.

# Be SMART™ About First-Strand cDNA Synthesis...continued

## perfect for all SMART Applications

SMARTScribe RT is the ideal enzyme for the most demanding SMART applications, and has been formulated specifically for use with all of our SMART Kits. For the assay in Figure 5, SMARTScribe was used with our **SMART RACE cDNA Amplification Kit** to amplify the 5' region of the transferrin receptor (TFR) gene from **Human Placenta Total RNA** (Figure 5). This kit contains a specially designed SMART Oligo that increases the likelihood of cloning entire gene sequences or upstream regulatory regions. Together, SMARTScribe RT and the SMART RACE cDNA Amplification Kit were able to amplify TFR cDNA from as little as 0.5 ng total RNA (Figure 5, Lane 3).

## which Clontech RT should I use?

Clontech now offers two RTs—SMART MMLV and SMARTScribe:

- Both are highly purified enzymes with all contaminating nucleases removed.
- *SMARTScribe RT* has been engineered to provide the best performance when amplifying rare or long transcripts, or in applications where it is important to faithfully maintain the proportions of the original RNA transcripts. For this reason, we recommend the use of SMARTScribe RT with more demanding SMART Kit applications.

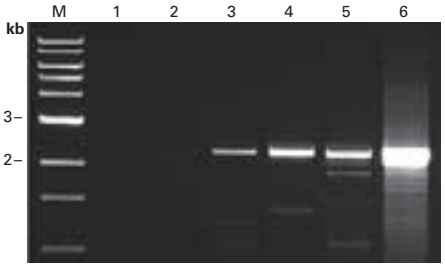


**Figure 5. SMARTScribe is specially formulated to complement all of our SMART Kits.** SMARTScribe was used in conjunction with our SMART RACE cDNA Amplification Kit to amplify transferrin receptor (TFR, 2.6 kb) cDNA from Human Placenta Total RNA. Template RNA was serially diluted 2X to obtain 0.25 ng–2 ng template per sample (Lanes 2–5, respectively). Control samples containing no template (Lane 1) and 50 ng template (Lane 6) were also used. Together, SMARTScribe and the SMART RACE cDNA Amplification Kit were able to amplify TFR cDNA from as little as 0.5 ng total RNA (Lane 3). Lane M: 1 kb ladder DNA size marker.

- *SMART MMLV RT* has been used with our SMART Kits by many scientists who have reported excellent results; it is an excellent enzyme for less demanding SMART applications. SMART MMLV RT has also been shown to provide higher yields for quantitative reverse transcription PCR (qRT-PCR) than do other enzymes.

In general, when performance is critical, we recommend using SMARTScribe RT with our SMART Kits, and SMART MMLV RT for qRT-PCR. However, this is not a definitive recommendation, as the enzymes may perform differently under the conditions required for a particular application.

SMARTScribe RT is the ideal high-performance reverse transcriptase for unbiased cDNA synthesis, mRNA amplification, or library construction from any RNA transcript. Try it by itself, or as the perfect complement to any of our SMART Kits.

| Product | Size | Cat. No. |
|---|---|---|
| SMARTScribe Reverse Transcriptase | | |
| | 40 rxns | 639536 |
| | 100 rxns | 639537 |
| | 400 rxns | 639538 |
| SMART MMLV Reverse Transcriptase | | |
| | 8,000 units | 639523 |
| | 20,000 units | 639524 |

NEW!

**Components**

- SMARTScribe™ Reverse Transcriptase
- 20 mM DTT
- 5X First-Strand Buffer

**Related Products**

- SMART™ RACE cDNA Amplification Kit (Cat. No. 634914)
- SMART™ PCR cDNA Synthesis Kit (Cat. No. 634902)
- Super SMART™ PCR cDNA Synthesis Kit (Cat. No. 635000)
- SMART™ cDNA Library Construction Kit (Cat. No. 634901)
- SMART™ mRNA Amplification Kit (Cat. No. 635001)
- Advantage® 2 DNA Polymerase (Cat Nos. 639201, 639202, 639206 & 639207)
- Human Universal Reference Total RNA (Cat. No. 636538)
- Human Placenta Total RNA (Cat. No. 636527)

**References**

1. Chenchick, A. *et al.* (1998) Generation and Use of High-Quality cDNA from Small Amounts of Total RNA by SMART™ PCR. In *Gene Cloning and Analysis by RT-PCR.* Eds. Siebert, P. & Larrick, J. (*Biotechniques* Books, MA) Ch 22.