Protocol-at-a-Glance

SMARTer™ RACE cDNA Amplification Kit
Protocol-at-a-Glance (PT4096-2)

Please read the User Manual for the SMARTer RACE cDNA Amplification Kit (Cat. Nos. 634923 & 634924) before using this Protocol-at-a-Glance. **This abbreviated protocol is provided for your convenience, but is not intended for first-time users.**

Generating RACE-Ready cDNA (Section V of the User Manual)

1. Prepare enough of the following Buffer Mix for all 5′- & 3′-RACE-Ready cDNA synthesis reactions and 1 extra reaction to ensure sufficient volume. For each 10 µl cDNA synthesis reaction, mix the following reagents and spin briefly in a microcentrifuge, then set aside at room temperature until Step 7:

2.0 µl	**5X First-Strand Buffer**
1.0 µl	**DTT** (20 mM)
1.0 µl	**dNTP Mix** (10 mM)
4.0 µl	**Total Volume**

2. Combine the following reagents in separate microcentrifuge tubes:

For preparation of 5'-RACE-Ready cDNA	**For preparation of 3'-RACE-Ready cDNA**
1.0–2.75 µl **RNA***	1.0–3.75 µl **RNA***
1.0 µl **5'-CDS Primer A**	1.0 µl **3'-CDS Primer A**

*For the control synthesis, use 1 µl of Control Mouse Heart Total RNA (1 µg/µl)

3. Add sterile H_2O to the tubes from Step 2 for a final volume of 3.75 µl for 5′ RACE and 4.75 µl for 3′ RACE.
4. Mix contents and spin the tubes briefly in a microcentrifuge.
5. Incubate the tubes at 72°C for 3 min, then cool the tubes to 42°C for 2 min. After cooling, spin the tubes briefly for 10 seconds at 14,000 g to collect the contents at the bottom.

   NOTE: This step can be performed in a thermocycler. While the tubes are incubating, you can prepare the Master Mix in Step 7.
6. To just the 5' RACE cDNA synthesis reaction(s), add 1 µl of the SMARTer IIA oligo per reaction. Mix well by vortexing and spin the tube briefly in a microcentrifuge.
7. Prepare enough of the following Master Mix for all 5′- & 3′-RACE-Ready cDNA synthesis reactions. Mix these reagents at room temperature in the following order:

4.0 µl	**Buffer Mix from Step 1**
0.25 µl	**RNase Inhibitor** (40 U/µl)
1.0 µl	**SMARTScribe™ Reverse Transcriptase** (100 U)
5.25 µl	**Total Volume**

8. Add 5.25 µl of the Master Mix from Step 7 to the denatured RNA from Step 5 (3'-RACE cDNA) and Step 6 (5' RACE cDNA), for a total volume of 10 µl.
9. Mix the contents of the tubes by gently pipetting, and spin the tubes briefly to collect the contents at the bottom.
10. Incubate the tubes at 42°C for 90 min in an air incubator or a hot-lid thermal cycler.
11. Heat tubes at 70°C for 10 min.

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12. Dilute the first-strand reaction product with Tricine-EDTA Buffer:
    - Add 20 µl if you started with <200 ng of total RNA.
    - Add 100 µl if you started with >200 ng of total RNA.
    - Add 250 µl if you started with poly A^+ RNA.
13. Samples can be stored at –20°C for up to three months.

Positive Control RACE PCR Experiment (Section VI.B of the User Manual)

Prior to performing 5'- and 3'-RACE reactions, we strongly recommend that you perform the positive control RACE PCR experiment in Section VI.B of the User Manual.

Rapid Amplification of cDNA Ends (RACE) (Section VI.D of the User Manual)

This procedure describes the 5′-RACE and 3′-RACE PCR reactions that generate the 5′ and 3′ cDNA fragments. We recommend that you also perform positive control 5′- and 3′-RACE using the TFR primers, UPM, and control RACE-Ready cDNAs as described in Section VI.B. Although the Nested Universal Primer A (NUP) is provided, nested PCR is generally not necessary in SMARTer RACE reactions.

Please note that all RACE PCR reactions have been optimized for use with the Advantage® 2 Polymerase Mix.

1. Prepare enough PCR Master Mix for all of the PCR reactions plus one extra reaction to ensure sufficient volume. The same Master Mix can be used for both 5′- and 3′-RACE reactions. For each 50 µl PCR reaction, mix the following reagents:

34.5 µl	**PCR-Grade Water**
5.0 µl	**10X Advantage 2 PCR Buffer**
1.0 µl	**dNTP Mix** (10 mM; in SMARTer RACE or Advantage 2 PCR Kit)
1.0 µl	**50X Advantage 2 Polymerase Mix**
41.5 µl	**Total Volume**

2. Mix well by vortexing (without introducing bubbles), then briefly spin the tube in a microcentrifuge.
3. **For 5′-RACE:** prepare PCR reactions as shown in Table I.
   For 3′-RACE: prepare PCR reactions as shown in Table II.
   Add the components to 0.5 ml PCR tubes in the order shown and mix gently.

Table I: Setting Up the 5′-RACE PCR Reactions

Component	Tube No. & Description				
	1 5′-RACE Sample	2 5′-TFR* (+ Control)	3 GSP1 + GSP2† (+ Control)	4 UPM only (– Control)	5 GSP1 only (– Control)
5′-RACE-Ready cDNA (experimental)	2.5 µl	2.5 µl	2.5 µl	2.5 µl	2.5 µl
UPM (10X)	5 µl	5 µl	—	5 µl	—
GSP1 (10 µM)	1 µl	—	1 µl	—	1 µl
GSP2 (10 µM)	—	—	1 µl	—	—
Control 5′-RACE TFR Primer (10 µM)	—	1 µl	—	—	—
H_2O	—	—	4 µl	1 µl	5 µl
Master Mix	41.5 µl	41.5 µl	41.5 µl	41.5 µl	41.5 µl
Final Volume	50 µl	50 µl	50 µl	50 µl	50 µl

* Skip this reaction if your RNA is nonmouse.
† Skip this reaction if your GSPs will not create overlapping RACE fragments.
For detailed descriptions of the control reactions, see Section VI.C of PT4096-1.

Table II: Setting Up the 3′-RACE PCR Reactions					
	Tube No. & Description				
Component	1 3′-RACE Sample	2 3′-TFR* (+ Control)	3 GSP1 + GSP2† (+ Control)	4 UPM only (– Control)	5 GSP1 only (– Control)
3′-RACE-Ready cDNA (experimental)	2.5 µl	2.5 µl	2.5 µl	2.5 µl	2.5 µl
UPM (10X)	5 µl	5 µl	—	5 µl	—
GSP1 (10 µM)	—	—	1 µl	—	—
GSP2 (10 µM)	1 µl	—	1 µl	—	1 µl
Control 3′-RACE TFR Primer (10 µM)	—	1 µl	—	—	—
H_2O	—	—	4 µl	1 µl	5 µl
Master Mix	41.5 µl	41.5 µl	41.5 µl	41.5 µl	41.5 µl
Final Volume	50 µl	50 µl	50 µl	50 µl	50 µl

* Skip this reaction if your RNA is nonmouse.
† Skip this reaction if your GSPs will not create overlapping RACE fragments.
For detailed descriptions of the control reactions, see Section VI.C.

4. Commence thermal cycling using one of the following programs (both programs 1 and 2 work with the positive control 5′- and 3′-RACE TFR and UPM Primers). Be sure to choose the correct number of cycles (as noted) based on whether you started with poly A+ or total RNA.

   NOTES on cycling: **Because the necessary number of cycles depends on the abundance of the target transcript, you may need to determine the optimal cycling parameters for your gene empirically. The optimal extension time depends on the length of the desired amplicon. For 0.2-2 kb amplicons, we typically extend for 2 min; for 2-4 kb amplicons, we extend for 3 min; and for 5-10 kb amplicons, we extend for up to 10 min.**

Program 1 (preferred; use if GSP T_m >70°C)

- 5 cycles:
  94°C 30 sec
  72°C 3 min*
- 5 cycles:
  94°C 30 sec
  70°C 30 sec
  72°C 3 min*
- 20 cycles (Poly A+ RNA)
  OR 25 cycles (Total RNA):
  94°C 30 sec
  68°C 30 sec
  72°C 3 min*

Program 2 (use if GSP T_m = 60–70°C)

- 20 cycles (Poly A+ RNA)
  OR 25 cycles (Total RNA):
  94°C 30 sec
  68°C 30 sec
  72°C 3 min*

*If fragments >3 kb are expected, add 1 min for each additional 1 kb.