

## SMART™ RACE cDNA Amplification Kit Protocol-at-a-Glance (PT3269-2)

Please read the User Manual for the SMART RACE cDNA Amplification Kit (Cat. No. 634914) before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

### First-Strand cDNA Synthesis (Section VII of the User Manual)

The two 10- $\mu$ l reactions described below convert 50 ng–1  $\mu$ g of total or poly A<sup>+</sup> RNA into RACE-Ready first-strand cDNA.

- Combine the following in separate microcentrifuge tubes:

For preparation of 5'-RACE-Ready cDNA	For preparation of 3'-RACE Ready cDNA
1–3 $\mu$ l RNA sample	1–3 $\mu$ l RNA sample
1 $\mu$ l 5'-CDS primer	1 $\mu$ l 3'-CDS primer A
1 $\mu$ l SMART II A oligo	

- Add sterile H<sub>2</sub>O to a final volume of 5  $\mu$ l for each reaction.
- Mix contents and spin the tube briefly in a microcentrifuge.
- Incubate the tube at 70°C for 2 min; then cool the tube on ice for 2 min.
- Spin the tube briefly to collect the contents at the bottom.
- Add the following to each reaction tube (already containing 5  $\mu$ l):

2 $\mu$ l	5X First-Strand buffer
1 $\mu$ l	DTT (20 mM)
1 $\mu$ l	dNTP Mix (10 mM)
1 $\mu$ l	MMLV Reverse Transcriptase*

**10  $\mu$ l Total volume**

\*Please see Addendum PT3980-4 for details on the choice of RT enzyme.

- Mix the contents and spin the tube briefly.
- Incubate the tube at 42°C for 1.5 hr in an air incubator or a hot lid thermal cycler.
- Dilute the first-strand reaction product with Tricine-EDTA Buffer:
  - Add 20  $\mu$ l if you started with <200 ng of total RNA.
  - Add 100  $\mu$ l if you started with >200 ng of total RNA.
  - Add 250  $\mu$ l if you started with poly A<sup>+</sup> RNA.
- Heat tubes at 72°C for 7 min.
- Samples can be stored at –20°C for up to three months.

### Positive Control PCR Experiment (Section VIII 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 VIII of the User Manual.

### Rapid Amplification of cDNA Ends (RACE) (Section IX of the User Manual)

- 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- $\mu$ l reaction, mix the following reagents:

34.5 $\mu$ l	PCR-Grade Water
5 $\mu$ l	10X Advantage 2 PCR Buffer
1 $\mu$ l	dNTP Mix (10 mM)
1 $\mu$ l	50X Advantage 2 Polymerase Mix

**41.5  $\mu$ l Total volume**

Mix well by vortexing (without introducing bubbles) and spin the tube briefly.



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TABLE III: SETTING UP 5'-RACE PCR REACTIONS

Component \ Tube No.	1 5'-RACE Sample	2 5'-TFR* (+ Control)	3 GSP 1 + 2† (+ 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 <sub>2</sub> O	—	—	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 nonhuman.

† Skip this reaction if your GSPs will not create overlapping RACE fragments.

TABLE IV: SETTING UP 3'-RACE PCR REACTIONS

Component \ Tube No.	1 3'-RACE Sample	2 3'-TFR* (+ Control)	3 GSP 1 + 2† (+ Control)	4 UPM only (- Control)	5 GSP2 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 <sub>2</sub> O	—	—	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 nonhuman.

† Skip this reaction if your GSPs will not create overlapping RACE fragments.

- For 5'-RACE and 3'-RACE: prepare PCR reactions as shown in Tables III & IV. Add the components in the order shown in PCR tubes.
- Overlay the contents of each tube with 2 drops of mineral oil and place caps firmly on each tube **Note:** Mineral oil is not necessary if you are using a hot-lid thermal cycler.

4. Commence thermal cycling using the appropriate program for touchdown PCR. If the  $T_m$ 's of your GSPs are less than 70°C, you cannot use touchdown PCR. Consult the User Manual for an alternative PCR program. Be sure to choose the correct number of cycles (as noted) based on whether you started with poly A<sup>+</sup> or total RNA.
- 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<sup>+</sup> RNA):  
OR
  - 25 cycles (Total RNA):  
94°C 30 sec  
68°C 30 sec  
72°C 3 min

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