Marathon-Ready™ cDNA Protocol-at-a-Glance

(PT1156-2)

Marathon cDNA amplification is a fairly complex, multiday procedure. Please read the *User Manual* before using this abbreviated protocol, and refer to it often for interpretation of results during the course of your experiments. The Protocol-at-a-Glance is provided for your convenience, but is not intended for first-time users.

VI. Rapid Amplification of cDNA Ends (RACE)

1. Prepare enough PCR master mix for all of the reactions plus one additional tube. The same master mix can be used for both 5'- and 3'-RACE reactions. For each 50-µl reaction, mix the following reagents:

36 µl	H_2O
5 µl	10X cDNA PCR Reaction Buffer
1 µl	dNTP Mix (10 mM)
1 µl	Advantage® 2 Polymerase Mix (50X)
43 µl	Final volume

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

2. **For 5'-RACE:** prepare PCR reactions as shown in Table II.
   For 3'-RACE: prepare PCR reactions as shown in Table III.
   Add the components in the order shown in 0.5-ml PCR tubes.

TABLE II: SETTING UP 5'-RACE REACTIONS

Component / Test Tube #: / Description:	1 Experimental Sample	2 G3PDH Pos. Ctrl *	3 GSP 1 + 2 Pos. Ctrl	4 AP1 only Neg. Ctrl	5 GSP1 only Neg. Ctrl
Marathon-Ready cDNA	5 µl	5 µl	5 µl	5 µl	5 µl
AP1 Primer (10 µM)	1 µl	1 µl	---	1 µl	---
GSP1 (antisense primer; 10 µM)	1 µl	---	1 µl	---	1 µl
GSP2 (sense primer; 10 µM)	---	---	1 µl	---	---
Control 5'-RACE G3PDH Primer (10 µM)	---	1 µl	---	---	---
H_2O	---	---	---	1 µl	1 µl
Master Mix	43 µl	43 µl	43 µl	43 µl	43 µl
Final volume	50 µl	50 µl	50 µl	50 µl	50 µl

* The G3PDH Positive Control should generate a 1.09-kb product.

TABLE III: SETTING UP 3'-RACE REACTIONS

Component / Test Tube #: / Description:	1 Experimental Sample	2 G3PDH Pos. Ctrl †	3 GSP 1 + 2 Pos. Ctrl	4 AP1 only Neg. Ctrl	5 GSP2 only Neg. Ctrl
Marathon-Ready cDNA	5 µl	5 µl	5 µl	5 µl	5 µl
AP1 Primer (10 µM)	1 µl	1 µl	---	1 µl	---
GSP2 (sense primer; 10 µM)	1 µl	---	1 µl	---	1 µl
GSP1 (antisense primer; 10 µM)	---	---	1 µl	---	---
Control 3'-RACE G3PDH Primer (10 µM)	---	1 µl	---	---	---
H_2O	---	---	---	1 µl	1 µl
Master Mix	43 µl	43 µl	43 µl	43 µl	43 µl
Final volume	50 µl	50 µl	50 µl	50 µl	50 µl

† The G3PDH Positive Control should generate a 1.2-kb product.

3. Overlay the contents of each tube with 2 drops of mineral oil and place caps firmly on each tube.

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Note: This is not necessary if you are using a hot-lid thermal cycler.

4. Commence thermal cycling using one of the following programs (programs 1 and 2 work with the Control G3PDH and AP1 Primers):

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

PE DNA Thermal Cycler 480:

- 94°C for 1 min
- 5 cycles:
  94°C 30 sec
  72°C 4 min‡
- 5 cycles:
  94°C 30 sec
  70°C 4 min‡
- 20–25 cycles:
  94°C 20 sec
  68°C 4 min‡

PE GeneAmp Systems 2400/9600 (or hot-lid thermal cycler):

- 94°C for 30 sec
- 5 cycles:
  94°C 5 sec
  72°C 4 min‡
- 5 cycles:
  94°C 5 sec
  70°C 4 min‡
- 20–25 cycles:
  94°C 5 sec
  68°C 4 min‡

Program 2 (if GSP T_m = 60–65°C):

PE DNA Thermal Cycler 480:

- 94°C for 1 min
- 25–30 cycles:
  94°C 30 sec
  68°C 4 min‡

PE GeneAmp Systems 2400/9600 (or hot-lid thermal cycler):

- 94°C for 30 sec
- 25–30 cycles:
  94°C 5 sec
  68°C 4 min‡

Notes on cycling:

You may need to determine the optimal cycling parameters for your gene empirically.

‡ The optimal extension time depends on the length of the fragment being amplified. We typically use 4 min for cDNA fragments of 2–5 kb. For 0.2–2-kb targets, we reduce the extension time to 2–3 min. For 5–10-kb targets, we increase the extension time up to 10 min.

5. When cycling is completed, analyze 5 µl from each tube, along with appropriate DNA size markers, on a 1.2% agarose/EtBr gel.
6. [Optional] If the primary PCR reaction fails to give the distinct band(s) of interest or produces a smear, you may wish to perform a Southern blot using a cDNA probe or a nested primer as a probe.

   Or, you may wish to perform a secondary, or "nested," PCR reaction using the AP2 primer supplied with Marathon-Ready cDNA and a NGSP. (See the discussion on Primer Design in Section V of the User Manual.)

   a. Dilute 5 µl of the primary PCR product into 245 µl of Tricine-EDTA buffer.
   b. Repeat steps 1–5 above, using:
      - 5 µl of the diluted primary PCR product in place of the Marathon-Ready cDNA.
      - 1 µl of the AP2 primer and 1 µl of your nested antisense GSP.
      - Fewer cycles (15–20 instead of 25–30).

VII. Characterization of RACE Products

At this point, we recommend that you characterize your RACE fragments and confirm that you have amplified the desired product. This can be done by (1) comparison of RACE products obtained with GSPs and NGSPs; (2) Southern blotting; and (3) cloning and sequencing. Characterization of your RACE products at this point can prevent confusion and wasted effort in your subsequent experiments, even when both RACE reactions produce a single major product.

After RACE products have been characterized by partial or complete sequencing, you have two options for generating the full-length cDNA:

1) Generation of Full-Length cDNA by PCR (Section VIII).
2) Generation of Full-Length cDNA by Cloning (Section IX).

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