

# Rapid Amplification of cDNA 3' Ends (3'- RACE)

## First-Strand cDNA Synthesis of 3'-RACE

(Omniscript RT kit, Cat No 205111)

- 1. Denature 12 $\mu$ l of the total RNA by incubation for 5 min at 65°C, then place immediately on ice.**

The protocol is optimized for use with 50 ng-2 $\mu$ g RNA. The concentration of total RNA isolated from the RNeasy 96 plate is 10 ng-100 ng / $\mu$ l.

**We use 12 $\mu$ l of total RNA as template for 20  $\mu$ l of RT reaction (6 $\mu$ l RNA for 10 $\mu$ l of RT reaction).**

- 2. Prepare fresh master mix in tube or reservoir on ice:**

<u>Component</u>	<u>1 reaction</u>	<u>8 reactions</u>	<u>96 reactions</u>
10 $\times$ buffer RT	2 $\mu$ l	18 $\mu$ l	206 $\mu$ l
dNTP mix (5 mM each)	2 $\mu$ l	18 $\mu$ l	206 $\mu$ l
3'CDS primer (10 $\mu$ M)	2 $\mu$ l	18 $\mu$ l	206 $\mu$ l
RNase inhibitor (10 units/ $\mu$ l)	1 $\mu$ l	9 $\mu$ l	103 $\mu$ l
Omniscript RT	1 $\mu$ l	9 $\mu$ l	103 $\mu$ l
Total		8 $\mu$ l	

Reduce half amount for total 10 $\mu$ l of reaction.

- 3. Add 8 $\mu$ l (4  $\mu$ l for 10  $\mu$ l of reaction) of master mix into the tubes containing denatured RNA template. Mix and spin briefly to collect the contents at the bottom.**
- 4. Incubate for 90 min at 37°C in PCR machine.**
- 5. For analysis of long cDNAs by PCR, do not need inactivated RT. For shorter cDNAs, Omniscript RT can be inactivated by heating the tubes to 93°C for 5 min, rapid cooling on ice. Store at -20°C.**

**Do not need to dilute the cDNAs, use < 10 $\mu$ l of finished reaction for 50 $\mu$ l PCR.**

**We do not inactivate and dilute the cDNA.**

**First round PCR** ( Simplified protocol based on Clontech's SMART- RACE )

- 1. Distribute 2.5  $\mu$ l of 3'-RACE-Ready cDNA in the PCR tubes, 8 strips or 96 plate on ice.**
- 2. Prepare enough PCR Master Mix on ice according to the table below:**

<b>Component</b>	<b>1 reaction</b>	<b>8 reactions</b>	<b>96 reactions</b>
<b>PCR-Grade Water</b>	34.5 $\mu$ l	311 $\mu$ l	3450 $\mu$ l
<b>10x PCR Buffer</b>	5	45	500
<b>10 mM dNTP Mix</b>	1	9	100
<b>10x UPM</b>	5	45	500
<b>10<math>\mu</math>M GSP1</b>	1	9	100
<b>Taq DNA Polymerase</b>	1	9	100

- 3. Add 47.5  $\mu$ l of Master Mix in the PCR tube containing 2.5  $\mu$ l of template and mix it on ice. Total 50  $\mu$ l of each reaction.**

- 4. PCR program ( Mastercycler gradient/ eppendorf):**

**3'RACE1:**

94 °C 2 min ( when 1 min past, put the PCR tube into the machine.)

5 cycles:

94 °C 30 sec

70 °C 30 sec

72 °C 3 min

30 cycles:

94 °C 30 sec

68 °C 30 sec

72 °C 3 min

1 cycle:

72 °C 5 min

- 5. Dilute 2.5  $\mu$ l of the first-PCR product into 122.5  $\mu$ l of Tricine- EDTA Buffer as the template for nested PCR.**

**Nested PCR** ( Simplified protocol based on Clontech's SMART- RACE )

6. **Distribute 5  $\mu$ l of diluted first PCR product in the PCR tubes, 8 strips or 96 plate on ice.**
7. **Prepare enough PCR Master Mix on ice according to the table below:**

<b>Component</b>	<b>1 reaction</b>	<b>8 reactions</b>	<b>96 reactions</b>
<b>PCR-Grade Water</b>	36 $\mu$ l	324 $\mu$ l	3600 $\mu$ l
<b>10x PCR Buffer</b>	5	45	500
<b>10 mM dNTP Mix</b>	1	9	100
<b>10 <math>\mu</math>M NUP ( Nest1)</b>	1	9	100
<b>10 <math>\mu</math>M GSP1</b>	1	9	100
<b>Taq DNA Polymerase</b>	1	9	100

We use our own Taq. and 10x PCR buffer.

8. **Add 45  $\mu$ l of Master Mix in the PCR tube containing 5  $\mu$ l of template and mix it on ice. Total 50  $\mu$ l of each reaction.**
9. **PCR program ( Mastercycler gradient/ eppendorf):**

**3'RACE2:**

94 °C 2 min ( when 1 min pass, put the PCR tube into the machine.)

5 cycles:

94 °C 30 sec

70 °C 30 sec

72 °C 3 min

25 cycles:

94 °C 30 sec

68 °C 30 sec

72 °C 3 min

1 cycle:

72 °C 5 min

- 10. Run 5  $\mu$ l of the reaction products on an 1% of agarose gel to check.**