

Reverse transcription and 3' RACE PROTOCOL

cDNA synthesis

Reverse transcription

Transcriptase reverse transcriptase (T-RT)(Roche) is used to synthesize a complementary DNA strand.

Total RNA 6 μ l/well

Incubate RNA at 65 °C for 5 min and quickly chill on ice for about 3 min.

Add

	μ l/well
5 x RT buffer	2
10 uM 3CDS	1
10 mM dNTP mix	0.5
RNase OUT (40U/ μ l)	0.125
Water (RNase-free)	0.125
T-RT (20U/ μ l)	0.25

Total mix/well	4 μ l
Final volume	10 μ l

Incubate at 55 °C for 30 min. Store at -20°C.

3' RACE

Polymerase: Taq polymerase (Taq, 5U/ μ l Invitrogen)

Primer sets:

	3' RACE1	3' RACE2	Sequencing
pUPA	pUPAtrap6 / UPM	pUPAtrap3 / Nest 1	pUPAtrap7

1st RACE PCR

1.5 μ l of cDNA is added into 23.5 μ l of 3'RACE1 mix	
Mix	/well
10 x PCR buffer (homemade)	2.5
10 mM dNTP mix (Fermantas)	0.5
10 μ M primer 1 (vector specific)	0.5
10x UPM primer	2.5
Taq (Invitrogen)	0.25
DDW	17.25

Total	23.5 μ l
Final volume	25 μ l

3'RACE1:

Start : 94°C 2 min
 5 cycles: 94°C 30 sec \rightarrow 70°C 30
 sec \rightarrow 72°C 3 min
 30 cycles: 94°C 30 sec \rightarrow 68°C 30
 sec \rightarrow 72°C 3 min
 1 cycle: 72°C 5 min
 End: 4°C

Add Mineral Oil to each well, seal plates with Aluminum cover, and then run Program 3RACE1. Keep at 4 °C.

Dilute 1st RACE product

5 ul of 1st RACE product is mixed with 25 ul of Tricine-EDTA buffer.

2nd RACE PCR

5 ul of 1/6-diluted 1st RACE product is added into 45 ul of 3'RACE2 mix

Mix	/well
10 x PCR buffer (homemade)	5
10 mM dNTP mix (Fermantas)	1
10 uM primer 2 (vector specific)	1
10 uM Nest1 primer	1
Taq (Invitrogen)	0.5
DDW	36.5

Total	45 ul
Final volume	50 ul

3'RACE2:

Start : 94°C 2 min

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

End: 4°C

Add Mineral Oil to each well, seal plates with Aluminum cover, and then run Program 3RACE2. Keep at 4 °C.

Load 3RACE2 product with Loading Dye onto 1.5~1.8% agarose gel, run gel in 1x TAE buffer. Stain gel with Ethelium Bromide. Take gel picture. Record picture clearly.

Primers:

3CDs (3'-RACE cDNA Synthesis Primer)

AAG CAG TGG TAA CAA CGC AGA GTA CTT TTT TTT TTT TTT TTT TTT TTT TTT TVN

pUPAtrap6

TAT AGC ATA CAT TAT ACG AAG TTA TCC GCT CGA C

pUPAtrap3

GCC ATG CAC CAT GGT TCG AAA TTC CAG

pUPAtrap7

ATT CCA GAC AAG TTT GTT GTT GGA TAT GCC

10X UPM (Universal Primer Mix)

Long (UPL, 0.4 μM)

CTA ATA CGA CTC ACT ATA GGG CAA GCA GTG GTA ACA ACG CAG AGT

Short (UPS, 2 μM)

CTA ATA CGA CTC ACT ATA GGG C

Nest1 (Nested Universal Primer)

AAG CAG TGG TAA CAA CGC AGA GT