

M-MLV Reverse transcriptase 200U/μl (Invitrogen cat #: 28025-013)

RT procedure:

1μl of 500 μg/ml Oligo(dT)₁₂₋₁₈ or 1μl of 50μM Oligo (dT)₂₀.

1ng-5 μg total RNA or 1-500ng mRNA

1μl 10mM dNTPs

DEPC treated water to 12μl

(1) Heat at 65°C/5min

(2) Chill on ice and collect the content by spinning (i.e. **pause**)

4μl 5X first strand buffer

2μl 0.1M DTT

1μl RNaseOUT (40U/μl)

(3) Incubate at 37°C/2min

(4) (Pause) Add * 1μl (i.e. 200U) M-MLV and pipette up and down

(5) Incubate at 37°C/50min (RT Enzyme action)

(6) Incubate 70°C/15min (Enzyme inactivation step)

Total volume 20μl

Optional: The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 μl (2 units) of *E. coli* RNase H and incubate at 37°C for 20 min.

* If less than 1 ng of RNA is used, reduce the amount of M-MLV RT in the reaction to 0.25 μl (50 units), and add the sterile, distilled water to 20-μl final volume.

PCR protocol:

Use only 10% of the first-strand reaction (i.e. 2 μ l of the RT reaction) for PCR. Adding larger amounts of the first-strand reaction may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR reaction tube for a final reaction volume of 50 μ l:

- 5 μ l 10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]
- 1.5 μ l 50 mM MgCl₂
- 1 μ l 10 mM dNTP Mix
- 1 μ l amplification primer 1 (10 μ M)
- 1 μ l amplification primer 2 (10 μ M)
- 0.4 μ l *Taq* DNA polymerase (5 U/ μ l)
- 2 μ l cDNA (from first-strand reaction)
- 38.1 μ l autoclaved, distilled water

Total volume 50 μ l

Add 50 μ l of mineral oil if you use old PCR machines without heated lid (like that in our lab).

Perform 15 to 35 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.

PCR protocol:

- (1) 94°C → 2min
 - (2) 94°C → 45sec
 - (3) 50-60°C → 45sec (annealing temperature)
 - (4) 72°C → 1min
 - (5) Go to (2) for 30-35 cycles
 - (6) 72°C → 10min
 - (7) 15C → 00:00:00 (or select End)
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