



The Power to Quantify

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# RT-PCR Protocol



## 1. cDNA Synthesis

- Prepare a solution containing -
  - a) 1  $\mu$ l oligo (dT)<sub>12-18</sub> (500  $\mu$ g/ml)
  - b) 1 ng-5  $\mu$ g total RNA
  - c) 1  $\mu$ l 10 mM dNTPs
  - d) and add RNase-free water to a final volume of 12  $\mu$ l
- Incubate at 70° C for 5 minutes to minimize RNA secondary structure, quick chill on ice and then add -
  - a) 4  $\mu$ l 5x reverse transcriptase buffer
  - b) 2  $\mu$ l 0.1 M DTT
  - c) 1  $\mu$  RNase inhibitor
- Incubate at 42° C for 2 minutes to anneal primer and template.
- Add 1  $\mu$ l reverse transcriptase (200 units) and incubate at 42° C for 50 minutes to extend the primer and then terminate the reaction by incubating at 70° C for 15 minutes.

**NOTE:** (As an optional step add 1  $\mu$ l RNase H (2 unit/ $\mu$ l) and incubate at 37° C for 20 minutes)

## 2. First PCR reaction

- Prepare a solution containing -
  - a) 5  $\mu$ l 10x PCR buffer (with or without\* MgCl<sub>2</sub>)
  - b) \*5  $\mu$ l 25 mM MgCl<sub>2</sub> (It may be necessary to vary the MgCl<sub>2</sub> concentration, 2.5 mM final concentration recommended.)
  - c) 1  $\mu$ l 10 mM dNTP
  - d) 1  $\mu$ l primer pair A
  - e) 1  $\mu$ l Taq DNA polymerase
  - f) 2  $\mu$ l cDNA and add water to 50  $\mu$ l
- Incubate at 94° C for 2 minutes to denature the cDNA.
- Perform 15-40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically for each template-primer pair.

## 3. Second PCR reaction

- Prepare a solution containing -
  - a) 5  $\mu$ l 10x PCR buffer (with or without\* MgCl<sub>2</sub>)
  - b) \*5  $\mu$ l 25 mM MgCl<sub>2</sub> (It may be necessary to vary the MgCl<sub>2</sub> concentration, 2.5 mM final concentration recommended.)
  - c) 1  $\mu$ l 10 mM dNTP
  - d) 1  $\mu$ l primer pair B
  - e) 1  $\mu$ l Taq DNA polymerase
  - f) 1-5  $\mu$ l first PCR product and add water to 50  $\mu$ l
- Incubate at 94° C for 2 minutes to denature the cDNA.
- Perform 15-40 PCR cycles. Annealing and extension conditions are primer and template dependent and must be determined empirically for each template-primer pair.
- PCR products are visualized on agarose gels stained with ethidium bromide.