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RT PCR PROTOCOL

I. RT/ cDNA synthesis:

Use 1 to 1.5 µg of RNA and add the following reagents to give a total volume of 20 µl:

25mM $MgCl_2$	4 µl
10x PCR buffer (PE)	2 µl
DEPC tx H_2O	1 µl · DTT 2µl
10mM dNTP's (mix)	8 µl
RNAase IN	25U · 6.5µl
(*) 3' Primer	200ng

DEPC tx H_2O to total volume of 20 µl.

(*) 3' primer could be a random primer (hexamer), oligo dT primer, or a specific 3' primer. Reverse transcriptase used is provided by PE in their RT PCR Kit.

Assemble above reagents in 0.5ml tube, incubate at :
42° C 60 mins.; 99° C 5mins.; 4° C 5 mins.

Amplification/PCR Protocol:

25mM $MgCl_2$	4 µl
10x PCR buffer (PE)	8 µl
5' Primer	200ng
(*) 3' Primer	200ng
Taq Polymerase	2.5 U

DEPC tx H_2O to total volume of 80 µl.

(*) If the 3' primer was used in the 1st reaction it is not necessary to add any more primer.

Cycle = 1	2 mins. 97° C
Cycle = 40	1 min. 95° C; 1 min. 37 to 65° C (check primer)
Cycle = 1	7 mins. 37 to 65° C

Check product on a 1 - 1.5% agarose gel.