RT-PCR
Small Scale

RT step: 2 λ RNA (0.5 μg/λ)
1 λ 5X Hyb buffer
1 λ 1 ng. downstream primer

Place in PCR machine
Heat to 90 degrees then cool to 43 degrees over 30 minutes

Add: 19.5 λ 1.25X RT mix (pre-warm to 43 ^{o}C and pre-mix
0.5 λ MMLV with enzyme)

In PCR machine incubate 30 min. at 43 ^{o}C
Then heat to 95 degrees for 5 min., then immediately to 4 degrees and hold until ready for next step

PCR step: To 5 λ from above reaction add
1 λ 15.0 ngs. downstream primer
1 λ 12.5 ngs. upstream primer
1 λ 2.5 ngs. ^{32}P -labeled upstream primer
1.5 λ RT-PCR buffer
10.3 λ H2O
0.2 λ 5 U/λ Taq polymerase

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94 ^{o}C 2 min. then X cycles at: 94 ^{o}C 1 min.
Y ^{o}C 1 min.
72 ^{o}C Z min.

(annealing temperature (Y) and extension time (Z) should be determined by your primers and length of predicted product. Cycle number (X) must be determined empirically for each transcript and is dependent on expression level of the RNA)
(For minigenes: X=16, Y=70, Z=1.5, for endogenous CD45: X=20, Y=70 and Z=2)

5x HYB

1.5 M NaCl

50 mM Tris pH 7.5

10 mM EDTA

1.25x RT Mix

1.25 mM ea. dNTPs

12.5 mM DTT

12.5 mM Tris pH 8.0

7.5 mM $MgCl_2$

To Make: 125 λ 1 M DTT

125 λ Tris pH 8

75 λ 1 M $MgCl_2$

9.175 mls H_2O

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aliquot in 950 ul per tube ➔ Freeze at –80ºC

Also make up 50 λ aliquots of 25 mM dNTPs ➔ Freeze at –80ºC

RT-PCR buffer

0.5 M KCl

0.1 M Tris pH 8.3

15 mM $MgCl_2$

0.01% gelatin