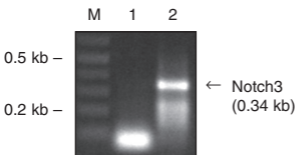


a



b

**Fig. 3. Specific detection of  $\beta$ -actin and Notch3 from human liver total RNA (100 ng), by two-step RT-PCR.**

(a) For many targets, such as 1.5 kb  $\beta$ -actin, specificity may be improved by decreasing the primer concentration in the PCR step. Compare results for 0.4 $\mu$ M versus 0.075 $\mu$ M primer. M: Marker; Lane 1: 0.4 $\mu$ M; Lane 2: 0.2 $\mu$ M; Lane 3: 0.1 $\mu$ M; Lane 4: 0.075 $\mu$ M. (b) For targets with high G+C contents, such as 0.34 kb Notch3 (G+C: 77%), increasing the temperature of the reverse transcription step and/or adding supplements for the RT and/or PCR steps, may improve specificity. RT reaction was carried out at 50 $^{\circ}$ C without supplements, and PCR was conducted with no supplements or with 1.0M betaine. The presence of betaine results in generation of desired product. M: Marker; Lane 1: No supplement; Lane 2: 1.0M Betaine.