Quantification of mRNA by non-radioactive RT-PCR and CCD imaging system

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The amount of PCR product increases exponentially in early cycles of the reaction, but subsequently reaches a plateau level (1). PCR product yield, therefore, does not quantitatively reflect the amount of the initial template DNA after the reaction reaches the plateau. This necessitates, for quantitative PCR analysis, the measurement of the product while the reaction occurs exponentially. We describe here a simple and widely applicable procedure for quantifying mRNA by non-radioactive RT-PCR avoiding the plateau effect.

We prepared PCR mixtures containing rabbit α globin primers and first strand cDNA libraries made from fibroblast RNA and serially diluted rabbit globin mRNA. PCR was then performed using capillary air thermo-cycler (Idaho Technology, USA) and one sample of the capillaries was picked up at every one or two cycles of reaction. Each sample was electrophoresed on agarose gel and stained with ethidium bromide to detect a 327 bp-band of amplified fragment (Figure 1A). The intensity of the ethidium-bromide luminescence was measured by CCD image sensor (Densitograph AE-6900-F; Atto, Japan) (2). Figure 1B shows the reaction cycle-intensity curves of each reaction mixture. All samples reached the same plateau level in ten cycles after the band intensity reached the limit of detection (Figure 1B).

For estimation of the initial amount of the template, regression equations of the form: $y = a \times b^n$, where y is the intensity and n is the number of cycles, were fitted to the data in the linear portion of the semi-logarithmic graphs (Figure 1B). Each constant 'a' and 'b' of the equations were expected to reflect the amount of the original template and the efficiency of amplification every cycle, respectively. As shown in figure 1C, the estimated values were in good agreement with the original amounts of template mRNA for a wide range (10 fg to 100 pg). For internal control, we also measured the mouse β actin mRNA using the same method, and no significant difference was found among the samples (data not shown).

REFERENCES

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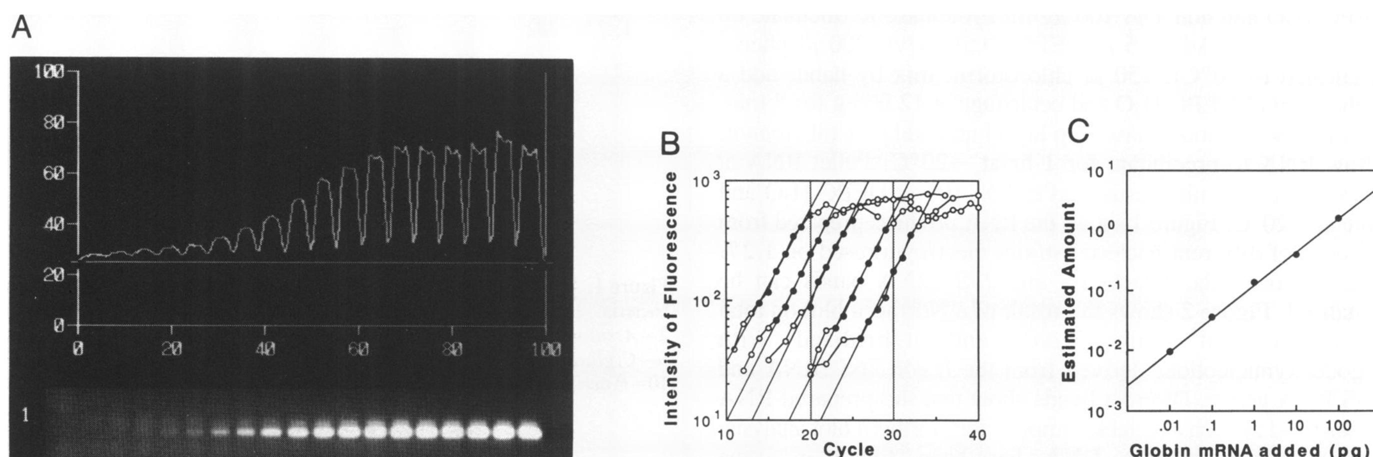

Figure 1. First-strand cDNA libraries were made by random priming from RNA samples containing 3 μg of mouse fibroblast (NIH/3T3) total RNA and serially diluted (100 pg, 10 pg, 1 pg, 100 fg and 10 fg) rabbit globin mRNA (Gibco BRL, USA) in 18 μl reaction. 4 μl of the cDNA solutions was added to 400 μl PCR reaction mixture containing 0.5 μM of rabbit α globin primers (5'-GCAGCCACGGTGGCGAGTAT-3' and 5'-GTGGGACAGGAGCTTGAAAT-3'). Each reaction mixture was divided into 10 μl aliquots in a microcapillary and PCR was performed for 10 to 40 cycles at 1- or 2-cycle intervals. A) 8 μl of products were electrophoresed on 1% of agarose gel in 0.5×TBE and 327-bp band was detected by ethidium bromide staining (lower panel). Fluorescence of each band was measured by CCD image sensor (upper panel). B) Number of cycles and increase of PCR product measured by CCD image sensor. Curves are for samples with 100 pg, 10 pg, 1 pg, 100 fg and 10 fg globin mRNA from left to right. Oblique lines show the regression equations determined by the linear portions of the curves. C) Relationship between estimated amounts of the template calculated by the regression equations (B) and relative concentrations of cDNA originally added to the solutions.