CORRESPONDENCE

Gel staining methods for detection of telomerase activity with the telomeric repeat amplification protocol (TRAP) assay

Recently, Wen *et al* reported an interesting study in this journal[1]: "A non-isotopic method for detection of telomerase activity in tumour tissue", suggesting that the silver staining assay (SS) is more convenient and sensitive than ethidium bromide staining (EB) for the detection of telomerase activity with the telomeric repeat amplification protocol (TRAP) assay.[2] The results prompted us to comment on results from our comparisons of staining methods such as EB, SS, and SYBR gold nucleic gel stain (SG).

The protein sample was isolated from a frozen tissue sample of colon adenocarcinoma resected by surgery, serially diluted, and aliquoted into separate tubes containing: 10 μg, 1.0 μg, 1.0×10^{-1} μg, 1.0×10^{-2} μg, 1.0×10^{-3} μg, 1.0×10^{-4} μg, 1.0×10^{-5} μg, and 1.0×10^{-6} μg protein. These samples were reacted with the solutions in a PCR based telomerase detection kit (TRAP-eze; Oncor Inc, Gaithersburg, Maryland, USA), according to the manufacturer's protocol. A 25 μl aliquot of each PCR product was applied to a 12.5% non-denaturing polyacrylamide gel and electrophoresed. The gels were stained and visualised as follows:

- The first gel was stained with 5.0 μl/ml of EB solution (Biorad, California, USA) in 200 ml double distilled water for 30 minutes and then destained with double distilled water. The gel was then viewed under UV transillumination and photographed.
- The second gel was stained with SS (DNA silver staining kit; Pharmacia Biotech, San Francisco, USA). The gel was stained with fixing solution for 30 minutes and then with staining solution for 30 minutes, according to the manufacture's protocol. It was then rinsed in double distilled water, developed in solution for 15 minutes, and immersed in the stopping and preserving solution for 30 minutes. The stained gel was then photocopied.
- The third gel was stained by the SG (Molecular Probes, Leiden, The Netherlands) method. The gel was immersed in the staining solution containing 20 μl of SG in 200 ml Tris boric acid EDTA (TBE) electrophoresis buffer for 30 minutes. The stained gel was viewed under UV transillumination and photographed.

Telomerase activity was present in all samples. In the gels stained by the SS and SG methods, a telomerase positive ladder was clear in most lanes. However, in the lane with a protein concentration of 10 μg (lane 1) stained by the SS and SG methods (fig 1B and C), high telomerase activity produced a smear, rather than a ladder, so that when high numbers of base pairs are present the ladder fuses into a smear using this method. In addition, there were no ladders in lanes 7 and 8 of the gel stained by the SS method, although the remaining lanes were the same for both SG and SS methods (fig 1B and C). When the telomerase activity was low, the sensitivity of detection was lower with the SS method than with the SG method. Ladders were seen in lanes 2 to 6 only in the gel stained with EB, and these were very faint (fig 1A). Therefore, this technique is fairly insensitive and can produce false negatives when telomerase activity is not very high.

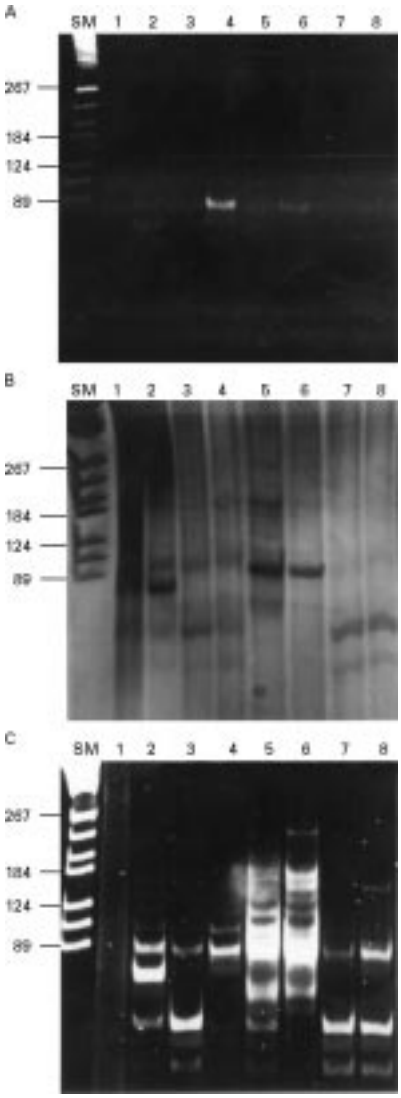

Figure 1 Telomerase activity in a colon carcinoma demonstrated by telomeric repeat amplification protocol (TRAP) assays. Three different staining protocols were used: (A) ethidium bromide, (B) silver staining, and (C) SYBR gold nucleic gel staining. TRAP assays were performed using extracts containing: lane 1, 10 μg; lane 2, 1.0 μg; lane 3, 1.0×10^{-1} μg; lane 4, 1.0×10^{-2} μg; lane 5, 1.0×10^{-3} μg; lane 6, 1.0×10^{-4} μg; lane 7, 1.0×10^{-5} μg; and lane 8, 1.0×10^{-6} μg protein. Lane SM, DNA molecular weight marker V.

In conclusion, the SG staining method is quicker, easier to perform, and more sensitive than the other two methods described here for detecting telomerase activity.

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1 Wen JM, Sun LB, Zhang M, *et al*. A non-isotopic method for detection of telomerase activity in tumour tissue: TRAP-silver staining assay. *J Clin Pathol: Mol Pathol* 1998;**51**:110–2.
2 Kim NW, Piatyszek MA, Prowse KR, *et al*. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994; **266**:2011–15.

Reply

Because 60–100% of malignant tumours display telomerase activity,[1] the detection of this activity is now considered to be a diagnostic marker for malignant tumours. A search for a non-isotopic telomerase repeat amplification protocol (TRAP) assay should benefit routine diagnoses. Although the non-isotopic silver staining TRAP assay developed by us is effectively detecting telomerase activity in malignant tumours, the sensitivity is still lower than that using the P^{32} isotopic labelling assay (Wen *et al* 1997, unpublished data). Fujita *et al* have also demonstrated another non-isotopic TRAP assay using the SYBR gold nucleic acid stain (SG) from Molecular Probes (Leiden, The Netherlands). It seems that staining by the SG method is better than by the silver staining method. On the other hand, because SG is a fluorescent dye, bands can be obscured in the gel (see fig 1B and C; lanes 5 and 6). It is not clear whether the SG method is more sensitive than the isotopic labelling TRAP assay. Nevertheless, we agree that the SG staining method, like the silver staining method, should be considered to be a quick, easy, and effective staining protocol for use with the TRAP assay.

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1 Wen JM, Sun LB, Zhang M, *et al.* Telomerase activation in malignant bone tumors. *Mol Diagn* 1998;**3**:29–35.

CORRECTION

Upregulation of ATM in sclerosing adenosis of the breast. Clarke RA, *et al*. *J Clin Pathol: Mol Biol* 1998,**51**:224–6.

The following footnote was omitted:
RA Clarke and R Kairouz contributed equally to the work and the order of these two authors should be considered arbitrary. The error is regretted.