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: “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. 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 x 10^{-1} µg, 1.0 x 10^{-2} µg, 1.0 x 10^{-3} µg, 1.0 x 10^{-4} µg, 1.0 x 10^{-5} µg, 1.0 x 10^{-6} µg, and 1.0 x 10^{-7} 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 10% PAGE gel (15 cm × 15 cm, 0.75 mm thick) using a Ladder Loading Kit (Fujifilm, Tokyo, Japan) 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 manufacturer’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.

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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Reply

Because 60–100% of malignant tumours display telomerase activity, 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 P² 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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Correction


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.