SYBR Green I staining of pulsed field agarose gels is a sensitive and inexpensive way of quantitating DNA double-strand breaks in mammalian cells

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ABSTRACT

Pulsed field gel electrophoresis (PFGE) is widely used to measure DNA double strand breaks (dsb). The DNA of cultured cells can be prelabelled with radioactivity, which helps greatly in detection and quantitation of DNA dsb. However, this approach cannot be used with non-cycling cells from biopsy material. We describe a method which uses SYBR Green I to stain DNA in dried agarose gels. DNA is detected and analysed using readily available camera equipment and image analysis software. This method is as sensitive as [3H]thymidine prelabelling of cells and allows DNA dsb to be measured simply and economically in non-cycling cells.

PFGE can be used to measure DNA double strand breaks (dsb) in mammalian cells (1). Cycling cells are incubated for at least two generations with [3H]- or [14C]thymidine to label the DNA. The cells are then embedded in agarose plugs and, following deproteination, the DNA is subjected to pulsed field gel electrophoresis (PFGE) under conditions where the proportion of DNA that leaves the plug and enters the gel is a measure of DNA dsb (2). Since the DNA is labelled with radioactivity, the proportion of DNA that leaves the plug is easily calculated by analysing gel slices by scintillation counting. DNA dsb in non-cycling cells can be measured using ethidium bromide (EtBr) to stain DNA, but this method is hampered by the insensitivity of EtBr at low DNA concentrations and the expensive detection equipment required (3).

Here we describe a method of measuring DNA dsb on PFGE gels using SYBR Green I nucleic acid stain (Molecular Probes, Eugene, OR).

DNA samples were loaded onto a 0.7% agarose gel (10 cm × 15 cm × 6 mm thick). Following electrophoresis, the gel was placed on a sheet of 3MM chromatography paper (Whatman, Maidstone, UK), precut slightly larger than the gel. The gel was then placed on a gel drier and covered in Saran® wrap (Dow) and dried under vacuum for 30 min without heat, followed by 60 min at 60°C. Experiments with [3H]thymidine-labelled DNA samples showed that there was no loss of DNA from the gel during the drying process. The Saran wrap was removed and the gel released from the 3MM paper by floating on distilled water until the 3MM paper sank. The gel was rinsed with distilled water and then placed in 100 ml prewarmed (50°C) SYBR Green I working solution (1 in 10 000 dilution in 0.5X TBE, pH 8.0) in an oven at 50°C for 3 h.

Gels were placed on a 312 nm UV transilluminator light source and imaged using a CCD TV camera (Ultraviolet Products), with a 16 mm lens and a Syb-100 SYBR green filter (Flowgen, Lichfield, UK) at F stop 11, focal length 62 cm.

Figure 1a shows a gel with known amounts of λ DNA (0–600 ng) after staining with SYBR Green I. Gel images were analysed using GelBase (Ultraviolet Products). The gel image is stored as a two-dimensional array of pixels, with each pixel being assigned a value from 0 to 255, depending on the fluorescence intensity at that point. Images are checked to ensure that all pixels have a value of less than 255 (i.e., the image was not saturated). Selected areas of the full gel image are analysed and assigned a value (area units) by the software. This value is dependent on the pixel values (i.e., fluorescence intensity) within the selected area, and is a measure of the amount of DNA present. The raw data were adjusted by subtracting background values derived from blank tracks.

Figure 1b shows the results of analysing independent duplicate gels with known amounts of λ DNA. If a gel is imaged under UV for a second time, there is a 30% reduction in image intensity, presumably due to exposure to UV light. However, the image obtained remains stable and reproducible to subsequent exposures to UV light (tested up to eight exposures), even if the gel is kept in the refrigerator for up to 1 week. When the results from all the images in Figure 1b are normalised by setting the value of 200 ng of λ DNA to 500 area units (and adjusting other values accordingly) the variations between the image intensity of different exposures is largely eliminated (Fig. 1c), indicating that the decrease in image intensity occurs uniformly across the gel from 0–600 ng of λ DNA. This type of normalisation procedure may be useful when comparing results from different gels, but is not necessary when the results are derived from individual gels, such as in the PFGE analysis of DNA dsb in mammalian cells.

To measure DNA dsb by PFGE, agarose plugs (37.5 µl), containing [3H]thymidine- labelled genomic DNA from 105 human fibroblasts, were irradiated with 137Cs γ rays (dose rate 3.1 Gy/min) and subjected to PFGE (4). After electrophoresis, the wells containing the agarose plugs were detached by cutting across the gel ~1 mm to the anode side of the wells. A further cut was made on the cathode side of the wells such that a 6 mm section containing the wells and agarose plugs was separated from the main body of the gel. This section was turned through 90° along its long axis so that the agarose plugs were lying with their maximum surface area uppermost. The whole gel was then

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transferred to 3MM paper, dried, stained with SYBR Green I and imaged (Fig. 2a).

For each dose of irradiation, the proportion of DNA that left the agarose plug and entered the gel (FAR, fraction activity released) was measured using GelBase software. After imaging by SYBR Green I staining, the gel was cut up and the proportion of \(^{3}H\)labelled DNA that had left the agarose plug and entered the gel was calculated by liquid scintillation counting. Representative results in Figure 2b show dose response curves for the induction of DNA dsb by \(\gamma\)-irradiation. These results, obtained from the same gel, were from analysis of SYBR Green I stained DNA or by scintillation counting. The excellent correlation shows that the SYBR Green I staining gives very similar results to radioactive prelabelling of genomic DNA. Therefore, the SYBR Green I staining technique allows the analysis of DNA dsb by PFGE without the need for radioactive prelabelling. In both techniques, the limit of detection of DNA dsb is \(\sim2.5\) Gy (Fig. 2b) using our standard PFGE conditions (4) and \(10^5\) cells per \(37.5\) \(\mu\)l agarose plug. Reducing the number of cells/plug reduces the sensitivity of the DNA dsb assay using SYBR Green staining because the background fluorescence becomes relatively more significant with lower amounts of DNA. The limit of detection is \(\sim10\) ng per track using the SYBR Green assay under the conditions described in the current work.

SYBR Green I offers significant advantages over EtBr staining methods. The most accurate EtBr staining methods for measuring DNA dsb after PFGE require a specialised silicon intensified tube camera connected to a digital signal processor. In quantitating DNA using this system, an ‘adjustment factor’ is required, and the value of this factor must be calculated for each individual gel (3). In addition, using EtBr can lead to significant underestimates of the amount of DNA. This effect is most apparent when low concentrations of DNA are spread out along the agarose gel track, as is the case for most PFGE methods for measuring DNA dsb (3).

In conclusion, SYBR Green I staining allows simple and accurate quantitation of DNA dsb using PFGE. This methodology will greatly simplify the study of the induction and repair of the critical DNA dsb lesion in non-cycling mammalian cells and primary biopsy material.

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