A SIMPLE AGAROSE-TRAP METHOD FOR DETECTION OF DNA FRAGMENTATION IN APOPTOTIC CELLS

Norazizah Shafee and Sazaly AbuBakar

Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

DNA fragmentation into oligonucleosomal DNA ladder is an important biochemical hallmark of a late stage apoptosis (1,2). It is normally demonstrated by a simple agarose gel electrophoresis of the total cellular DNA. The method widely used for preparation of the DNA, however, is tedious and is often complicated by the presence of high molecular mass DNA which makes the sample very viscous. The viscous DNA is difficult to handle and it often obscure the presence of the DNA ladders. Various methods to overcome this problem have been suggested. These include breaking up the DNA by repeated pipetting or by heating the DNA samples to 50°C. A number of commercial kits for harvesting apoptotic cells DNA are also available but most of them are costly and involve the use of hazardous chemicals such as phenol, chloroform and ethanol. We report here a rapid, simple, and environment-friendly method for harvesting DNA samples for detection of apoptotic oligonucleosomal DNA fragments.

Cells to be analyzed (1 x 10^5) were pelleted by centrifugation at 800 x g. This was followed by addition of 50 µl lysis solution containing 1% N-lauryolsarcosine (Sigma, USA), 0.2% sodium deoxycholate (Sigma, USA) and 1 mg/ml proteinase K (Sigma, USA) in L-buffer (0.01 M Tris-HCl, pH 7.6, 0.02 M NaCl, 0.1 M EDTA). The samples were then incubated overnight at 37°C. Following incubation and inactivation of proteinase K in the samples, RNase A (Sigma, USA; 1 mg/ml) was added and the samples were incubated for another 4 hours at 37°C. Since the purpose of the exercise was to separate the lower molecular weight DNA (<10 kb), to see the oligonucleosomal DNA ladders, it was desirable that the presence of the high molecular weight genomic DNA be reduced from the samples. DNA concentration was estimated using the GeneQuant (Pharmacia Biosciences, Sweden) and the samples (100 µl) was resuspended with cut-off pipet tips in an equal volume of 1.0% agarose (Promega, USA) kept molten at 42°C. The samples were immediately chilled at 4°C for 5 minutes or until the agarose solidified. The agarose-embedded samples were minced into small pieces using pipette tips and sterilized nuclease free water (1/10th volume) was then added. The samples were centrifuged at 20,000 x g for 2 minutes and the resulting supernatant was electrophoresed at 80 V for 1 hour in a 1.2% agarose gel. To visualize the DNA fragments, the gel was stained with ethidium bromide and observed under ultraviolet light at wavelength of 302 nm.

Comparisons between untreated (Figure 1A) and agarose-treated (Figure 1B) samples performed using similar DNA samples clearly demonstrated the benefits of the latter method. Lane 2 in both figures showed DNA samples from non apoptotic cells. In Figure 1A, the quantity of the high molecular weight DNA was not consistent with the remaining samples, though equal amount of DNA was supposedly loaded into the well. This could be due to inaccurate volume of sample was actually added as a result of the difficulty in pipetting the highly viscous DNA. Whereas, in Figure 1B a sharp band corresponded to the expected amount of DNA was noted. In a similar study, samples obtained from

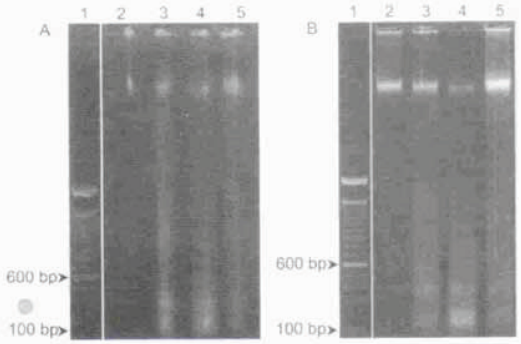

Figure 1. Agarose gel electrophoresis of DNA samples comparing the effects of trapping the high molecular weight DNA in agarose for detection of oligonucleosomal DNA fragments. Apoptotic and non-apoptotic DNA samples harvested as described in the text were electrophoresed in a 1.2% agarose gel at 80V for 1 hour. Samples untreated with agarose showed DNA smearing which obscured the DNA ladder (A). In comparison, samples electrophoresed following agarose treatment showed distinct DNA ladder (B). Lane 1: 100 bp ladder DNA marker; lane 2: DNA samples from non apoptotic cells; lanes 3-5: DNA samples from apoptotic cells.

Corresponding author:
Sazaly AbuBakar Ph.D.
Department of Medical Microbiology, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur, MALAYSIA
Tel: 603-7967-5756, Fax: 603-7967-5757
email: sazaly@ummc.edu.my

three different apoptotic cell preparations (Figure 1A, lanes 3, 4, 5) showed the presence of DNA smearing but not the expected DNA ladders. The corresponding samples, when embedded in agarose, showed distinct apoptotic DNA ladders. The DNA smearing and DNA ladders seen in both figures could not be due to the mincing of the agarose since no DNA smearing or ladders were noted in the similarly treated samples (Figures 1A and 1B, lane 1).

In conclusion, the agarose entrapment method described herein allowed accurate amount of DNA sample to be loaded into the agarose gel wells since the sample was non viscous and easy to be pipetted. Furthermore, the procedure was easy to perform without needing any specialized equipment or requiring organic extraction steps. Finally the technique is highly reproducible, thus, is suitable for isolating apoptotic DNA fragments in most cell types.

Acknowledgment

This study was supported partly by the Malaysian Government IRPA grant No. 06-02-03-0618.

References

1. Wyllie, A.H. Cell death: The significance of apoptosis. Int Rev Cytol 1980; 68: 251-306.
2. Arends M. J., R. G. Morris, A. H. Wyllie. Apoptosis and the role of endonuclaese. Amer J Pathol 1990; 136: 593-608.