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Microscope Slide for Enhanced Analysis of DNA Damage Using the Comet Assay


The comet assay is a powerful tool for evaluating damage to nuclear DNA. It is a relatively quick test and is sufficiently sensitive to detect small differences in DNA strand breaks (1,3). The technique has a wide range of applications and protocols (1–3). Generally, cells are embedded in low-melting-point agarose and layered on microscope slides. The cells are lysed, electrophoresed, stained with propidium iodide and examined using a fluorescence microscope.

Typically, fully frosted microscope slides that are uniformly roughened on one side are used to promote gel adhesion to the slide. These slides are troublesome in that they accumulate stain residue and create a level of background fluorescence that diminishes contrast. Removing the stain from the slides for reuse is labor-intensive, while consistent use of new slides is expensive and does not eliminate background fluorescence.

A simple but effective technique has been developed by our laboratory to maintain the adhesive advantage of the frosted slide while allowing for enhanced sensitivity. Using 19-mm-wide masking tape, standard microscope slides were carefully taped across the center of a metal tray in sets of five. These slides were cautiously sandblasted in a Zero-brand, Blast-in-peon sandblasting cabinet to roughen both ends of the slide without removing too much material. The tape was removed, and the slides were washed to eliminate residues. The resulting slides have rough ends for gel adhesion and a window of smooth glass in the center for analysis with augmented sensitivity.

We performed several comet assays to illustrate the advantage of the custom-frosted slides. Cells were damaged with 0.2 J/cm² of UV light to demonstrate the enhanced sensitivity. Along with controls, the damaged cells were assayed on both fully frosted and custom-frosted...
Benchmarks

slides. Three replications were performed with over 200 comets analyzed per slide type. The results, obtained by computerized analysis, showed that the damaged DNA was significantly more quantifiable on the custom slides ($p = 0.0154$). Cells receiving the UV treatment registered an average of 1.42 tail-moment units (see Reference 1 for an explanation of tail moment) higher on custom slides (3.82 vs. 2.40). Because only tail moments greater than 2 are regarded as significantly damaged, and typical undamaged controls are approximately 0.5, this kind of increase could discriminate mildly damaged cells from undamaged ones. Other experiments, using more considerably damaged DNA, exhibited as high as 5.21 U difference in favor of the custom configuration. We attribute this enhanced sensitivity to the decreased background fluorescence, which provides greater contrast and allows for increased gain settings during analysis.

REFERENCES


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The SSCP Phenomenon: Addition of HEPES Buffer Dramatically Affects Electrophoretic Mobility


To our knowledge, analysis of single-strand conformation polymorphism (SSCP) (5) is the most widely used means of mutation scanning. SSCP reveals single-base sequence changes because altered electrophoretic migration of one or both single strands on a non-denaturing gel can be detected. SSCP does not detect all sequence changes with one set of electrophoresis conditions, and sensitivity of detection is a complex function of parameters such as sequence and size (1). The sensitivity of mutation detection by SSCP can be increased by using two electrophoresis conditions, such as electrophoresing at 20° and 8°C. This approach has some disadvantages including: (i) smearing of segments at 8°C (especially if the GC content is ≥55%); and (ii) inconvenience of loading samples and performing experiments in a cold room.

We show that the addition of 20 mM HEPES to standard 50 mM TBE buffer (HTBE) substantially alters SSCP mobilities, allowing segments to be analyzed under two convenient and essentially independent room temperature conditions.

To compare HTBE and TBE electrophoresis buffers, we analyzed a 1-kb region from exon H of the human factor IX gene [from bp 30 646 to 31 645 using the numbering system of Yoshitake et al. (7)]. Twenty-six overlapping

![Figure 1. Schematic of overlapping PCR and SSCP fingerprinting. A 1000-bp genomic DNA region of exon H is first amplified with primers A and B. The first nucleotide of the amplified segment corresponds to No. 30 738 in the numbering system of Yoshitake et al. (7). An overlapping PCR is performed with six downstream and six upstream primers to produce the twenty-six double-stranded overlapping DNA segments. Each primer contains a short sequence-specific region and a noncomplementary 5' tail that prevents short segments from megaprimering to produce longer segments in subsequent cycles of PCR (6). The locations of the 5' end of the six downstream primers and the six upstream primers are indicated; e.g., downstream primer D2 begins at nucleotide 116 from the PCR product AB, while the 5' end of the upstream primer U1 is at nucleotide 840. The 26 amplified segments are shown along with their sizes.](image-url)