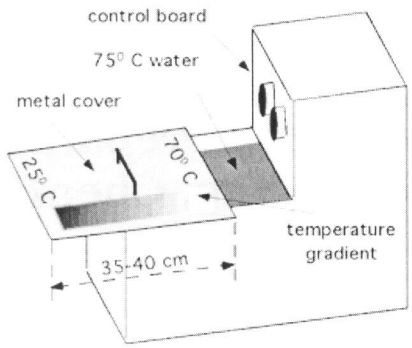

FIG. 1. Waterbath image showing metal plate position, allowing both the temperature gradient formation and access to the hot steam.

MATERIALS AND METHODS

Equipment

The equipment (Figs. 1,2) required for the metaphase spreading protocol includes (1) a source of hot steam (waterbath at 75–80°C); (2) a heated metal plate, ideally with a temperature gradient across its surface. To achieve this, the metal plate/lid covers the waterbath only partially (Fig. 1) and carries a temperature gradient between the hot end and a cold end. A 2–3-mm thick metal plate is ideal. We tested plates made of stainless steel (3 and 1.5-mm thick) and copper (1-mm thick). When the plate rests about 2 cm from the hot water surface, the part of the plate in contact with the hot steam shows roughly 70°C, whereas the other end of the plate (10–15 cm long) is at room temperature. If such a metal plate is not available, a minimum of two surfaces, one at 65–75°C (a simple heat block), and one at room temperature are required; and (3) good quality slides. Two brands of precleaned slides, Gold Seal (Becton Dickinson, Portsmouth, NH) and Superfrost (Erie Scientific, Portsmouth, NH), were commonly used. They did not require any extra cleaning steps or washes prior to use. Cell suspensions were dropped on slides taken directly out of their original boxes. Lower quality glass slides can be passed through successive washes in acetone, HCl/ethanol, and triple distilled water and their quality compared.

Chromosome Preparation

Cell culture and fixation. Cell suspensions from peripheral blood, fibroblasts, bone marrow, lymphoblastoid cell lines, and germ cell tumors were used to prepare metaphases for G-banding and FISH. Cell culture was performed according to standard protocols (1). For harvesting, the hypotonic buffer used was 0.075 M KCl, at 37°C for 10–20 min (incubations were shorter for peripheral blood cultures, longer for tumors). Hypotonic treatment increases cell volume and disrupts the cell membrane of the red blood cells (allowing their removal).

After hypotonic treatment, usually in 15-ml centrifuge tubes, cells were pelleted by centrifugation 10 min at 1,000 rpm, resuspended in 1–1.5-ml fixative (3:1 methanol-to-acetic acid concentration), and transferred into 1.5-ml vials. All subsequent fixative washes were done in these small vials, with centrifugations performed in a tabletop microfuge for 1–2 min at 6,000–7,000 rpm.

Chromosome spreading and G-banding. Cells in fixative were diluted at the appropriate density, empirically determined in any cytogenetic laboratory, to produce a reasonable number of well-spread metaphases in each microscopic field. With an automatic pipette, 25–35 μl of cell suspension was evenly distributed on several locations on the slide and the liquid was spread by gently moving the pipette tip parallel to the surface. As the fixative gradually evaporated, the surface of the slide became grainy (cells visible). At that moment, the slide was placed face down into the steam of the hot water bath (75°C or more) for 1–3 s, then dried by placing the slide on the metal plate (carrying a gradient of temperature across its surface; Fig. 1). The degree of spreading is adjusted using the different temperatures of the heated plate, with higher temperatures increasing chromosome spreading. For difficult-to-spread cells, after the surface became grainy, the slide was passed briefly through the water vapors, then four to six droplets of acetic acid were placed on the slide. After the acetic acid slowly spreaded and covered the surface, the slide was held 3–5 s in the steam of the waterbath, then quickly dried on the hottest area of the metal plate or the metal block (65°C). After overnight incubation at 65°C (aging), G-banding was performed according to a standard laboratory procedure (1).

FISH: Chemical Aging and Slide Denaturing

Chemical aging. A freshly prepared slide was placed on the metal block of a thermocycler (a polymerase chain reaction [PCR] machine; Fig. 2). Ethanol (150–200 μl) was pipetted on the slide, covered with a coverslip, and ethanol-soaked gauze was placed on top to prevent ethanol evaporation. A plastic lid (for example, from a box of pipette tips) was used to cover the slide-gauze assembly. The block was programmed to increase the temperature to 94°C, to keep it for 2–20 s, and to cool it to room temperature. Depending on the machine, the heating and cooling speed was 1–2°C/s. Alternatively, the slide can be incubated 10–15 s each, in jars with ethanol at 50, 75, 94, 75, and 50°C, followed by drying at room temperature.

After aging, the slides were subjected to brief (30–60 s) pepsin pretreatment, using 0.005% pepsin in 0.01 N HCl. This was followed by rinsing in PBS, ethanol series and air drying.

Gradual denaturing. In (a) simultaneous protocol (Fig. 6c), 11–12 μl labeled DNA probe in hybridization buffer was pipetted on the slide, covered with a 22 × 22-mm coverslip, and sealed with rubber cement. The slide was placed on the metal block of a thermocycler, which was programmed to gradually (within 90 s) heat the slide to 75°C, to keep that temperature for 90–120 s, and to gradually (90 s) cool to room temperature.

In (b) the separate protocol (Fig. 6f,g), labeled DNA probe was denatured 5 min at 75°C in a waterbath. For slide denaturing, 150 μl of 70% formamide/2 × SSC was

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