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High-quality CGH slides irrespective of the lymphocyte suspension used

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▼Comparative genomic hybridization (CGH) is a highly efficient tool for acquiring a genome-wide screening of chromosomal copy number changes within a single experiment (for first description, see Ref. 1; for a recent review of CGH results in solid tumors, see Ref. 2) without the need for mitotic cells. The quality of the metaphase chromosomes used for CGH is crucial, whereas the technical equipment (e.g. CCD camera or image analyzing system) are not that important (Ref. 3,4). CGH chromosomes have to be much 'harder' than chromosomes used for usual metaphase fluorescence *in situ* analysis (FISH) (Ref. 5); 'weak' chromosomes have no shape after denaturation, whereas 'hard' chromosomes still look like chromosomes after the heat treatment.

CGH slides are normally produced by introducing not very reproducible variations into the chromosome preparation protocol until suitable 'optimal' chromosomes are achieved. To overcome this problem, a simple and highly efficient method for 'hardening' of chromosomes for CGH approaches has been developed. It consists of a combination of a modified slide pretreatment procedure (Ref. 6) and a rapid denaturation assay (Ref. 5). As shown in Fig. 1, this kind of pretreatment hardened even the most fragile chromosome preparations, as tested on ten suspension samples that were previously unsuitable for any kind of CGH (an example of a CGH result is shown in Fig. 2). However, it has to kept in mind that some laboratories have an opposite problem, with chromosomes that are too 'hard'; this technique is not suitable for these labs.

In one CGH study on ovarian cancer (Ref. 7), commercially available CGH slides (Vysis) were compared with those produced by the method mentioned above. The commercially available slides have quality problems (differences between batches can appear) and so the comparison was performed on a high-quality batch. No differences in the resulting CGH pattern could be observed.

A detailed description of the chromosome-hardening procedure has not been given in detail before. In our hands, lymphocytes subjected to the hardening procedure are not suitable for other FISH approaches. Conversely, the technique is very helpful in hardening 'fragile' chromosomes from amniocytic fluid. Single-copy probes, centromeric probes and whole and partial chromosome painting probes hybridize well after the pretreatment described below.

1. Protocol

1.1. Slide pretreatment

Place drops of any chromosome preparation on slides, let them air dry and carry out prefixation the following day.

Prefix metaphase chromosomes on the slide surface by incubation in 100 ml formalin buffer for 10 min at room temperature, in a coplin jar. Formalin buffer contains 3% v/v acid-free formaldehyde (37%; Roth 4979.1) in 1× PBS pH 7.0 (Dulbecco 9.55 g l^{-1}).

Put the slides in 2× SSC (prepared from 20× SSC; Gibco-BRL 15557-036) in a 100 ml coplin jar for 5 min at room temperature.

Remove slides from the coplin jar, add 100 μl of RNase solution to each slide and cover with a 24 × 50 mm coverslip. RNase solution contains 100 μl 2× SSC plus 1 μl RNase stock solution, made fresh as required. RNase stock solution contains 5 μg μl^{-1} RNase type A (Boehringer 109142) dissolved in filtered, double-distilled water.

Incubate the slides in a moist chamber for 15 min at 37°C.

Put slides back into the coplin jar with 100 ml 2× SSC at room temperature and remove the coverslips using forceps. Leave slides in 2× SSC solution for 3 min.

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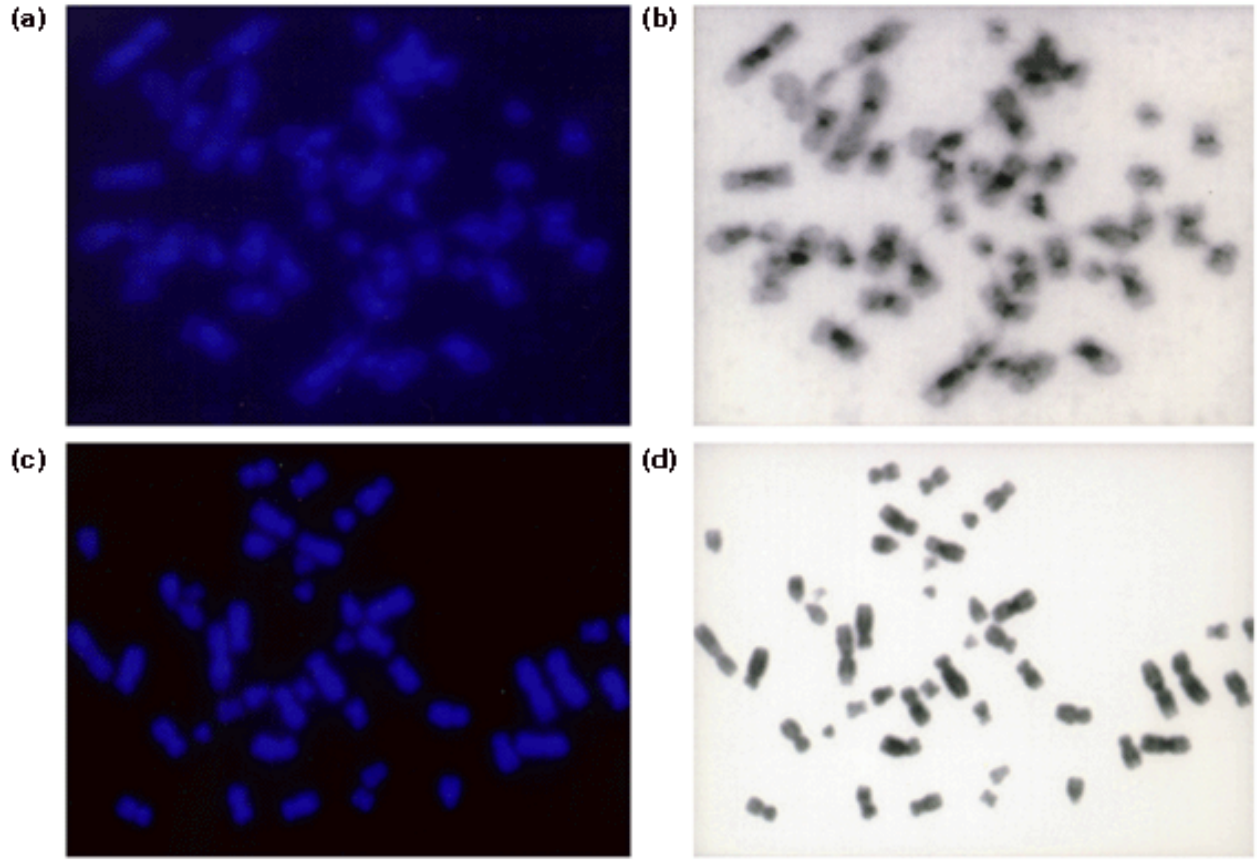

FIGURE 1. Typical result of one of the ten tested lymphocyte suspensions. Images were captured with the ISIS digital imaging system (MetaSystems, Altlussheim, Germany) using a XC77 CCD camera with on-chip integration (Sony) and a Zeiss Axiophot microscope. (a, b) A DAPI counterstained metaphase spread without prefixation after a denaturation time of only 2 min. (c, d) Another metaphase spread of the same suspension after prefixation and a prolonged denaturation time (6 min). (a, c) Blue diamidinophenylindol (DAPI) counterstaining; (b, d) inverted DAPI banding. In the metaphase preparations without prefixation, no DAPI banding pattern can be obtained and there is a high plasma background. In the prefixed metaphases, no disturbing plasma background can be observed. Moreover, an easily evaluated DAPI banding pattern is visible, which is necessary for a reliable CGH analysis, even though the denaturation time has been extended from 2 min to 6 min, which is necessary for bright CGH signals (Fig. 2).

Discard 2× SSC and replace it with 100 ml 1× PBS at room temperature for 5 min.

Replace 1× PBS with 100 ml prewarmed fresh pepsin buffer (37°C) and incubate the slides for 10 min at 37°C, without agitation. Pepsin buffer is produced by adding 1 ml of 1 M HCl to 99 ml distilled water and incubating at 37°C for ~20 min, after which 50 μl of 10% (w/v) pepsin stock solution is added. The 10% pepsin stock solution contains 100 mg pepsin (Serva 31855) in 1 ml filtered, double-distilled water, dissolved at 37°C, aliquoted and stored at −20°C.

Replace fluid with 100 ml 1× PBS, incubate at room temperature for 5 min with gentle agitation.

Postfix nuclei on the slide surfaces by replacing 1× PBS with 100 ml of formalin buffer for 10 min at room temperature.

Replace formalin buffer with 100 ml 1× PBS and leave for 2 min at room temperature.

Dehydrate the slides in an ethanol series (70%, 90%, 100%, 3 min each) and air dry.

1.2. Suitability test for CGH

To test the suitability of the slides for CGH, add 100 μl denaturation buffer to each slide surface and cover with 24 × 50 mm coverslips.

Incubate slides on a warming plate for 6 min at 75°C.

Remove the coverslips using forceps and place slides in a coplin jar filled with 70% ethanol, dehydrate slides in an ethanol series (90%, 100%, 3 min each) and air dry.

Counterstain the slides by adding 15 μl antifade (Vectashield, Camon Vector Laboratories, H1000) and diamidinophenylindol (DAPI; Serva, 18860) at 200:1,

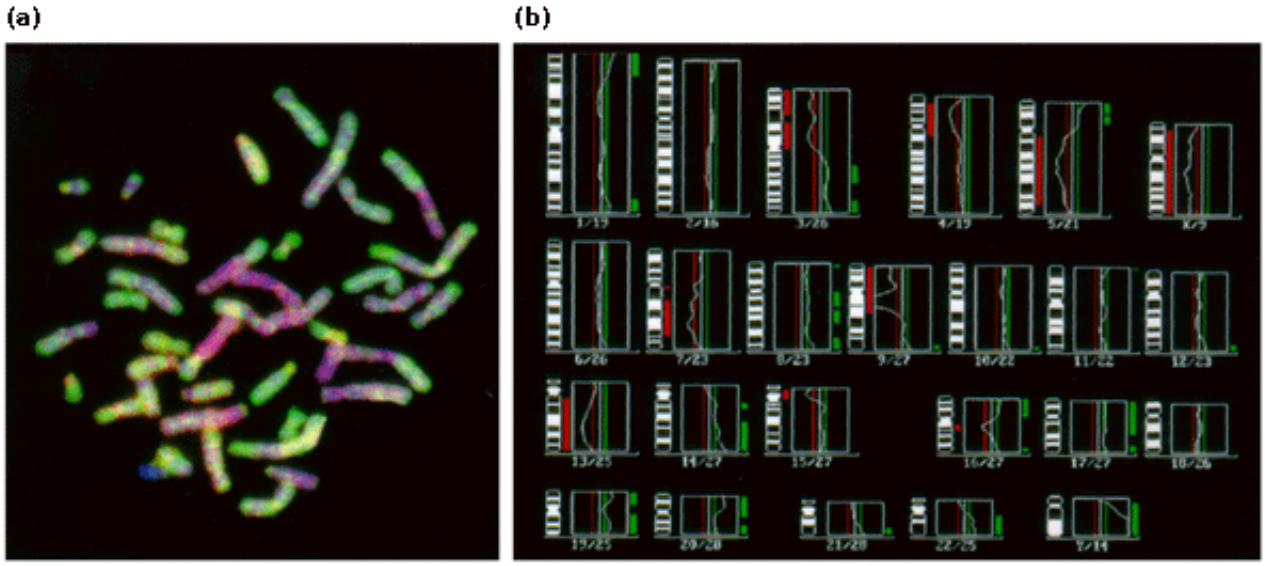

FIGURE 2. CGH using metaphase spreads pretreated as described. (a) A metaphase spread pretreated as described, after CGH using 'male' tumor DNA of an oral squamous cell carcinoma (internal number K98), labeled in green, and 'female' control DNA labeled in red. (b) The summarizing CGH profile for K98 of 15 metaphase spreads is shown as produced by the ISIS/IKAROS digital imaging system (MetaSystems, Altlussheim, Germany). More detailed CGH results on oral squamous cell carcinomas are summarized in Ref. 2.

cover with coverslips and look at the results under a fluorescence microscope.

A counterstained standard slide can be compared to the test slides. When they match, the samples can be used in a standard CGH experiment (e.g. Ref. 7).

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Products Used

ISIS digital imaging system: ISIS digital imaging system from Jackson ImmunoResearch Laboratories Inc

CCD camera: CCD camera from Photometrics

Axiophot: Axiophot from Carl Zeiss

RNase: RNase from Sigma

vectashield: vectashield from Vector Laboratories Inc

antifade: antifade from Oncor Inc

DAPI: DAPI from Sigma