Benchmarks

obtained without a phenol/chloroform extraction; however, fewer errors, greater signal strength and longer reads are obtained from the inclusion of a phenol/chloroform extraction before the butanol precipitation (Figure 1, A and C).

In conclusion, this paper presents a simple dye terminator sequencing product purification protocol. This protocol is standardly used in our laboratory and provides a number of advantages over the ethanol precipitation procedure. These include higher quality sequencing data, the requirement of less time and pipetting steps, and it avoids the possibility of salt precipitation and thus, the need for a 70% ethanol wash.

REFERENCES

stead have based the identification of a binucleate cell on the close proximity of two nuclei each having roughly the same size and shape (4). Without cytoplasm to distinguish one cell from another, these scoring criteria are highly subjective with respect both to the identification of binucleate cells and to the origins of a given micronucleus. In addition, this difficulty necessitates low cell density as a means of reducing ambiguities in association, greatly reducing scoring efficiency and increasing costs. These concerns prompted the development of the in situ protocol described here that allows a directly labeled whole-chromosome paint to hybridize with interphase nuclei and micronuclei while maintaining binucleate cell cytoplasm.

Cell Culture and Fixation

The procedure was developed using the normal human male cell line, AG1522 (National Institute on Aging [NIA] Cell Culture Repository at the Coriell Institute for Medical Research, Camden, NJ, USA). Cells were grown at 37°C in flasks containing Dulbecco’s modified Eagle medium (DMEM) or Ham’s F-12 culture medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 15% fetal calf serum (Life Technologies), 2 mM glutamine and 25 µg/mL gentamicin sulfate (Life Technologies) and incubated in a humidified atmosphere containing 2% CO₂ in air. To form binucleate cells, fibroblasts were cultured in 5 µg/mL Cytochalasin B (Sigma, St. Louis, MO, USA) for 72 h. Note that 72 h incubation was necessary to generate a sufficiently high binucleate cell frequency (typically 45%–55%). The cells were then detached using 1× trypsin-EDTA solution (Sigma) and separated from the medium by centrifugation. Following one wash in phosphate-buffered saline (PBS, pH 7.4), the cells were resuspended in 1% sodium citrate and incubated for 20 min at 37°C. The cells were recovered by centrifugation, resuspended in 5% glacial acetic acid and incubated for 10 min at room temperature (RT). The suspension was centrifuged, and the cells resuspended at RT in 3:1 methanol, glacial acetic acid Fixative Solution. Samples were held for 10 min at RT then stored at -20°C for future use.

Slide Preparation

Premium Microscope Slides (Fisher Scientific, Pittsburgh, PA, USA) were first cleaned by soaking in a solution of 62.5% ethanol, 22.5% H₂O and 15% HCl for 24 h and then dried and stored in 100% ethanol at -20°C until needed. Fixed cells were recovered by centrifugation and resuspended in a minimum volume of fresh Fixative Solution to yield the desired cell concentration. Slides were removed from the ethanol and dipped in H₂O that had been treated with a Milli-Q® Ultrapure Water System (Millipore, Bedford, MA, USA) at 0°C until the water sheeted.

Figure 1. Fixed binucleate fibroblast probed with SpectrumGreen whole chromosome paint specific for chromosome 4 (C) and counterstained with DAPI (A) and propidium iodide (B). (D) Composite image of chromosome-4 probe (C) and DAPI (A). Arrowhead indicates a micronucleus containing a portion of chromosome 4. Arrows show micronuclei lacking signal. Images were captured under epifluorescence using the appropriate single band-pass filter set in an Axioshot 2 epifluorescence microscope with a DXC-950i Sony® 3CCD Color Video Camera attached. Processing and image overlay (D) was done using Northern Eclipse 2.0 Image Analysis Software (Empix Imaging, Mississauga, ONT, Canada).
cleanly from the surface. The concentrated cell suspension was then aliquotted (20–30 µL) directly into the sheeting water, and the slide was flushed with Fixative Solution, blotted and dried over a 60°C water bath for 1–2 min. Phase contrast microscopy was used to confirm the presence of intact cytoplasm and desired cell density. Slides prepared in this manner should be used for FISH within one week. Optimal FISH is achieved using 0–2-day-old slides, whereas slides could be stored for FISH within one week. Optimal results in lymphocytes require the cells to be preserved with a fixative containing formalin, and cellular denaturation might then require up to 10 min at 71°C.

The reaction was quenched by dipping the slides sequentially for 2 min in each of 70%, 80% and 95% ethanol solutions at 0°C followed by air drying. The hybridization mixture contained a probe for chromosome 4 (Whole Chromosome Paint 4 SpectrumGreen®; Vyysis, Downers Grove, IL, USA) and was prepared and then applied according to the manufacturer’s recommendations. The slides were then placed in a sealed box humidified with the Denaturating Solution and incubated at 37°C for 18 h. Following hybridization, unbound probe was removed by sequential 5-min, 46°C washes in 50% formamide, 2× SSC solution (pH 7.0) and then in 2× SSC (pH 7.0). After a final 2.5-min wash with 2× SSC, 0.10% Nonidet® P-40 (pH 7.0) at 46°C, the slides were removed and immediately counterstained with a 3:1 mixture of 4′,6-diamidine-2-phenylindole (DAPI; 0.1 µg/mL) and propidium iodide (2.5 µg/mL) (both from Oncor, Gaithersburg, MD, USA).

Figure 1 shows an example of a binucleate-fibroblast cell prepared and probed under these conditions. The DAPI, propidium iodide and SpectrumGreen fluorophore (Figure 1, A, B and C, respectively) were visualized individually under 630× magnification using the appropriate single band-pass filter set in an Axioshot® 2 Epifluorescence Microscope (Carl Zeiss Canada, Don Mills, ONT, Canada). The use of DAPI, a DNA-specific fluorophore that fluoresces blue, was found to assist with accurate identification of micronuclei. Propidium iodide was used to identify the preserved cytoplasm (Figure 1B), staining it and the nucleus red/orange.

**REFERENCES**


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