

TECHNICAL NOTE

SIMPLIFIED AG-STAINING TECHNIQUE FOR NUCLEOLAR ORGANIZING REGIONS AND NUCLEOLI

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The Ag-staining technique now used for nucleolar organizing regions (NORs) and nucleoli was first described by Goodpasture and Bloom (1975) and Howell *et al.* (1975). NOR specificity to this type of staining was shown in nine mammal species previously submitted to *in situ* hybridization with ribosomal RNA (Goodpasture and Bloom, 1975). The use of Ag-staining rapidly became widespread, leading to a great accumulation of data on the morphological and physiological patterns presented by these structures in many organisms.

Several modifications of the Ag-staining technique have been developed since that time. Today the most commonly used is that of Howell and Black (1980). In this technique two solutions are used. One is prepared by completely dissolving one g of gelatin in 50 ml of deionized hot water and adding 0.5 ml of formic acid (solution A). The other is prepared by dissolving one g of silver nitrate in two ml of deionized water and filtering through filter paper (solution B). Both solutions are kept in dark bottles, wrapped in aluminum paper. For staining, two drops of solution B and one of solution A are dropped onto the prepared slides, covered with a coverslip and placed in an incubator or on a hot plate at 70°C for about eight minutes, after which the mixture turns a golden brown color.

Boquist (1990) mixed the two solutions described in Howell and Black (1980) and filtered them through a 0.22 μm Millipore filter under safelight conditions. After filtering, the mixture is immediately dropped onto the slides and these are incubated for 35 minutes at room temperature in a dark-room.

We have modified the Boquist technique as follows: the A and B solutions, prepared as in the Howell and Black, are dropped onto the slides in the same proportions of 1A:2B, the drops mixed by moving each slide carefully (the coverslip is unnecessary) and the slides put into a dark chamber for about 40 minutes. The dark chamber may be any box which closes well. The slides are then washed in running, deionized water and air-dried before mounting. Since slides are not heated chromosome structure is better preserved.

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REFERENCES

- Boquist, L.L.V. (1990). Nucleolar organizer regions in normal, hyperplastic and neoplastic parathyroid glands. *Virchows Archiv. A. Pathol. Anat.* 417: 273-241.
- Goodpasture, C. and Bloom, S.E. (1975). Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma* 53: 37-50.
- Howell, W.M., Denton, T. and Diamond, J.R. (1975). Differential staining of the satellite regions of human acrocentric chromosomes. *Experientia* 31: 260-262.
- Howell, W.M. and Black, D.A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: 1-step method. *Experientia* 36: 1014-15.

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