

University of Groningen

Isolated metaphase chromosomes stabilized by DNA-intercalation or polyamine additon

Buys, Charles H. C. M.; ATEN, JA; Koerts, T; Osinga, Jan; van der Veen, Anneke Y

Published in:
Cell Biology International Reports

DOI:
[10.1016/0309-1651\(84\)90149-8](https://doi.org/10.1016/0309-1651(84)90149-8)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1984

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Buys, C. H. C. M., ATEN, JA., Koerts, T., Osinga, J., & van der Veen, A. Y. (1984). Isolated metaphase chromosomes stabilized by DNA-intercalation or polyamine additon: A comparison. *Cell Biology International Reports*, 8(4), 273-273. [https://doi.org/10.1016/0309-1651\(84\)90149-8](https://doi.org/10.1016/0309-1651(84)90149-8)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

ISOLATED METAPHASE CHROMOSOMES STABILIZED BY DNA-INTERCALATION OR POLYAMINE ADDITION: A COMPARISON

Charles H.C.M. Buys¹, Jacob A. Aten², Trijntje Koerts¹, Jan Osinga¹, and Anneke Y. van der Veen¹

¹ Department of Human Genetics, State University of Groningen, The Netherlands

² Laboratory for Radiobiology, University of Amsterdam, The Netherlands

Interest in the isolation of metaphase chromosomes has increased by the recent application of flow cytometry to chromosome suspensions to detect aberrations or to sort specific chromosomes. A most critical aspect of chromosome isolation procedures is the stabilization of chromosomes upon release from the cells. A recent procedure includes addition of polyamines to the surrounding medium for stabilization (1,2). In a very simple procedure we use DNA-intercalators for the same purpose (3-5). Here, we compare the morphological and biochemical characteristics of chromosomes stabilized by these methods, as well as their suitability to flow karyotyping.

The intercalated chromosomes were much longer than those stabilized by polyamines. Using a Chinese hamster cell line, DON Wg3-h, which contains one easily recognizable subtelo-centric chromosome we measured the length of this chromosome after isolation, fixation on a slide, and air-drying. Dependent on the intercalator, this resulted in a 26% to 76% difference between intercalated chromosomes and those isolated with polyamines (Fig. 1).

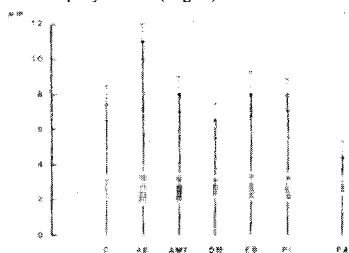


Fig. 1. Length measurements (mean \pm S.D., $n=20$) of a specific chromosome isolated in the presence of the intercalators adriablastin (AB), a psoralen derivative (AMT), daunomycin (DM), ethidium bromide (EB), and propidium iodide (PI), and of the same chromosome isolated in the presence of polyamines. After isolation the chromosomes were fixed on slides. As a control (C) the same chromosome fixed within cells was measured.

The less condensed appearance of the intercalated chromosomes did certainly not detract from their suitability for flow karyotyping. Irrespective of the isolation procedure, a very good resolution of peaks in the flow histogram was obtained (Fig. 2).

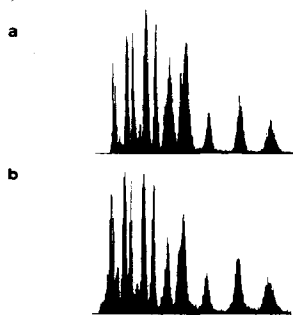


Fig. 2. Flow karyograms of chromosomes isolated in the presence of the intercalator propidium iodide (a) or in the presence of polyamines (b) from V79 Chinese hamster cells.

Received: 13th February 1984.

DNA extracted from samples of chromosomes isolated by each of both methods and subsequently electrophoresed in agarose appeared to be about 100 kb long. (Fig. 3). Patterns of chromosomal proteins resulting after SDS-polyacrylamide electrophoresis, fixation, and silver staining were very similar with the exception of a few specific bands. The propidium iodide-chromosomes gave an extra band at about MW 27,000 which was absent in the pattern from the polyamine-chromosomes, whereas the latter gave extra bands at MWs 33,000 and 52,000 (Fig. 4).

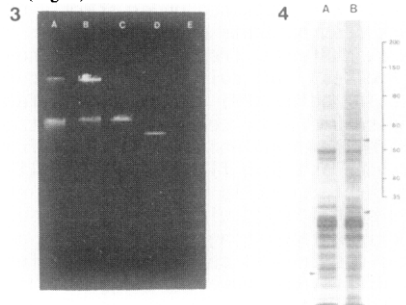


Fig. 3. Size determination of DNA extracted from chromosomes isolated in the presence of the intercalator propidium iodide (A) or in the presence of polyamines (B) in 0.25% agarose. For comparison DNA from bacteriophages HI1 (120 kb) and lambda (49 kb), and an Eco RI digest from the latter DNA (largest fragment 23 kb) were coelectrophoresed (C, D, and E, respectively).

Fig. 4. Silver-stained pattern of chromosomal proteins extracted from chromosomes isolated in the presence of propidium iodide (A) or polyamines (B) after electrophoresis in a 10% SDS-polyacrylamide gel. Molecular weights $\times 10^{-3}$.

In conclusion, both methods are equally suitable for the isolation of metaphase chromosomes to be used for biochemical studies. Although they can also both be applied very successfully for flow karyotyping, the intercalation method should be preferred because - in contrast to the polyamine method - it has been shown to be compatible with banding of the chromosomes allowing the direct identification of the vast majority of them (4,5).

References

- Blumenthal, A.B., Dieden, J., Kapp, L.N. and Sedat, J.W. (1979) Rapid isolation of metaphase chromosomes containing high molecular weight DNA. *Journal of Cell Biology* **81**, 255-259
- Sillar, R. and Young, B.D. (1981) A new method for the preparation of metaphase chromosomes for flow analysis. *Journal of Histochemistry and Cytochemistry* **29**, 74-78
- Aten, J.A., Kipp, J.B.A. and Barendsen, G.W. (1980) Flow-cytofluorometric determination of damage to chromosomes from X-irradiated Chinese hamster cells. *Acta Pathologica Microbiologica Scandinavica Supplement* **1980**, 287-292
- Buys, C.H.C.M., Koerts, T. and Aten, J.A. (1982) Well-identifiable human chromosomes isolated from mitotic fibroblasts by a new method. *Human Genetics* **61**, 157-159
- Buys, C.H.C.M., Koerts, T. and van der Veen, A.Y. (1984) Banding of unfixed mitotic chromosomes in suspension after release from human lymphocytes and fibroblasts. *Human Genetics*, in press

Accepted: 26th March 1984.