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Photomap of the polythenic chromosomes of Drosophila and in situ mapping

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We use this protocol and it's working

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- 1 Making the photomap of polythenic chromosomes
- 2 1 The slides of politenic chromosomes of *D. malerkotiana* of the population of Parque Dois Irmãos (Recife) were prepared by crushing the dissected salivary glands of zero-hour pre pupae, fixed with a solution of acetic acid, water and lactic acid, in a 3:2:1 ratio. The material was stained with aceto-lactic orcein (1g of orcein-MERCK, 45ml of acetic acid, 25ml of lactic acid 85%, and 30ml of distilled water), according to ASHBURNER (1967). The best slides were photographed under Leica Microscope in increase of 100 X 1.25 with Kodak TMAX Iso 100 film.
- 3 In situ hibridization
- 4 For in situ hybridization, the ENGELS et al. (1986) technique was used. After dissection of the larvae, the salivary glands were fixed in 45% acetic acid and crushed with cover slip in a lactic-acetic solution (lactic acid, water and acetic acid, 1:2:3). The slides were stored at 4°C for at least 18 hours. The slides were removed and the slides were submitted to several washes in 2X SSC and ethanol. The slides were air dried and stored at 4°C. After these steps, the best slides were selected and only those with intact chromosomes, spread properly, were stored for further hybridization.
- 5 For the marking and mapping of the Hsp83 locus in *D. malerkoltiana*, the clone lambda 6 of the Hsp83 gene of *D. melanogaster* (HOLMGREN et al., 1981), inserted in the plasmid pBR322, was used as probe. The plasmid was amplified by the transformation of the Xm1 strain of the bacterium *Escherichia coli*, cultivated in LB medium with ampicillin (100°g/ml) (SAMBROOK et al., 1989) and was extracted by non-phenolic method (PHILIPPSEN et al., 1991).
- 6 To prepare the probe, 1µg of the plasmid containing the gene fragment, marked with biotin by nick-translation with gibco's Bionick kit, was used. The probe marking was tested in "dot blot" with revelation with Estreptavidin – Alkaline Phosphatase (SA-AP), Nitroblue tetrazolium chloride (NBT), 5-bromine-4-chloro-3-indolylphosphate p-Toluidine salt (BCIP). 500ng of probe per blade was used under high astringency conditions (temperature of 36° C and 50% of formamide) for 48 hours. The revelation was performed with SA-AP, NBT and BCIP, followed by the lacto-acetic orcein counter-dye at 0.2%. After drying the blades, the material was permanently assembled with Entellan.