COPAS Select can collect the non-fluorescent embryos, thereby producing a nearly pure population of homozygous embryos. Use of a less stable form of GFP (Xianqiang *et al*., 1998) and a stage specific promoter should allow collection of embryos at a specific developmental stage. These embryos can then be allowed to grow to the developmental stage of interest and examined. COPAS Select technology also allows the use of GFP, YFP or RFP fluorescence markers. These markers can be detected simultaneously, allowing one to select for multiple characteristics in a single sorting run.

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References: Bell, L.R, E.M. Maine, P. Schedl, and T.W. Cline 1988, Cell 55: 1037-1046; Casso, D., F. Ramirez-Weber, and T.B. Kornberg 2000, Mech. Dev. 91: 451-454; Frohman, M.A., M.K. Dush, and G.R. Martin 1988, Proc. Natl. Acad. Sci. USA 85: 8998-9002; Halfon, Marc S., S. Gisselbrecht, J. Lu, B. Estrada, H. Keshishian, and A.M. Michelson 2002, Genesis 34: 135-138; Keyes, L.N., T.W. Cline, and P. Schedl 1992, Cell 68: 933-943; Samuels, M.E., P. Schedl, and T.W. Cline 1991, Mol. Cell Biol. 11: 3584-3602; Xianqiang, L., Z. Xiaoning, Y. Fang, X. Jiang, T. Duong, C. Fan, C.C. Huang, and S.R. Kain 1998, J. Biol. Chem. 273: 34970-34975.

A simple method to prepare DNA fibres in the male germ line.

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Cytologic analysis of the male germ line of *Drosophila melanogaster* has been extensively developed in the past years (Lindsley and Tokuyasu, 1980; Cenci *et al.*, 1994). In slides prepared in this way, cellular structures are preserved before and after fixation procedures, and DNA organization in germ cells is largely maintained. A critical step during slide preparation is to avoid excessive spreading of the tissue and the consequent loss of cell identity. For this, testes must be gently squashed between slides and coverslips. To obtain that, usually a small drop (3 to 4 µl) of buffer is put on the coverslip, then testes are carefully transferred inside it after dissection, and finally the slide is put on it upside down; capillarity permits obtaining good squashes in a few seconds. In case of too small a drop, waiting up to 1-2 minutes is still sufficient for a good spreading. However, this kind of preparation does not permit analysis of single DNA fibres, since DNA organization inside nuclei is preserved. Fibres of DNA must be prepared by stressing cells, to break down nucleus organization.

Several attempts using different experimental conditions permitted the conclusion that it is possible to achieve this result by strongly pressing the coverslip over the slide, after the previously described tissue spreading. Best results are obtained by an orthogonal pressure, without moving the coverslip laterally; in this way a sufficient number of DNA fibres become visible. Instead, lateral movements completely destroy the tissue and avoid using it any further. The strength to be used in these preparations should be similar to that used for larval brain squashes. During this preparation, cellular organization is no more visible under a phase contrast microscope, and tissues generally appear like a layer of uniform material. However, most of the nuclei preserve their organization and are comparable to those described by Cenci and coworkers (1994) after DAPI or Hoechst staining (Figure 1 A and B). The most delicate cells in the male germ line are primary spermatocytes, which undergo quite a long maturation stage lasting 90 hours, during which the nuclear volume increases its size 30 times. Inside mature primary spermatocyte nuclei, DNA is less compact that in other cells, and it is organized in three chromatin clumps corresponding to the three couples of the major

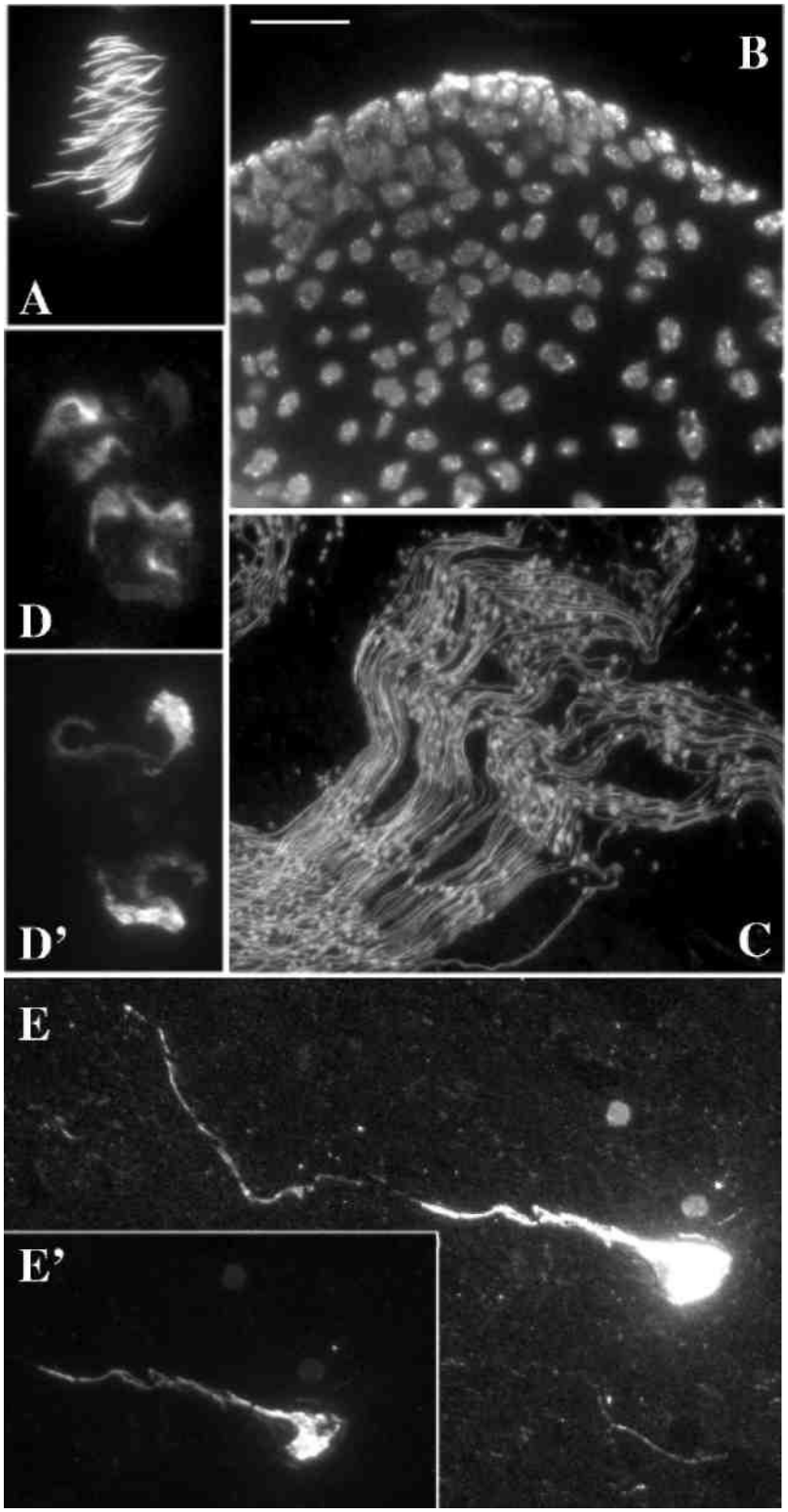

Figure 1. Microphoto-graphs of slides prepared for the analysis of DNA fibres. Most cells of the germ line, such as sperm heads (A), spermatogonia and young primary spermatocytes (the tip of a testis is shown in B) are not altered under these experimental conditions. T53-1 antibody is still able to decorate kl-3 loop (data not shown) as well as sperm tails (C). On the contrary, DNA of mature primary spermatocytes is easily spread, although it is still possible to find almost normal nuclei (D). Also S5 staining remains normal, permitting sometimes to discriminate between kl-5 and ks-1 loops (D'). In E a kl-5 loop is shown, from which a DNA fibre is released; note that in order to evidentiate the fibre, the microphotograph was over-exposed (in E' the normal exposure is shown). All pictures were taken at the same magnification. Bar represents 20 µm.

chromosomes. Spaces among the three clumps are filled by the nucleolus and by the three lampbrush like loops, namely kl-5, kl-3 and ks-1, which are the cytological evidence of the activity of the corresponding fertility factors mapping on the Y chromosome (Bonaccorsi *et al.*, 1988). Slides for fibres usually show completely crushed primary spermatocyte nuclei and in many cases it is possible to observe isolated DNA fibres even at low magnification (Figure 1 D and E).

In order to evaluate the possibility of performing immunostaining of these fibres, two different antibodies were used, T53-1 (Pisano *et al.*, 1993) and S5 (Saumweber *et al.*, 1980; Risau *et al.*, 1983). T53-1 specifically decorates sperm tails and the kl-3 loop, while S5 strongly stains kl-5 and, more faintly, ks-1 loops. There are four reasons these two antibodies were chosen: (i) they recognize DNA binding proteins, which (ii) are very abundant during this phase of germ line development, but (iii) they react with a specific portion of DNA, so it is possible to see if the organization of this subset of chromatin is still preserved during fibres preparation; besides (iv) it is also possible to evaluate if cross-reactions occur after this mechanical stress. As shown in the picture, the conclusions of this study are that (i) both T53-1 (data not shown) and S5 (Figure 1 D' and E-E') antibodies are still able to bind to Y loops fibres, and (ii) there is no cross reaction with other DNA fibres (Figure 1 D-D'), indicating that this method is able to preserve the DNA-proteins interaction and its specificity. Moreover (iii) in many cases also intact loops are preserved, and S5 is still able to discriminate between kl-5 and ks-1 (Figure 1 D').

It is noteworthy that, in the described situation, this method also adds some new knowledge about the molecular organization of the Y loops. In fact it is known that the kl-3 loop has a filamentous aspect while the other two, after S5 immunostaining, show a more compact appearance. Preparation of DNA fibres indicates that also the last two loops have a filamentous organization (Figure 1 E-E'), and that their compactness in standard preparations is probably due to their higher order, three-dimensional organization, which is lost after the stress induced by squashing. Sometimes, as illustrated in Figure 1 E-E', the kl-5/ks-1 loops show a variable thickness of the fibre, which size increases from one extremity to the other. At the moment it is not possible to assess if this increasing thickness is a chance or if it reflects a real organization of the fibre. However, it would be intriguing to argue that the accumulation of the protein bound to the loop (a protein which recognizes nascent RNAs; Saumweber *et al.*, 1980) increases towards the 3' end of the filament, resembling the ultrastructural organization of the amphibian oocytes' lampbrush-like loops from which they take their name.

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References: Bonaccorsi, S., C. Pisano, F. Puoti and M. Gatti 1988, Genetics 120: 1015-1034; Cenci, G., S. Bonaccorsi, C. Pisano, F. Vernì, and M. Gatti 1994, J. Cell Sci. 107: 3521-3534; Lindsley, D.L., and K.T. Tokuyasu 1980, Spermatogenesis. In: *The Genetics and Biology of* Drosophila, (Ashburner, M., and T.R.F. Wright, Eds.). Vol. 2, pp. 225-294. Academic Press, New York; Pisano, C., S. Bonaccorsi, and M. Gatti 1993, Genetics 133: 569-579; Risau, W., P. Symmons, H. Saumweber, and M. Frash 1983, Cell 33: 529-541; Saumweber, H., P. Symmons, R. Kabish, H. Will, and F. Bonhoeffer 1980, Chromosoma 80: 253-275.

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