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# Localizing multiple probes on the same chromosome preparation of *Drosophila*: A multi-well *in situ* hybridization technique.

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*In situ* hybridization techniques, along with immunocytochemistry allow detection of specific DNA or RNA sequences or proteins in morphologically preserved chromosomes, cells or tissue sections. This technique was originally developed by two independent groups, Pardue and Gall (1969) and John *et al.* (1969). Since then it has been modified and refined for different applications. Conventional *in situ* hybridization methods use a single probe on a single chromosome slide. Further, while using enzyme based detections, co-analysis of multiple sequences usually require a number of such slides. However, there can be variations among different chromosome preparations, and it may be difficult to analyze the results together. Here, we describe a "multi-well" technique for *in situ* hybridization of DNA probes on metaphase and polytene chromosome of *Drosophila melanogaser*. We have used three genes from *Drosophila melanogaster,* which have been identified and are being characterized in our laboratory (P28, Acc. No.Z29571; Aminopeptidase P, Acc.No.AJ131920; and Hexokinase, Acc.No.AJ271350) for hybridization on *Drosophila* chromosome spreads.

This method uses polypropylene plastic rings as 'wells' around different areas of chromosome spreads, on a single slide, so that multiple probes can be used in different wells at the same time. As shown in Table 1, commercially available or custom made polypropylene rings (which are autoclavable) of different diameters can be used. The metaphase and polytene chromosome spreads were prepared according to standard protocols (Ashburner, 1989). The chromosomal DNA was denatured by incubating the slides for 90s in 0.1N NaOH at room temperature, followed by washing with 2×SSC and subsequent dehydration in increasing alcohol grades. Once air dried, the slides were immediately screened under a microscope to locate areas having good chromosome spreads (such areas can also be pre-located and marked). A thin layer of a special adhesive was applied to the lower plastic surface of the wells and using forceps and using a forceps, they were carefully placed on the slide (Figure 1), encircling good spread. The smallest well used (3mm inner diameter) could cover around 25-30 metaphase chromosomes and 5-10 polytene chromosomes, depending on the method and quality of preparation. After about 30 seconds of setting, the slides were pre-warmed to the hybridization temperature and the denatured probes [labeled with either Digoxygenin (Roche, Germany) or the fluorescent nucleotide Cy3 (Amersham, UK)] were added to individual wells

Table 1. Dimensions of the wells used and respective hybridization buffer volumes.

| *Wells* | | Hybridization buffer volumes |
|---|---|---|
| *Inner diameter* | *Height* | (maximum needed) µls |
| 3 mm | 5 mm | 10-20 µls |
| 5 mm | 5 mm | 20-50 µls |
| 8 mm | 5 mm | 50-100 µls |

and the wells sealed with a small piece of Parafilm (American Can Co., USA) creating a 'localized humid chamber'.

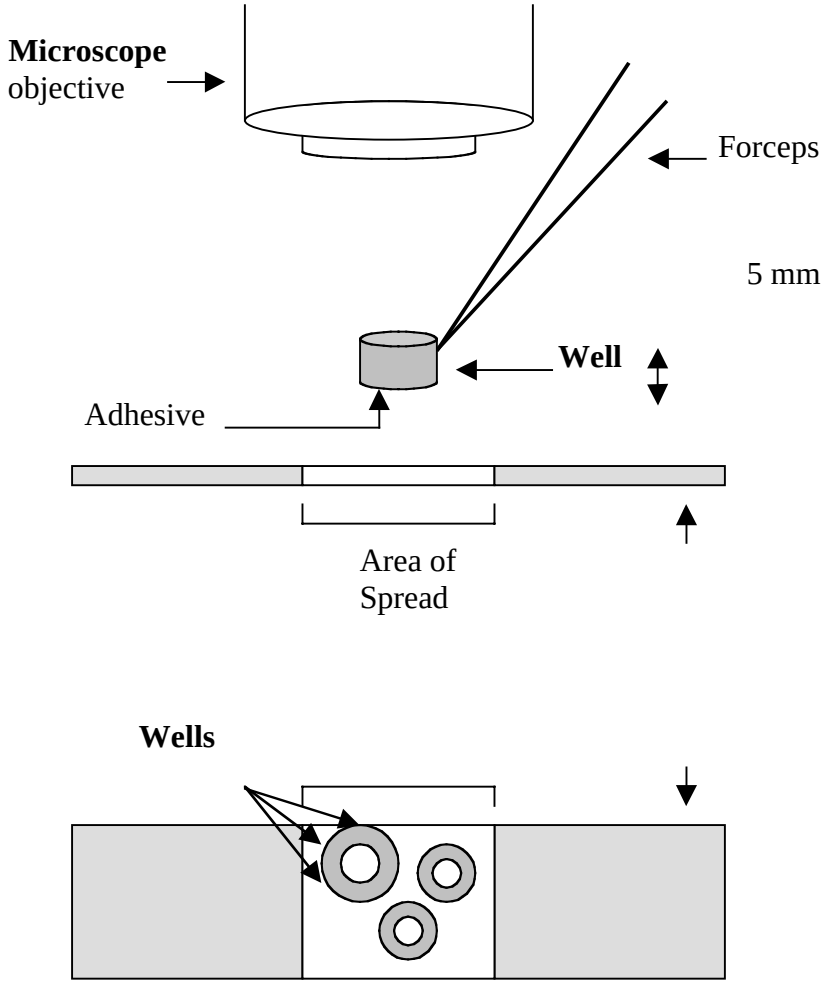


Figure 1. Diagrammatic representation of the multi-well technique.

Table 1 describes the volumes of hybridization buffer (the probe concentration can be from 2-5 ng/µl, depending on the type of probe) that can be used, according to the different well sizes. Hybridization was carried out under appropriate conditions for 5-6 hours. The excess hybridization solution was carefully taken out using a micropippet and stored at –20ºC (this probe can be reused several times). The wells were removed by gently lifting them using forceps and the slides were processed further according to procedures described earlier (Langer-Safer, *et al.*, 1982). Good hybridization signals were observed and the positions of all DNA sequences used were confirmed (Data not shown). Thus, three probes were localized to the respective positions (P28-21DE/2L; Aminopeptidase P-36A/2L; and Hexokinase-97B/3R) and could be easily compared on the same slides, under identical conditions.

This technique of using multiple wells for *in situ* hybridization offers several advantages. Multiple probes, along with proper controls can be used on a single slide, under identical conditions. Relatively less volume of hybridization solutions (Table 1) and small quantity of probes could be used, so that relative concentration of probes are high. Further, the unused probe could be recovered and reused without significant loss of volume. This is convenient when the probes are available in limited quantities. Simultaneously, absolute concentration of probe in a well could be high enough for specific spread to favor hybridization driven by probe concentration.

Also, the chromosomes that are not covered by any well (/ probe) can serve as a control for chromosome morphology during the experiment. Since control as well as various experimental hybridizations on the chromosomes could be visualized under the identical condition of detection of antibody binding or enzyme activity, the comparisons are more natural.

We have also used this technique for *in situ* hybridization on metaphase and polytene chromosomes of malarial vector mosquito *Anopheles stephensi,* along with those of *Drosophila melanogaster*, for comparative analysis of repetitive DNA sequences being characterized in our laboratory; namely Asm9 (Acc No.AJ238282), Asm12 (Acc No.AJ238404) and Asm 19 ( Acc. No. AF454465). (data not shown). Being closely related insects, the information available from *Drosophila* can be very useful for identifying and localizing the homologous sequences in less characterized and medically important organisms like mosquitoes. The method described above could be applied and further refined for use in such studies.

References: Ashburner, M., 1989, Drosophila*: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, USA; John, H., M. Birnstiel, and K. Jones 1969, Nature 223: 582-587; Langer-Safer, P.R., M. Levine, and D.C. Ward 1982, Proc. Natl. Acad. Sci. USA 79: 4381-4385; Nonradioactive *in Situ* Hybridization Application Manual, Boehringer Mannheim (now Roche Molecular Systems), Germany; Pardue, M.L., and J.G. Gall 1969, Proc. Natl. Acad. Sci. USA 64: 600-604.