Chromosome Microdissection on Semi-Archived Material

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Abstract
Glass needle-based chromosome microdissection (midi) is a standard approach developed in the 1980s and remains more frequently applied in testing than the comparable technique using laser-based platforms. As the amount of DNA extracted by this technique is minimal and often in the range of picograms, the isolated DNA must be further amplified prior to use; the isolated amplified product can be readily utilized in multiple molecular research and diagnostic investigation. DNA libraries created by midi are either chromosome- or chromosome-region-specific. However, a critical component to this process is the need for timely chromosome preparation via the air-drying method not to exceed a ~2–3 h before midi is performed. Failure of this time-sensitive step often results in the chromosomes drying out after dropping, and upon initiation of the midi technique, the dissected material can jump away while touching by the needle, and collection of a suitable sample is inhibited. Herein, we demonstrate with a simple adaptation of the standard procedure, midi can be performed on semi-archived material stored for longer periods at −20°C. Thus, the critical step to obtain well-spread chromosome preparations can be completed under established conditions, for example, in the primary laboratory, stored at −20°C, and sent directly to specialized reference laboratories offering midi. In our study, we were able to obtain high-quality DNA libraries, as verified by gel electrophoresis and reverse fluorescence in situ hybridization, via midi extracted chromosome spreads derived from human, fish, snake, lambrush, and insect stored for up to 6 months. © 2019 The Authors. Cytometry Part A published by Wiley Periodicals, Inc. on behalf of International Society for Advancement of Cytometry.

Key terms
chromosome; molecular cytogenetics; DNA; glass-needle-based chromosome microdissection (midi); semi-archived material; air-drying method

Since its first application in polytenic chromosomes of Drosophila at the beginning of the 1980s (1), glass needle-based microdissection (midi) remains a robust method to isolate DNA from whole chromosomes or chromosomal segments under an inverted microscope. Midi or flow-sorting (2) represent the most common applied methods to produce whole chromosome probes (WCPs) or partial chromosome probes (PCPs). Following the development and widespread availability of polymerase chain reaction (PCR) based amplification of either microdissected (3) or flow-sorted chromosomes (4) using a degenerate universal primer (DOP) (5) allowed for the application of midi in fluorescence in situ hybridization (FISH). In the past three decades, WCPs and PCPs have been widely used in clinical diagnostics for the application in prenatal, postnatal, and cancer genetics as well as in comparative cytogenetic studies of a great variety of animal groups (6–15).

Although flow sorting generates paints of higher complexity since multiple chromosome (~500) copies are isolated in a rather automated procedure (2), there are many reasons why the technique is not widely utilized given it is not suitable for most research and many clinical cases: (1) the higher costs of the equipment, (2) the
need for high-quality chromosome preparation, and (3) most species present highly uniform karyotypes, therefore interfering their proper sorting. Comparatively, midi is a low-cost procedure, requires few chromosomal copies and with the distinct advantage that PCPs can be generated. One of its disadvantages, however, is the need for the definite identification of species and storage times of chromosomes fixed on coverslips of samples are listed in Table 1.

| SPECIES                              | CHROMOSOME PREPARATION DERIVED FROM | STORAGE TIME 30 DAYS | STORAGE TIME 60 DAYS | STORAGE TIME 180 DAYS |
|--------------------------------------|-------------------------------------|-----------------------|----------------------|-----------------------|
| Homo sapiens                         | Peripheral blood                    | Two tests             | Two tests            | Two tests             |
| Lebiasina bimaculata                 | Kidney                              | Three tests           | Three tests          | Three tests           |
| Boa constrictor                      | Peripheral blood                    | Two tests             | Three tests          | three tests           |
| Pelophylax esculentus                | Oocytes                             | Five tests            | Three tests          | Three tests           |
| complex                              |                                     |                       |                      |                      |
| Omophoita octoguttata                | Gonads                              | Four tests            | Three tests          | Four tests            |

Figure 1. FISH with WCPs using probes prepared after midi procedures performed on Giemsa-stained coverslips previously stored at −20 °C for 30 (a, d, and g), 60 (b, e, and h), and 180 days (c, f, and i). Bar = 5 μm. (a, b, c) Probe derived from the pair 1 of fish species Lebiasina bimaculata (2n = 36). (d-f) Probe derived from the pair 2 of snake species Boa constrictor (2n = 36). (g) Probe derived from pair 1 of human (2n = 46). (h) Probe derived from pair 2 of human (2n = 46). (i) Probe derived from pair 3 of human (2n = 46). [Color figure can be viewed at wileyonlinelibrary.com]
of the target chromosomes, and therefore, well-spread metaphases are required. Alternatively, laser-based chromosome microdissection is only rarely used for animal chromosomes, and the establishment of PCPs has to the best of our knowledge, not been reported (16).

Conventionally, midi coverslips containing chromosomal preparations are prepared no longer than a few hours before undertaking midi. Herein, we tested the effectiveness of probes obtained with midi on semi-archived material stored for a range of time periods from several days to months at −20°C.

MATERIAL AND METHODS

Chromosome-Spread Preparation

Chromosome spreads were prepared from 20 μl of cell suspension (i.e., chromosomes in Carnoy’s fixative = 3:1 methanol/glacial acetic acid) of different target species, including human, fish (Lebiasina bimaculata) and snake (Boa constrictor) dropped on to 24 × 60 mm coverslips (Menzel Glaeser, Braunschweig, Germany). In addition, several days to weeks old preparations of lampbrush chromosomes from frog (Pelophylax esculentus complex) provided by Prof. Alla Karlskova (St. Petersburg, Russia) and insect (beetle: Omaphoita octoguttata) provided by Prof. Roberto Artoni (Ponta Grossa, Brazil) were used (Table 1). Coverslips were stored in a 50 ml tube containing 10% SDS and rinsed thoroughly with ultra-distilled water right before the dropping procedure. Right after cell suspension-dropping, while still wet, 20 μl of Carnoy’s fixative was dropped onto the coverslip. The coverslip was then placed on a hot plate at 50°C for 30 s to support spreading and drying. Afterward, the metaphase chromosomes on coverslip were stained with Giemsa solution (35 ml of phosphate buffer and 3.5 ml of Giemsa) for 3 min, immediately rinsed thoroughly with ultra-distilled water and allowed to air-dry for 5 min. The Giemsa-stained coverslips were then stored at −20°C for 30, 60, and 180 days until the midi procedure (Table 1). Interestingly, there are not really any special conditions for storage and defrosting of coverslips.

Figure 2. The steps of midi procedure in semi-archived material derived from human chromosomes previously stored at −20°C for 6 months. Target chromosome touched by the glass needle tip. Initiation of collecting the whole target chromosomes by the glass needle; chromosome may be easily manipulated and shows no tendencies to bursting. Target chromosome with no strong adhesion to the surface and this it can be dissected in one piece (not fragmented). The final collection of the target chromosomes and its complete attachment to the glass needle after capture. [Color figure can be viewed at wileyonlinelibrary.com]
Following defrosting, stiffness of the chromosomes on the coverslip may be tested by touching one of them using the tip of the glass needle. If chromosomes seem unsuitable for midi procedure, the coverslip may be rinsed for 60 s in distilled water and wiped dry on the side without metaphases. This rinse allowed midi on all tested scenarios similar to the process on freshly prepared slides. Methods for microneedle preparation, DOP-PCR amplification, probe labeling, and hybridization followed the protocols described in the study by Kosyakova et al. (17).

RESULTS AND DISCUSSION
Herein, we successfully adapted the conventional procedure for chromosomal preparations before midi experiments. Previously, three major technical issues supported the need for freshly prepared coverslips prior to the midi procedure: (1) the better quality of chromosomal preparations (often associated with fresh coverslips) in order to facilitate the proper identification of the target chromosome, (2) a weaker adhesion of the chromosomes on the coverslips, which facilitates their removal during midi and (3) the need for humidity adjusted yet still readily manipulated chromosomes that do not jump away when touched by a needle. These three issues were thought critical to enable glass needle-based midi procedure and highly dependent upon the fresh preparation of metaphase chromosomes on the coverslips.

However, here midi was successfully performed on Giemsa-stained coverslips previously stored at −20°C for 30, 60, and 180 days, without any technical issues. In all tested scenarios, the DNA amplifications [here performed through DOP-PCR reactions using T7 DNA polymerase (Sequenase) as described in the study by Kosyakova et al. (17)] were successfully performed, producing a smear pattern of approximately 300–1,500 bp after gel electrophoresis (results not shown). Accordingly, after additional DOP-PCR-labeling steps, high-quality probes were obtained in all tested species (Fig. 1). Two possible problems that could theoretically occur and were anticipated during the midi procedures including a higher adhesion of the chromosomes to the surface and a repulsion of the dissected fragments from the needle after its capture were not observed in any tested scenario (Fig. 2).

CONCLUSIONS
The methodological adaptations tested here demonstrated that glass-needle-based chromosome microdissection can be performed on semi-archived material previously stored at −20°C for up to 6 months. The good quality yield from these experiments provides support that storage of coverslips at longer periods may also be conducive to a successful midi. This observation affords an opportunity for researchers that do not have the infrastructure for performing midi experiments to prepare a large set of coverslips containing the target chromosomes and keep them at −20°C to be sent in batches to specialized laboratories performing midi. Overall, this new technical development may stimulate research in comparative genomics that had previously been deemed impossible.

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CONFLICT OF INTEREST
There are no such COIs to report.

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