EDORIUM Journals

Video J Clin Res 2020;2:100004VAM08AR2020.
www.videojournalofclinicalresearch.com

TECHNICAL REPORT PEER REVIEWED | OPEN ACCESS

Glass needle-based chromosome microdissection—How to set up probes for molecular cytogenetics?

Ahmed Al-Rikabi, Leon B Liehr, Thomas Liehr

ABSTRACT

Aims: Molecular cytogenetics is basically the use of fluorescence in situ hybridization (FISH). To perform FISH, among others species-specific DNA-probes are required. Glass needle-based chromosome microdissection is a feasible approach to establish corresponding probes. More specifically, by glass needle-based chromosome microdissection whole chromosome and partial chromosome painting probes can be established. **Methods:** Glass needle-based chromosome microdissection is still much more successful and cost-effective for production of FISH probes, than comparable laser-based chromosome microdissection approaches. Thus, here we present the first video protocol how to practically perform this approach. **Results:** Technical details on necessary solutions have been previously published elsewhere and are also referred to in the video, however, the practical manual work cannot be better described than done here, by a short movie. **Conclusion:** Based on this video, it should be much easier now for other laboratories to establish this technology.

Keywords: Fluorescence in situ hybridization (FISH), Glass needle-based chromosome microdissection, Partial chromosome paint, Whole chromosome paint

How to cite this article

Al-Rikabi A, Liehr LB, Liehr T. Glass-needle based chromosome microdissection—How to set up probes for molecular cytogenetics? Video J Clin Res 2020;2:100004VAM08AR2020.

Ahmed Al-Rikabi[1], Leon B Liehr[1], Thomas Liehr[1]

Affiliation: [1]PhD, Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Am Klinikum 1, D-07747 Jena, Germany.

Corresponding Author: Dr. Thomas Liehr, Institut für Humangenetik, Am Klinikum 1, D-07747 Jena, Germany; Email: Thomas.Liehr@med.uni-jena.de

Received: 17 June 2020
Accepted: 12 July 2020
Published: 19 August 2020

Article ID: 100004VAM08AR2020

****

doi:10.5348/100004VAM08AR2020TR

INTRODUCTION

Glass needle-based chromosome microdissection (midi) was first described in the 1980s [1]. It is a straightforward approach for setting up species specific whole and partial chromosome painting probes suited for fluorescence in situ hybridization (FISH) [2, 3]. Furthermore, DNA-isolation by midi can be the starting point for many other studies, including exact breakpoint characterization by next generation sequencing [4–6].

Compared to chromosome flow sorting [7], midi needs less sophisticated and expensive equipment and is not only restricted to production of whole chromosome paints. Concerning laser-based microdissection compared to midi the former is not regularly applied yet for preparation of whole and not at all for partial chromosome painting probes [8]. Furthermore, just recently we published a protocol how to use semi-archived material in midi [8].

Accordingly, midi is a very timely approach. Even though we provided earlier detailed protocols on how to do midi [3], practical guidance on how to do hands on steps of this procedure in more detail still lack. Thus, in this article, supplied with the accompanying movie, we are sure to be able to close this gap and to give advice on how to do midi under real conditions.

THE MIDI PROCEDURE AS SHOWN IN THE ACCOMPANYING MOVIE

The midi procedure as shown here is based on the protocol published in ref. [3]. Special emphasis is given in this paper on some working steps of midi procedure, which were not outlined in detail there.

HOW TO DO GLASS NEEDLE AND PIPETTE PREPARATION?

The preparation of glass needles and pipettes must be done by using a pipette puller. This machine is shown in Figure 1 and works with a heating unit and weights and balances. Glass rods or pipettes can be fixed in there and pulled in length as shown in Video 1.

Also the necessary silanization procedure of extended pipettes (Figure 2) is explained and shown in Video 1. This step is essential to avoid sticking of the microdissected DNA to pipettes, instead of being regularly processed during the procedure.

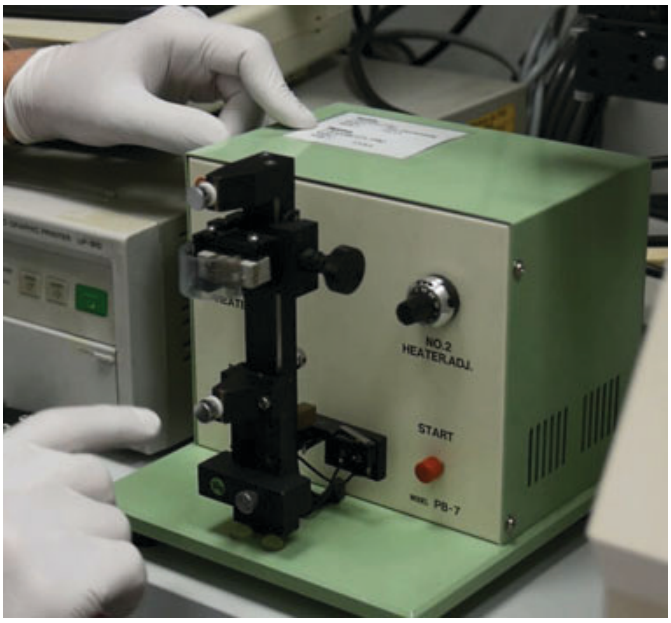


Figure 1: Pipette puller.

Video 1: Technical details on the practical manual work in preparation of and during glass needle-based chromosome microdissection.

Video 1 URL: http://www.videojournalofclinicalresearch.com//archive/article-full-text/100004VAM08AR2020

Access Video on other devices

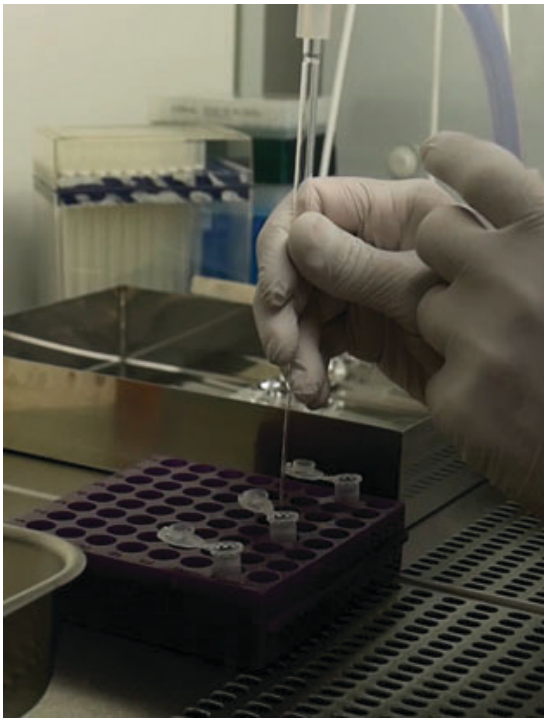


Figure 2: Silanization of the extended glass pipette.

HOW TO PREPARE COVERSLIPS AS CARRIERS OF CHROMOSOMES?

Due to optical features of applied inverted microscopes one cannot use microscope slides for midi. Instead, only sodium dodecyl sulfate (SDS)-treated coverslips (Figure 3) can be applied to spread and fix chromosomes on them. The spreading of the chromosomes on the coverslips can be improved by holding them in the steam of a water bath. Very important is to mark the orientation “up-side” and “down-side” of the coverslip, which can be done by breaking off an edge (Figure 4). Before optical control of metaphase spreading, coverslip goes for 4 minutes on a heating plate (60 °C).

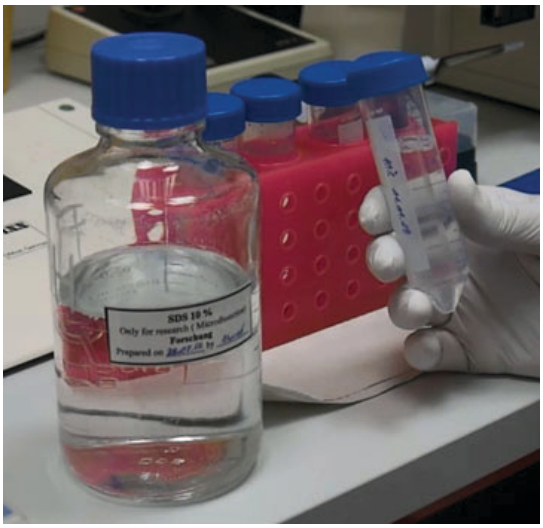


Figure 3: Coverslips SDS solution.

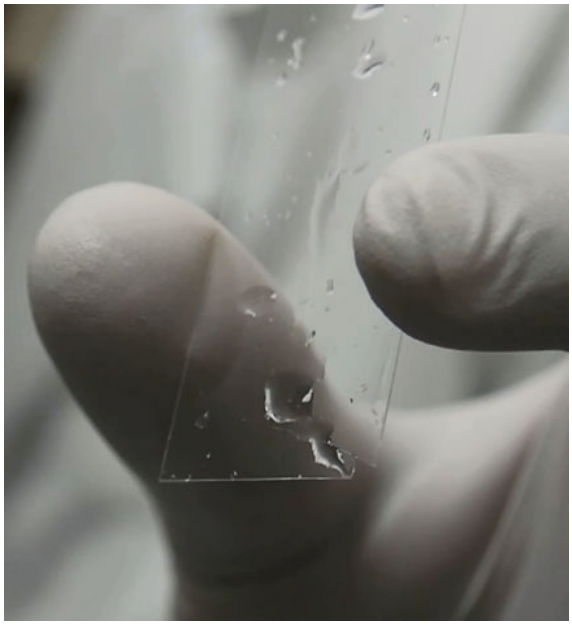


Figure 4: Coverslip after spreading chromosomes on it.

HOW TO DO CHROMOSOME G-BANDING ON THE COVERSLIP?

G-banding, if necessary, is done according to standard protocols [9]. Main difference to regular protocols is that coverslip staining is done here in 50 mL tubes (Figure 5).

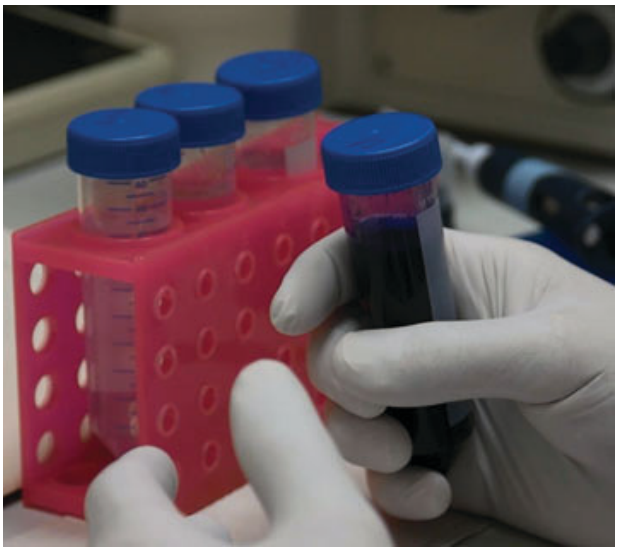


Figure 5: Solutions necessary for G-banding.

HOW TO PREPARE THE INVERTED MICROSCOPE?

The inverted microscope used, being equipped with one, or better two micromanipulating units, has to be prepared by mounting elongated pipette and glass needle at the corresponding spots. The pipette has to be loaded with the collection drop [3] before mounting (Figures 6 and 7).

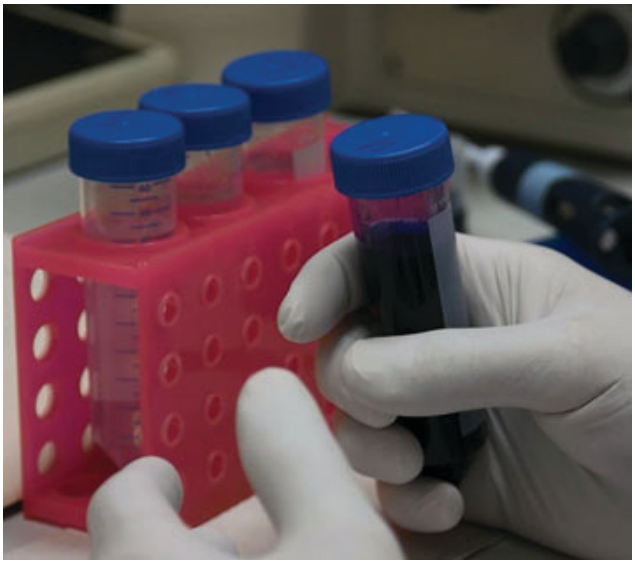


Figure 6: Extended glass needle for midi.

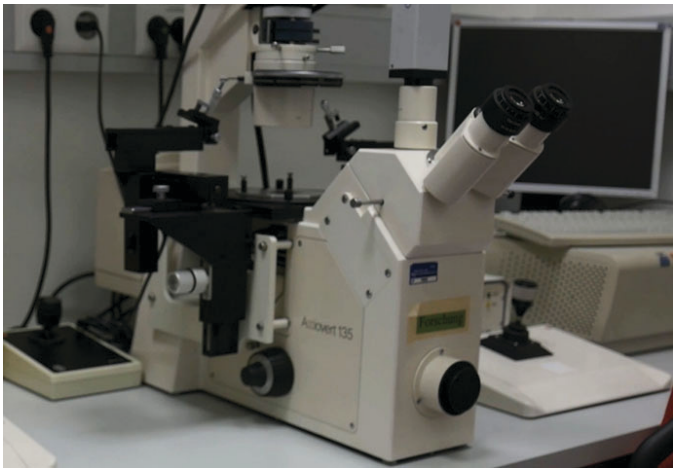


Figure 7: Inverted microscope for midi.

HOW TO DO MIDI?

The exact use of the micromanipulators is a matter of personal training. However, the principle is simple: just cut out a chromosomal band/region or scrape together one whole chromosome to a single spot. Afterward pick up the microdissected DNA by the glass needle. Then the DNA sticking to the glass needle is transferred to the collection drop in the pipette and washed off.

DNA AMPLIFICATION

The DNA accumulated in the collection drop after 1–15 midi steps is then transferred into a reaction tube. This is subjected to DNA probe amplification by a specific PCR procedure [degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR)] [3].

CONCLUSION

Midi is a laborious, manual, and sophisticated approach, needing some preparation to be performed. However, it is still the best suited, most straightforward, and most cost-efficient way to establish whole or partial chromosome painting probes for molecular cytogenetic studies or other studies including sequencing.

REFERENCES

1. Scalenghe F, Turco E, Edström JE, Pirrotta V, Melli M. Microdissection and cloning of DNA from a specific region of Drosophila melanogaster polytene chromosomes. Chromosoma 1981;82(2):205–16.
2. Kosyakova N, Hamid AB, Chaveerach A, et al. Generation of multicolor banding probes for chromosomes of different species. Mol Cytogenet 2013;6(1):6.
3. Kosyakova N, Liehr T, Al-Rikabi AB. FISH-microdissection. In: Liehr T, editor. Fluorescence In Situ Hybridization (FISH) – Application Guide. 2ed. Berlin: Springer; 2017. p. 81–99.
4. Jancuskova T, Plachy R, Stika J, et al. A method to identify new molecular markers for assessing minimal residual disease in acute leukemia patients. Leuk Res 2013;37(10):1363–73.
5. Zlotina A, Kulikova T, Kosyakova N, Liehr T, Krasikova A. Microdissection of lampbrush chromosomes as an approach for generation of locus-specific FISH-probes and samples for high-throughput sequencing. BMC Genomics 2016;17:126.
6. Zamariolli M, Di-Battista A, Moysés-Oliveira M, et al. Disruption of PCDH10 and TNRC18 genes due to a balanced translocation. Cytogenet Genome Res 2020;160(6):321–8.
7. Ferguson-Smith MA, Yang F, O'Brien PCM. Comparative mapping using chromosome sorting and painting. ILAR J 1998;39(2–3):68–76.
8. Al-Rikabi ABH, Cioffi MB, Liehr T. Chromosome microdissection on semi-archived material. Cytometry A 2019;95(12):1285–8.
9. Weise A, Liehr T. Pre- and postnatal diagnostics and research on peripheral blood, bone marrow chorion, amniocytes, and fibroblasts. In: Liehr T, editor. Fluorescence In Situ Hybridization (FISH) – Application Guide. 2ed. Berlin: Springer; 2017. p. 171–80.

****

Author Contributions

Ahmed Al-Rikabi – Design of the work, Acquisition of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Leon B Liehr – Design of the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Thomas Liehr – Conception of the work, Design of the work, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Guarantor of Submission

The corresponding author is the guarantor of submission.

Source of Support

None.

Consent Statement

Written informed consent was obtained from the patient for publication of this article.

Conflict of Interest

Authors declare no conflict of interest.

Data Availability

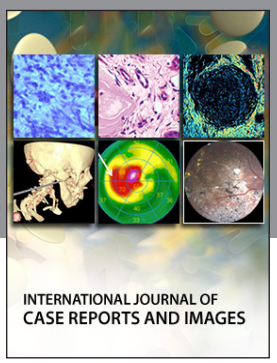
All relevant data are within the paper and its Supporting Information files.

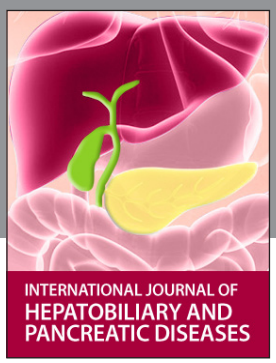
Copyright

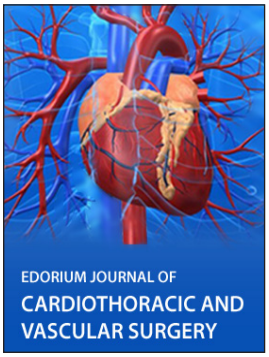
© 2020 Ahmed Al-Rikabi et al. This article is distributed under the terms of Creative Commons Attribution License which permits unrestricted use, distribution and reproduction in any medium provided the original author(s) and original publisher are properly credited. Please see the copyright policy on the journal website for more information.

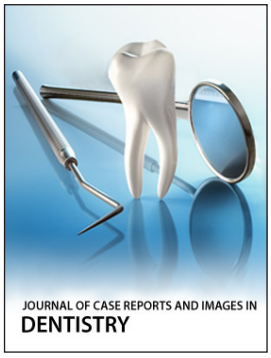
Access full text article on other devices

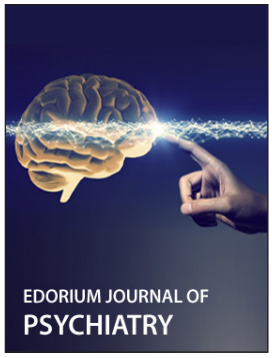
Access PDF of article on other devices

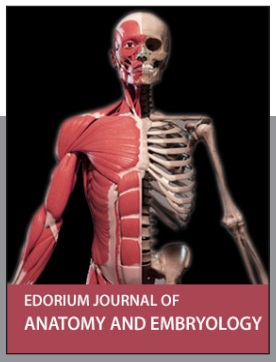


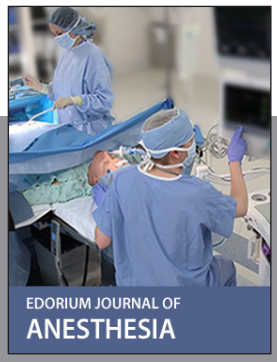












Submit your manuscripts at

www.edoriumjournals.com