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Molecular Analysis of Individual Chromosomes Enabled through Microdissection

PROTOCOL #6

Chromosome Application

Chromosomes are structural units that exist within the nucleus of eukaryotes. Chromosomes carry genes, the genetic blue-prints of life. Individual chromosomes carrying specific genes can be identified based on their morphological characteristics.

A considerable amount of scientific research has been done in the area of cytogenetics, the scientific field that studies the relationship between chromosome structure and genetic phenomena. Alteration of chromosome structure or function can result in serious genetic defects in organisms through improper segregation of chromosomes during cell division or due to abnormal gene expression. Chromosome spreads done at the metaphase stage of cells (called "metaphase spreads") are one of the primary techniques employed in clinical and molecular cytogenetics to study chromosome structure and function.

In this report, we review a method for dissecting specific chromosomes from metaphase spreads using laser microdissection for down-stream molecular analysis.

Tissue Preparation

Metaphase spreads of human peripheral blood cells were prepared by standard karyotyping methods onto PEN membrane slides. A Giemsa stain was used to identify chromosomes in the preparation.

Giemsa Stain Materials

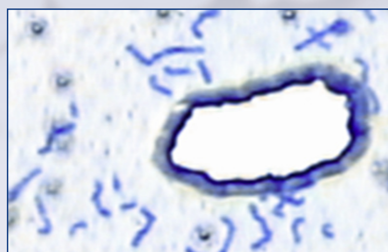
Material	Quantity
Working Giemsa stain	
Giemsa stain, stock	2.0ml
Phosphate buffer, pH 6.8	25.0ml
0.1M Phosphate Buffer, pH 6.8 or Gurr Phosphate Buffer, pH 6.8	
0.1M NaH_2PO_4	96ml
0.1M Na_2HPO_4	104ml
Check pH. If necessary adjust pH by adding 0.1M NaH_2PO_4 or 0.1M Na_2HPO_4	

Staining Procedure

Step	Process	Time
1.	Phosphate buffer, pH6.8	1 minute
2.	0.025% Trypsin in Phosphate Buffer, pH 6.8	2 minutes
3.	Phosphate Buffer, pH 6.8	1 minute
4.	Giemsa stain, working solution	15 minutes
5.	Distilled water	2-3 dips
6.	Shake off excess water, dry backside of slide with a kimwipe and air dry. Alternatively, to speed up drying process, place slide in an oven set at 50-60°C for 1-2 minutes to dry	

Figure 1. Microdissection of human chromosome with the Veritas™ Microdissection System:

A: Giemsa stained metaphase spread before microdissection.



B: After microdissection.

C: Captured chromosome on the cap.

Laser Microdissection

Collection of individual chromosomes from metaphase spreads are done as follows:

Microdissection Procedure

Step	Procedure
1.	Set Laser Cutting power to lowest possible setting that will still allow full cutting through the PEN membrane.
2.	Set Laser Cutting properties such that no tabs will be generated during cutting process.
3.	Set capture properties such that no automatic LCM spots are generated after the cutting process.
4.	Scan slide for optimal chromosome spreads. While scanning, store the location of selected spread(s) to be retrieved later for collection by using "Stored Position" feature in the "Microscope" window.
5.	Place a CapSure Macro LCM cap onto sample.
6.	Draw around chromosome(s) that will be collected.
7.	Activate the laser cutting process with LCM cap on top of sample.
8.	Manually place a single LCM spot to attach the chromosome to LCM cap.
9.	Remove the CapSure Macro LCM cap from the slide.

DNA Extraction

DNA from the microdissected chromosome on the CapSure Macro LCM cap is extracted using PicoPure DNA Extraction Kit following the kit protocol. The DNA extract can now be used for DNA analysis, an example of which is described by Gribble, et al (2004)[1].

Reference

[1] Gribble, S.M., Fiegler, H., Burford, D.C. Prigmore, E., Yang, F., Carr, P., Ng, B.L., Sun, T., Kamberov, E.S., Makarov, V.L., Langmore, J.P. and Carter, N.P. 2004. Applications of combined DNA microarray and chromosome sorting technologies. *Chromosome Research*. 12:35-43.

Acknowledgements

This study was conducted by Arcturus Bioscience in collaboration with US Labs.

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