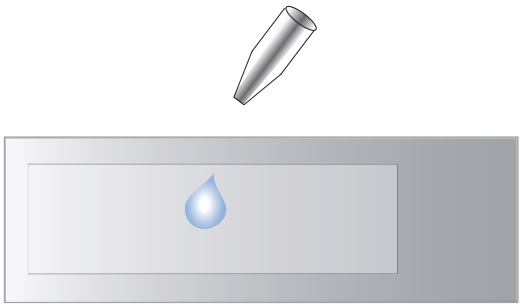
Protocol ♦ Chromosome LCM

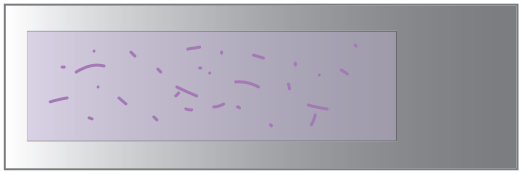
The precision of the MMI CellCut system enables the user to simply locate and precisely microdissect structures smaller than single cells, for example chromosomes. The following protocol demonstrates how to prepare chromosome spreads for laser microdissection. It is used frequently in clinical and molecular cytogenetics to study chromosome structure and function.

Materials:

- Cell culture
- Pipettes
- Colchecin, Colcemid
- Centrifuge
- Centrifuge tubes
- Sodium citrate solution
- Cell culture media
- Distilled water
- Fixative solution
- Giemsa stain
- MMI Membrane Slides (PN: 50102, 50103)
- Cover glass
- Glass slides
- Xylene
- MMI Isolation Cap (PN: 50202-50212)
- Incubator



Allow the suspension to drop from at least 10 cm height



The chromosome spread must be on the flat side of the slide (where the membrane is attached to the metal frame)

Method:

Establishing Cells in Metaphase:

1. Add 0.5 mM Cocemid (Sigma D1925) or Colchine (Sigma C3915) to rapidly dividing cells
2. Harvest and transfer cells to a 15 ml tube (with Colcemid after ca. 2 - 8 hrs, with Colchine after ca. 72 hrs)
3. Pellet cells for 15 min at 500 *g*
4. Resuspend cells in 10 ml hypotonic saline solution (e.g. growth media diluted 1:4 with ddH_2O)
5. Allow cells to swell for 15 - 20 min at 37 °C in an incubator
6. Pellet cells for 15 min at 500 *g*, then resuspend in 5 ml fixative solution (e.g. ice cold methanol:acetic acid at 3:1)
7. Fix for 5 minutes

Staining:

Giemsa is the most common stain used for chromosomes, however, other stains, such as reverse-FISH analysis can be applied as well.

1. Dilute Giemsa stain 1:10 with distilled, filtered H_2O
2. Incubate at room temperature for up to 30 minutes
3. Sample should be ready for slide preparation

Note: To enhance adherence of sample to membrane slide, incubate MMI Membrane Slides with 0.1 % Poly-L-Lysine solution at 37 °C for at least 1 h (alternatively, gelatine or agarose can be used)

Slide Preparation:

1. Incubate membrane slide for 1.5 - 24 hrs in ethanol, then rinse with distilled water before use
2. Place slide at 45° angle, dispense 1 - 3 drops of cell suspension onto the slide from a height of minimum 10 cm
3. Allow to air dry
4. Ready for LCM