Laser Capture Microdissection

Pictures: Before, After, Cap

. Dis-enable laser before taking pictures.
. Re-focus image before each shot.

Spot Size: 7.5µm, 15µm, 30 µm

. Focus spot using lowest power magnification (10 X) first.
. 7.5 µm: .most effective for removal of smaller sections within the sample.
  .Power: 50-55 mW
  .Duration: 3.50- 4.00 ms
. 15 µm: .most effective for removal of epithelium.
  .Power: 80-85 mW
  .Duration: 1.5-2 ms
.30 µm: .Power: 45-55 mW
  .Duration: 5.00 ms +

Sections: Frozen

. Thin sections work best (~ 5µm thick, max 10 µm).
. Place section in center of slide.

Staining: LCM Kit, H&E, Quick Stain.

. For best results, perform LCM immediately following staining.
. Slides must be completely dry for effective LCM.
. H&E staining yields least favorable results, while LCM kit staining (modified protocol) allows for optimal removal.
. Re-Hydration of previously stained slides are not as effective for LCM.

LCM Staining: Protocol I

*Take precautions to ensure RNAse free conditions
*Ensure that solutions are fresh (changed daily)

LCM Staining Kit (modified)

1) Remove sections from freezer, and allow them to thaw for 1 minute. If performing several rounds of staining at once, slides may be allowed to thaw in fumehood for up to 5 minutes.

2) Place slides in 75% ethanol for 30 seconds.

3) Wash with distilled water for 30 seconds.

4) Using a staining rack, dip slides in Haematoxyllin solution for 15-20 seconds (~ 10 dips)

5) Wash with distilled water for 30 seconds.

6) Place slides in 75% ethanol for 30 seconds.

7) Place slides in 95% ethanol for 30 seconds.

8) Place slides in 100% ethanol for 30 seconds.

9) In the fumehood, place slides in Xylene for 3 minutes.

10) Dry slides in fumehood for 10 minutes. Gently remove any surrounding moisture with a kimwipe.

11) Store slides in a slide box containing dessicant.

** Ensure that slides are completely dry before starting LCM.

LCM Staining: Protocol II

*Take precautions to ensure RNAse free conditions

H & E staining

1) Place slides in 100% ethanol for 4 seconds.

2) Transer slides to 90% ethanol solution for 4 seconds.

3) Tranfer slides to 70% ethanol for 4 seconds.

4) Wash slides with distilled water

5) Place slides in Haematoxyllin for 60 seconds.

6) Wash slides with distilled water.

7) Place slides in Lithium Carbonate for 10 seconds

8) Wash slides with distilled water

9) Place slides in Eosin for 20 seconds.

10)Place slides in 70% ethanol for 10 seconds.

11)Place slides in 90% ethanol for 10 seconds.

12)Place slides in 100% ethanol for 10 seconds.

13)Place slides in 100% ethanol for 10 seconds.

14)In fumehood, directly transfer slides to Xylene for 3 minutes.

15)Leave slides to dry in the fumehood for 10 minutes.

16) Place slides in a slide box with fresh dessicant.

** Ensure that slides are completely dry before starting LCM.

LCM Staining: Protocol III

*Take precautions to ensure RNAse free conditions.

Quick Staining

1) Place slides in Fixative solution for 5 seconds (or 5 dips).

2) Place slides in Eosin for 5 seconds.

3) Place slides in Methylene blue for 5 seconds.

4) Wash slides under the water tap, on the opposite side of staining.

5) Allow slides to dry in fumehood for 10 minutes.

6) Place slides in slide box containing fresh dessicator.

**Ensure that slides are completely dry before starting LCM.

LCM Re-Hydration: Protocol

1) Place slides in 95% ethanol for for 60 seconds.

2) Place slides in 100% ethanol for 30 seconds.

3) In the fumehood place cells in Xylene for 3 minutes.

4) Allow slides to dry for 10 minutes in fumehood.

LCM- Pre-mRNA Isolation: Protocol

1)Place cap into 500 μl microcentrifuge tube with 100 μl Buffer RLT.

2) Invert tube several times to digest material off cap. (Alternative: incubate inverted a 42 C for 30 minutes).

3) Vortex cap end of tube.

4) Centrifuge at 2000 x g for 60 seconds.

5) Remove cap.

6) Adjust the volume to 350 μl with Buffer RLT.

7) Add one volume of 70 % ethanol.

8) Proceed using Rneasy Mini kit for isolation of total mRNA from <u>animal cells</u>.

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To further concentrate the Sample (optional)

1) Concentrate the sample with 0.5 ammonium acetate and 5 μg glucogen.

2) Centrifuge

3) Wash pellet with 75% and 100% ethanol.

4) Allow to air dry, briefly.

5) Solubilize in 10 μl Rnase-free water.