Staining of histological sections for microdissection Expression analysis

Dr. Klaus Bendrat, MEDEEA Forschungs-GmbH
e-mail contact: k.bendrat@hamburg.de

Preparations
Cool cryostat down to –22°C
Use PALM® MembraneSlides for microdissection experiments

NOTE: PALM® MembraneSlides have to be hermetically sealed, no fluid is allowed to trickle between glass and membrane to assure that the catapulting procedure will be successful.

Accomplishment

1. Put 5 µm or 12 µm thick sections, resp. onto the PALM® MembraneSlides und dip immediately into 70% ethanol for 60 seconds.

2. Acccording to publications sections are stable up to 72 hours at these conditions and at 4°C. On the strength of our past experience the sections are stable a few weeks.

3. Remove imbedding medium by dipping into water 5 times

4. Stain section for 15 s in **Mayer´s Hematoxylin**

5. Dip into water 10 times (15 s)

6. Perform blueing for 15 s in **„Automation"-buffer** (Biomeda, 1:10) (dip 10 times)

7. 15 s **water** (dip 10 times)

8. 30 s in **70% Ethanol** (dip 20 times)

9. 30 s in **96% Ethanol** (dip 20 times)

10. [Optional: 15 s **Eosin** (1:2 diluted in water plus 1 drop of glacial acetic acid /50 mL)]

11. 15 s **96% Ethanol** (dip 10 times)

12. 30 s **96% Ethanol** (dip 20 times)

13. 30 s **99% Ethanol** (dip 20 times)

14. 30 s **99% Ethanol** (dip 20 times)

15. 30 s **Xylene** (dip 20 times)

16. 3 min **Xylene**

17. 3 min **Xylene**
    RNA yield is worse if a waterfree alcohol like methanol, ethanol or isopropanol is used for steps15 to 17.

18. Let section air dry (2 min under a fume hood). To get high yields of RNA avoid microdissection times longer than 20 minutes!