Slide Processing For Paraffin Embedded Tissue Samples

This procedure describes the steps involved in slide processing of paraffin embedded tissue. The pretreatment process de-paraffinizes and pre-treats the sample prior to denaturation and hybridization with appropriate probes. Following pretreatment, the slides and appropriate probes are denatured, the slides are hybridized, washed and counterstained prior to analysis.

Pretreatment:

1. Prewarm 40 ml 0.01N HCL (or 0.85% NaCL) in a jar in 37°C water bath.
2. Age slide at 90°C for 25 min.
3. De-paraffinize the slide in Xylene (in flow hood) 15 min, and repeat 1 more time.
4. Dehydrate slide in 100% Ethanol 5 min, and repeat 1 more time.
5. Air dry slide.
6. Pre-warm a jar with 10 mM Citric acid to 80°C in water bath.
7. Place slide into jar for 55 min.
8. Add 80 mg of Pepsin into the jar with 0.01N HCL (0.2% Pepsin working concentration. Different tissues may need different Pepsin concentration).
9. Place slide into Pepsin solution for 30 min.
10. Rinse slide in 70% ethanol 30 second.
11. Air dry slide and check slide for proper digestion: reveal dark distinguishable cells
12. Dehydrate slide through70%, 85%, and 100% Ethanol each 2 min.
13. Air dry slide and proceed to hybridization:

Hybridization:

Prepare 10 ul of probe mix per hybridization for 22 mm^2 coverslip. Add the probe on the processed tissue slide and place coverslip taking care that there are no air bubbles.

Hybridize the slides in Thermobrite with following program:

Denaturation: 83°C for 3 min.
Hybridization: 37°C for 24-48 hours

Post Hybridization wash:
In 0.3% Igepal, CA-630, Sigma (or NP40)/ 0.4XSSC @ 73°C for 2 min.
In 0.1% Igepal CA-630, Sigma (or NP40)/2XSSC @ RT for 90 seconds.

Counterstain with DAPI II.