IHC on frozen sections before Laser Capture Microdissection and subsequent RNA-analysis

Laser capture microdissection (LCM) from Carl Zeiss is the method of choice to isolate specific single cells and small structures from complex heterogeneous tissues.
Prerequisite for successful LCM is good microscopic imaging together with precise recognition and definition of interesting target structures.
Unfortunately immunohistochemistry (IHC) - the ideal method for highly specific marker detection – requires conditions where RNA can be rapidly degraded by intrinsic RNases in unfixed sections. Therefore immunohistochemical staining on frozen sections before LCM with subsequent RNA extraction will always be a very sensible procedure.

The antibody incubation has to be performed in aqueous solutions which allow RNase activity. In frozen sections, even after short ethanol "fixation", the intrinsic degrading activity of RNases will come back to life as soon as the tissue is rehydrated and not frozen anymore. Thus the results in RNA quality will strongly depend on the amount of intrinsic RNase activity of the specific tissue itself, the time and temperature for incubation with the antibody and the possible RNase content of the antibody-solution/serum. In theory a very short and cold incubation with a single antibody, preferably directly labeled with some fluorescent dye, would therefore be recommendable.

Up to now only few publications show antibody staining on frozen sections before laser microdissection with still useful RNA analyses (please see attached list). These papers will give some insight in specific successful examples but nevertheless the situation may be different for any other combination of tissue and antibody.

Recommended reading:

- The publication by *von Smolinski et al* (2006) addresses main pitfalls of IHC and RNA. It offers a possible solution focused on time reduction and a special labeling process.

- *Brown* and *Smith* (2009) present an astonishingly simple principle for strong RNA stabilization by high salt conditions applied during normal antibody incubation. A limitation can be the requirement for quite robust antibodies. (Stability of the AB-binding against 2M NaCl and dehydration by ethanol) .
If this methodology will proof to be applicable also in other tissues and with other antibodies it could revolutionize the application of IHC with LCM.

Citations:

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