

## I. Tissue dehydration

1. Fix 8  $\mu\text{m}$  frozen sections in 70% ethanol for 15 sec
  - *Move slides directly from  $-80^{\circ}\text{C}$  to ethanol (do not allow slides to warm to room temperature)*
  - *Process no more than four slides simultaneously*
  - *Use slide forceps to transfer slides from jar to jar. Do not use a rubber slide holder (the holder transfers liquid from jar to jar, which prevents dehydration).*
2. Dehydrate in 95% ethanol for 15 sec, then in 100% ethanol for 1 min
3. Remove ethanol with a xylene dip for 2 min
  - *A second xylene clearing is not necessary and will increase the likelihood of collateral pickup during microdissection*
4. Air dry 5–10 min and store in a dessicator
  - *Another set of four slides can be started at this point, if needed*
  - *Ethanol can be reused for multiple slide sets, but should be discarded at the end of staining*
  - *Tissue should appear white, indicating light adherence to the slide*
5. Optional: verify tissue fluorescence with microscope
6. Proceed immediately to laser capture microdissection

## II. Laser capture microdissection

1. Transport slides in dessicator to microdissector
2. Turn on the instrument and spray hands with RNase Away
3. Load Capsure HS LCM caps (Arcturus #LCM0214) onto the instrument
4. Detach LCM cap, focus laser, and begin dissecting at power = 50 mW and duration = 20 (tenths of milliseconds)
  - *If the sample has been appropriately dehydrated, this laser power should allow good capture and resolution (1–2 cells per laser shot)*
  - *If the polymer does not wet after many shots, increase the laser power in 5 mW increments*
  - *Imaging settings*
    - *Phase A (no phase contrast)*
    - *Illumination tab: intensity = 50, gain = 0, diffuser = in*
    - *Fluorescence tab: microscope lamp intensity = 45, microscope lamp camera gain = 100, fluorescence intensity = 25.*
5. If there is extensive collateral pickup from adjacent nondissected cells, press LCM cap lightly on the adhesive of a Post-It note
  - *Try to use the least adhesive Post-It note available, and press the cap as lightly as possible to minimize unintended sample loss*
6. Load LCM cap onto ExtracSure adaptor (included in #LCM0214) and store upside down at room temperature
7. Proceed immediately to single-cell mRNA amplification