Laser-capture microdissection of 3D structures Entered by Kevin Janes
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I. Rapid nuclear-fast-red staining

1. Fix 8 μm frozen sections in 75% ethanol for 30 sec
   - *Move slides directly from –80°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. Transfer to distilled water for 30 sec
3. Stain with a few drops of nuclear fast red (Vector Laboratories #H-3403) containing 1 U/ml RNAsin Plus (Promega #N2611) for 30 sec
   - *100 μl nuclear fast red + 2.5 μl RNAsin Plus is sufficient for four slides containing two sections per slide*
4. Transfer to distilled water for 15 sec (dip slide, remove slide, dip slide again)
5. Repeat Step 4 with a second jar of distilled water
6. Dehydrate in 70% ethanol for 30 sec, 95% ethanol for 30 sec, and finally 100% ethanol for 30 sec
7. 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*
8. Air dry 5–10 min and store in a dessicator
   - *Another set of four slides can be started at this point, if needed*
   - *Replace the distilled water from Steps 2, 4, and 5 after each set of slides (the ethanol can be reused for multiple slide sets, but should be discarded at the end of staining)*
9. Proceed immediately to laser capture microdissection

II. Laser capture microdissection

1. Transport slides in dessicator to microdissector
2. Turn on the instrument, spray hands with RNAse Away, and clean slide with a PrepStrip (Arcturus #LCM0207)
   - *Each PrepStrip can be cut in half lengthwise to save reagents*
   - *Do not press down too hard with the PrepStrip (intermediate pressure is sufficient)*
3. Load Capsure HS LCM caps (Arcturus #LCM0214) onto the instrument
4. Detach LCM cap, focus laser, and begin dissecting at 0.175 V, 50–65 mW, 750 μs laser power
   - *If the sample has been appropriately dehydrated, this laser power should allow good capture and resolution (1–2 cells per laser shot)*
   - *Multiple shots are often required to cause polymer wetting at this laser power*
   - *If the polymer does not wet after many shots, increase the laser power in 5 mW increments*
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