**Protocol #21**

**Cryosection**

**LIANG TING (2022.07.20)**

1. Fix the embryos subjected to WISH with indicated riboprobes with fix solution (8% PFA and 2x fix solution [8% sucrose, 1 M CaCl2 0.03% and 5% 20x PBS]) O/N at 4°C.
2. Embed the enbryos in 1.5% agarose dissolved in 5% sucrose, kept in a sealed 15-ml tube with 30% sucrose in PBS O/N at 4°C.
3. Freeze the resulting agarose block containing the embryos with butanol (JUNSEI, Tokyo, Japan) chilled with liquid nitrogen.
4. The embryos were then serially sectioned to slices (20 μm in thickness) using a microtome (CM3000S, Leica, Wetzlar, Germany), the slide will be ready for imaging under microscope.