Embedding of cryofixed 3D structures Entered by Kevin Janes
Janes Lab Protocols 1/5/07

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1. Cut plastic coverslips (VWR #48376-049) to fit at the base of each chamber on an eight-chamber slide.
2. Sterilize coverslips with 100% ethanol and place at the bottom of a sterile eight-chamber slide.
3. After the ethanol has evaporated, coat the chamber slide with matrigel according to standard procedures. Make sure to cover the matrigel beyond the edge of the coverslip to the edge of the chamber without forming a meniscus.
4. Plate and culture MCF-10As according to standard procedures for the desired number of days.
5. Transfer the assay medium from the chamber slide to individual wells on a 24-well plate, crack open the chambers, and transfer the coverslips face-up to the 24-well plate containing the assay medium. Make sure to detach any coverslips that have stuck to the plastic chamber walls.
6. Aspirate the assay medium and fix the 3D structures with 0.5 ml freshly prepared 3.7% PFA. Incubate for 15 min at room temperature
   - *Stock solutions of 37% PFA should be prepared according to Wang et al.* Meth. Enzymol*. 85:514 (1982) and stored in single-use aliquots at –20°C*
   - *Fix slides in a fume hood and dispose of PFA as hazardous waste*
7. Wash coverslips 3 × 5 min in 1 ml PBS
8. Incubate coverslips in 15% (w/v in PBS) sucrose for 15 min, followed by 30% (w/v in PBS) sucrose for 15 min
   - *Sucrose incubations dramatically improve the morphology of the 3D structures after freezing and sectioning*
9. While the samples are equilibrating in the sucrose solutions, cover the base of small cryomolds (VWR #25608-922) with ~1 mm thickness of Neg-50 embedding medium (VWR #84000-154).
10. After the embedding medium has settled uniformly at the base of the cryomold, snap freeze the cryomold in a dry ice-isopentane bath. Keep the frozen cryomolds on dry ice.
11. Remove the coverslip from the 30% sucrose solution with a pair of forceps and place face up inside a frozen cryomold. This step can be done quickly on a benchtop before the Neg-50 thaws.
12. Fill the remainder of the cryomold with Neg-50 and snap freeze the cryomold in a dry ice-isopentane bath. Keep the embedded coverslips on dry ice and embed the remaining coverslips.
13. Wrap the embedded samples in tinfoil and store at –80°C for 6–12 months or more. Isopentane can be stored at room temperature and reused indefinitely (it should not be disposed of down the sink).