Embedding of fresh 3D structures for laser-capture microdissection Entered by Kevin Janes
Janes Lab Protocols 5/15/11

When referring to this protocol, please cite: Janes KA, Wang CC, Holmberg KJ, Cabral K, Brugge JS. (2010) Identifying single-cell molecular programs by stochastic profiling. *Nat Methods*, 7, 311-7.

1. Cut plastic coverslips (VWR #48376-049) to fit at the base of each chamber on an eight-chamber slide.
   - *The coverslips should fit a little loosely at the base of each chamber so that the matrigel flows around the edge of the coverslip during the 3D setup; this prevents the matrigel from delaminating during processing for fixed frozen sections*
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 MCF10As 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. Cover the base of small cryomolds (VWR #25608-922) with ~1 mm thickness of Neg-50 embedding medium (VWR #84000-154).
   - *Make sure that the Neg-50 embedding medium lies as flat as possible at the base of the cryomold; if a meniscus forms, this will create an air pocket in later steps that will cause the block to crack in half during sectioning*
7. 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.
8. Remove coverslips individually from the assay medium 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.
   - *Try to lie the coverslip as flush as possible on the frozen Neg-50; if an air pocket forms underneath the coverslip, the block may crack during sectioning*
9. 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.
10. Wrap the embedded samples in tinfoil and store at –80^{o}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).