Embedding Protocol for MCF-10A Frozen Sections

Notes:

- *OCT will also work as an embedding medium (the ability to section with OCT can depend upon the cryostat)*
- *Isopentane can be reused repeatedly (should not be disposed of down the sink)*

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 ethanol has evaporated, coat chamber slide with Matrigel™ per standard protocol. 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 per standard protocol for the desired number of days.
5) Cover the base of small cryomolds (VWR #25608-922) with ~1 mm thickness of NEG 50 embedding medium (VWR #84000-154).
6) 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.
7) Aspirate the assay medium from the chamber slide and crack open the chambers. Make sure to detach any coverslips that have stuck to the plastic chamber walls.
8) Lift a coverslip 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.
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°C.