Embedding of fresh brain samples for laser-capture microdissection Entered by Matt Sutcliffe
Janes Lab Protocols 6/13/18

1. After brain isolation, confirm tdTomato fluorescence using a stereomicroscope equipped with a fluorescence lamp and collect images if desired.
2. 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*
3. After the embedding medium has settled uniformly at the base of the cryomold, place the hemisphere medial side up into the embedding medium with a pair of forceps.
   - *Try to avoid air pockets underneath the sample; if an air pocket forms, the block may crack during sectioning*
4. Fill the remainder of the cryomold with Neg-50 and snap freeze the cryomold in a dry ice-isopentane bath. Keep the embedded samples on dry ice and embed the remaining samples.
5. 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).