Cryopreservation of Human Tissue in OCT (TCGA Project)

Note: This protocol is intended as a way to preserve in OCT tissue in a manner that allows the specimen to be used in multiple analyses without compromising structural integrity of the tissue, or the quality of the sample for biochemical and/or genetic investigations and the subsequent tissue sectioning on a cryostat.

Things you will need:
- OCT Embedding Media
- Embedding molds, large
- 2L Dewar ¾ full with liquid nitrogen
- Freezing basket, pre-cooled in liquid nitrogen
- Disposable forceps
- Container of dry ice
- Heavy-duty aluminum foil squares, 3" x 3"
- Sample labels

1) Fill the bottom of the embedding mold with just enough OCT to cover the entire area. Place at -80°C or in the vapor phase of liquid nitrogen – store at -80°C until use.
2) Prepare a good number of squares of heavy-duty aluminum foil roughly 3" x 3".
3) Have the sample labels ready and pre-made (if applicable).
4) Before collection, be sure to have a Dewar (2L) of liquid nitrogen ready, as well as a Styrofoam container of dry ice.
5) Place the appropriate number of OCT molds on the dry ice and proceed to collection.
6) Collect tissue/biopsy specimens per approved protocol.
7) Keep all specimens on ice until embedded to avoid degradation.
8) Quickly make note of the specimen weight – TCGA requests at least 100mg of tissue.*
9) Using disposable forceps, carefully remove an OCT raft from the dry ice, and quickly place a single specimen directly onto the frozen OCT. Make sure the specimen is laying flat and that the largest surface is in full contact with the OCT layer.
10) Quickly cover the specimen with additional OCT. Use just enough OCT to completely cover the sample.
11) Quickly place the OCT-embedded specimen in the basket and suspend the sample in the vapors of the liquid nitrogen until frozen.
12) Carefully remove the frozen specimen from the basket and wrap in one of the foil squares.
13) Quickly apply the completed label to the wrapped specimen and place in the liquid nitrogen, or on dry ice.
14) Repeat as necessary. Bank all tissue.
15) When all of the specimens have been frozen, they should be stored at -80°C.

* If there is plenty of tissue (more than 100mg) excise a small portion to be flash-frozen (see GMB)