**Mayo Clinic Brain Tumor Patient-Derived Xenograft (PDX) National Resource**

**Instructions For Cryopreservation of PDX Flank Tumor Tissue**

**A few important notes:**

* Users are responsible for acquiring the appropriate institutional approvals before engraftment.
  + These institutional approvals will likely influence how the below protocol can be executed. It is the user’s responsibility to modify the below generic protocol to meet their institution’s requirements.
* With each tumor passage, tissue samples are routinely archived in liquid nitrogen and/or paraffin.
  + Cryopreservation in early generations is important to build up stores for restoration purposes.
* Each PDX line provided will have a unique lineage which is highlighted and explained below. You should commit the lineage(s) you receive from us to your records somewhere should there be a need to refer back to this in the future.
  + Example: 12, 16, 14, 10
    - 12: The GBM PDX line. Came from the 12th patient tumor xenografted.
    - 16: Mouse number with the GBM flank tumor
    - 14: Previous mouse flank number. #14 flank tumor was passaged to #16 flank.
    - 10: Tumor generation. Number of times the tumor was passed from mouse-to-mouse.
    - VF: Virus-Free. At one point, this line was cleared of the LDEV mouse virus.
      * We no longer test for this as the virus does not affect our studies.
    - G: Our abbreviation for Glioblastoma or GBM

Cryopreservation of xenograft tissue

Materials:

* Mouse bearing tumor measuring 1 to 1.5 cm in greatest dimension (for cryopreservation)
* Freezing Media:
  1. DMEM (Mediatech)
  2. Penicillin/Streptomycin (Cellgro; 5000 I.U./mL Pen, 5000 μg/mL strep (P/S))
  3. DMSO (Fisher Scientific)
  4. Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals)
* 150-mL sterile filter (Nalgene)
* CO2 source
* Betadine
* #10 Scalpels
* Sterile Culture Plates or petri dishes
* 1-cc syringe
* 1.8-mL Cryogenic vial (cryo-vial) (Corning or Nunc)
* Cryo 1°C Freezing Container (Nalgene)
* -80oC Freezer
* Liquid Nitrogen tank

1. Identify an appropriate mouse tumor for use, preferably measuring 1 to 1.5 cm in greatest dimension. Label the cryo-vials with the lineage information for the tissue that is being preserved prior to euthanizing the mouse.
2. Make up freezing media by combining:

* 50 mL of FBS
* 15 mL DMSO
* 85 mL of DMEM media supplemented with 10% FBS, 1% penicillin, and 1% streptomycin.
* Sterile filter and store at 4°C for up to 3 months.

1. Prior to euthanizing the animal, label all specimen containers/cryo-vials with tumor lineage and passage information. Recording the date, lineage, and passage information data in a file will be important for experimental tracking and future studies.
2. Euthanize the tumor-bearing mouse by CO2 and swab the tumor area with Betadine.
3. Cut out the tumor using a sterile scalpel, separate the tumor from the skin, and process as previously described by mincing the tumor with sterile scalpels in a sterile culture or petri dish.
4. Use a 1cc syringe to break the tumor into smaller chunks, pull up ~0.5cc of tumor into the syringe, and then pull up ~0.5 cc of freezing media.
5. Place tumor and freezing media into a pre-labeled cryo-vial (from step 3). Set tissue aside on ice.
6. Place cryo-vials into a freezing container and place into a −80°C freezer overnight. Transfer the cryo-preserved tissue from the −80°C freezer into a liquid nitrogen storage tank the next day for long-term storage.
7. Record the pertinent tumor information on the tissue preserved and the archival location in a file exclusively for tracking xenograft information (e.g., an Excel spreadsheet).