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

**Instructions for Establishment, Restoration, and Passaging of PDX Flank Tumor Lines**

**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.
* Engraftment must be done in immunodeficient mice.
  + Our lab serially passages tumors in athymic nude mice to avoid cells ever seeing plastic.
  + At the time of passaging, short-term explant cultures can be created for experimental use. These cultures should be used within two weeks.
* After implantation, tumors can take up to one year to develop but typically grow faster as models are passaged.
* The passage of tumors can be staggered to provide a near continuous supply of cells for experimental use.
* 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

Materials:

* Tumor sample
  + Ideally 1 cm3 in size but can be as small as 0.125 cm3
* Immunodeficient mice
  + We use 4- to 5-week-old female athymic nude mice (Charles River, strain code 553)
* Centrifuge
* 1 cc syringe
* 16-gauge 1½ inch needles
* Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
* Bell jar-type desiccator for anesthesia
* Fume hood
* Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230)
* 10% povidone-iodine (Carefusion #29906-016)
* Scalpels
* Petri dish
* Wet ice
* Dry ice

Establishment

PDXs were established through injection of human surgical tissue into the flank of female athymic nude mice 6-8 weeks of age (Envigo; following steps similar to 4-13 below) in accordance with the Belmont Report and U.S. Common Rule with approval from the Mayo Clinic Institutional Review Board and written consent from participating patients. All animal experiments were approved by the Mayo Clinic Institutional Animal Care and Use Committee Protocol #A00003130-17.

Restoration:

1. Place cryo-preserved PDX tissue at -80°C if sample will be used within a few days. Otherwise, store in liquid nitrogen until ready.
2. Pull the sample and place it on dry ice. Thaw sample in 37°C water bath.

* If removing from liquid nitrogen storage, loosen the top of the cap slightly to allow any liquid nitrogen to escape or the tube may explode.

1. In the meantime, thaw Matrigel on wet ice as well.

* 200 µL per animal
* Xenografts are typically passaged into three mice for the first passage, and then a tumor line is subsequently maintained in three mice at any one time.

1. Spin down the sample in a centrifuge at 320 RCF for 3 min, aspirate off the freezing media from the specimen, and re-suspend the entire contents of the tube in 200 μL of sterile cold PBS.
2. Draw up 200 μL of tumor into the 1cc syringe, insert the syringe into a capped 16-G needle and place it on wet ice, then draw up an equal volume of Matrigel through the needle. Mix the tumor and Matrigel in the syringe by rapidly pulling the plunger back and forth and then return the syringe to the ice. If injecting more than one mouse, prepare individual syringes for each mouse.

* Dispel all air bubbles from prep.
* When mixing tumor and Matrigel, do not expel through the needle as this could sheer the tissue. Limit mixing to within the syringe itself.
* If tumor is difficult to draw up in the syringe, it can be moved, after dicing, to a 1.5-mL microcentrifuge tube, and the syringe can be used as a “pseudo” pestle to further disaggregate the tumor.

1. Mice are anesthetized with isoflurane in a plastic desiccator. Place the desiccator into an externally vented fume hood.
2. Place a paper towel in the bottom of the desiccator and add 1–2 mL of isoflurane to the towel.

* Add additional anesthetic as needed to maintain the required effect.

1. Place an individual mouse in the desiccator. Once the mouse is unconscious and not moving, remove it from the desiccator and mark the ear using an ear punch or other method of animal identification.
2. Swab the back of the mouse and the injection site with Betadine or rubbing alcohol.
3. Inject all 400 μL of the tumor/Matrigel mixture into the flank of the mouse.

* We inject on the posterior, lateral aspect of the lower rib cage. Insert the needle through the skin into the subcutaneous space to inject. You should lift up the skin with your needle prior to injecting to insure that you are not in the muscle. The needle should be inserted approximately 5mm beyond the end of the needle bevel.

1. While removing the syringe, pinch the injection site for 15 to 30 sec to preserve tumor placement.
2. Place the animal back in their cage and repeat the process until all animals are injected. Label the cage with the appropriate xenograft number and record the appropriate information in a laboratory book or computer file.
3. Observe the animals weekly for visible tumor growth.

* Using our cryopreservation technique, approximately 90–95% of cryopreserved tumor samples are successfully restored within 2-3 months.

Serial passage of flank tumor xenografts

For tumors measuring 1-1.5 cm in greatest dimension for passage into two to three recipient mice.

Materials:

* Mouse bearing tumor
* CO2 source
* Forceps
* Betadine
* #10 Scalpels
* 100-mm Sterile Culture Plates or petri dishes
* Tissue Path Disposable Base Molds (Fisher Scientific)
* 2 ounce Specimen Containers (Kendall)
* OCT Media (Sakura Tissue-Tek)
* 10% Buffered Formalin (Fisher Scientific)
* 1.8-mL Cryogenic vial (cryo-vial. Nunc and Corning)
* 1-cc syringe
* Hypodermic 16-G needles
* Matrigel
* Isoflurane (Novaplus)
* 1.5-mL micro-centrifuge tube
* Dry ice
* Wet ice

1. Sterilize surface with Spore-Klenz or a similar disinfectant and allow enough contact time for sterilization to occur per manufacturers recommendations.
2. Euthanize the tumor-bearing mouse by CO2.
3. Swab the skin around the tumor with Betadine to minimize infection risk.
4. Dissect the tumor using a sterile #10 scalpel and separate it from the skin. Place tumor into a sterile 100mm culture or petri dish.

* Tissue is routinely archived for future studies from each tumor generation using the following techniques, as outlined in steps 5 through 7 below. This archived tissue can be very useful for molecular and histopathological studies.
* Cryopreservation in early generations is important to build up stores for restoration purposes. For steps on this process, refer to our Cryopreservation of Flank Tumor protocol.
* If no tissue is to be archived, proceed to step 8 below.

1. Using sterile scalpel, cut a cross-section of the tumor (~1–2 mm thick slice) for OCT embedding. Add a small amount of OCT to the base mold, place the tissue section on the OCT bed. Using forceps, press the tissue into the OCT, such that the tumor is lying flat in the base mold, and then encase the rest of the tissue with OCT to ensure that the tissue is not degraded over time in the −80°C freezer. Place OCT sample between two blocks of dry ice for freezing and then transfer to a −80°C freezer.
2. Cut another cross-section of the tumor (~1–2 mm thick) and place it in a specimen jar filled with formalin. Optimal fixation can be achieved with overnight fixation prior to paraffin embedding. Fixation in formalin for more than two weeks prior to paraffin embedding is sub-optimal, at which point the tissue can become brittle, section poorly, and affect antigen recognition for IHC staining.
3. Place another portion of tumor in a labeled 1.8-mL cryo-vial for fresh frozen tissue. Immediately place this sample on dry ice or into liquid nitrogen, and subsequently transfer the specimen into a −80°C freezer.
4. After the appropriate tissues are archived, thaw Matrigel on wet ice and mince the remaining tissue into small pieces using scalpels in a sterile 100-mm culture or petri dish.
5. Use a 1-cc syringe to break up the tumor into even smaller pieces by repeatedly drawing up and expelling the tissue. Finally, draw up 100 μL of tumor into the syringe, put 16G needle on, chill on ice, and then draw up an equal volume of Matrigel. Mix the tumor and Matrigel in the syringe and then return the syringe to the ice.

* Using a syringe without a needle to break up the tissue reduces the risk of tissue shearing.
* If there is limited tumor available or it is difficult to draw up in the syringe, the tumor can be moved, after dicing, to a 1.5-mL microcentrifuge tube, and the syringe can be used as a “pseudo” pestle to further disaggregate the tumor.

1. Anesthetize the mice and inject tumor cell mixture subcutaneously as described above.