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

**Cryomush (cryopreserved tissue) Implantation Instructions**

**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.
	+ Short-term explant cultures should be used within approximately two weeks.
* Processing of patient tumor samples for implantation into immunodeficient mice is the first step toward developing primary xenografts. For development of our model, we have exclusively relied on direct implantation of primary patient tumor samples into the flank of nude mice. Tumors are passaged directly from mouse to mouse without touching plastic.
	+ Tumor propagation via prolonged cell culture promotes loss of EGFR amplification and gain of MGMT promoter methylation.
	+ ~1% of the time a tumor will not grow after passage
* After implantation, tumors can take up to one year to develop but on subsequent tumor passage, the growth rates of the tumors typically increase. Experience in multiple labs suggests that approximately one-third of tumors implanted will establish viable xenografts.
* The passage of tumors can be staggered to provide a near continuous supply of tumors for use in laboratory studies.
* With each tumor passage, tissue samples are routinely archived in liquid nitrogen and/or paraffin, and, at least in early generations, cryopreserved as well to facilitate restoration of early passage tumors.

**Materials:**

* Tumor sample
	+ Ideally 1 cm3 in size but can be as small as 0.125 cm3
* Sterile 50 mL conical tube (Falcon)
* Hanks Balanced Salt Solution (HBSS, Irvine Scientific)
* 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

**Care upon receipt:**

1. Place the cryo-preserved PDX sample 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.
	1. 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.
3. In the meantime, thaw Matrigel on wet ice as well.
	1. 200 µL per animal
	2. 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.
4. 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 PBS.
5. Using a 1-cc syringe, pull up 200 μL of the tumor into the syringe and insert into a capped 16G needle. Return to wet ice.
6. Draw up 200 μL of Matrigel into each syringe/needle and mix with the tumor by rapidly pulling the plunger back and forth.
* 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 cryo-preserved tumor samples are successfully restored within 2-3 months.
	1. If injecting more than one mouse, make up individual syringes for each mouse.