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

**Instructions for Flank Tumor Implantation Using PDX Explant Cultures**

**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.
    - More indolent lines may be kept for longer periods, but the maximum culture time should be four weeks.
  + We do not recommend passaging short-term explant cultures *in vitro* prior to injection.
* After implantation, tumors should be closely monitored for growth.
* 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:

* Short-term explant cultured tumor cells
* Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
* Trypsin-EDTA (Corning #25-052-CI; 0.05% trypsin/0.53 mM EDTA in HBSS) **OR** TrypLE (Thermo Fisher # 12604-013)
* Complete DMEM (10% FBS and 1% P/S) **OR** Complete StemPro media (Knockout DMEM/F-12 and supplements)
  + For complete information on these medias, refer to fresh cell culture protocol.
* 15 mL and 50 mL Conical Tubes
* Sterile phosphate-buffered saline (PBS: Corning #21040CV)
* 1½ inch 21-gauge hypodermic needles
* 1 cc syringes
* Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
* Centrifuge
* Hemocytometer
* Trypan Blue (0.4%: Corning #25-900-CI)
* Wet ice

1. Warm the appropriate media that will be needed for the cells.

* Pay close attention to the type of cultures you are using for injections: FBS or stem cell cultures.

1. Gather PBS, trypsin (for FBS cell cultures) or TrypLE trypsin replacement (for stem cell cultures), and media into a clean, biological hood.
2. Carefully aspirate media out the flask, add 5-10ml of sterile PBS into each flask, and rock to wash off excess media.

* Dispense PBS down the side of the flasks to keep cells adherent.

1. Remove PBS and add 5-10ml of trypsin or TrypLe to each flask. Incubate at room temperature for 3-10 minutes. Once cells begin rounding up and can be dislodged from the plate when tapping the outside of the flask, proceed to the next step.

* If cells are not releasing from the plastic, the flasks can be incubated at 37°C with careful monitoring of cell lifting.
* Avoid over-trypsinizing cells.

1. Add Complete DMEM (FBS cell cultures) OR Complete StemPro (Stem cell cultures) to inactivate the trypsin. Use media to rinse any adherent cells off the flask and transfer cell/media mix to a conical tube, and centrifuge the cells at 320 RCF for three minutes.

* Keep the volume at an even number (e.g., 10 ml) to help with math.

1. Spin cells down in the centrifuge at 1200rpm for 3 minutes before resuspending cell pellet in 10-30ml of PBS depending on how many flasks were trypsinized.
2. Mix 10ul of resuspended cells with 10ul of Trypan blue in a 1.5ml tube. Mix with pipette and transfer 10ul of the mixture to a hemocytometer. Count cells in the top left and lower right quadrant. Multiply the total number of cells counted in both quadrants by 10,000. That calculation will give you cells/ml.
3. Calculate the number of cells and volume needed for the injections.

* We typically use 2 million cells in 50ul (40,000 cells/ul or 40 million cells/ml) which will be combined with 50ul of matrigel right before the injection.
* A typical way to do the calculation would be as follows:
  + How many mice needed for the study? N=10 mice.
    - Always mix up enough cells for at least 5 extra mice, so in this case, calculate for 15 mice.
  + 15 mice x 50ul = 750ul. We need 750ul of cells at 40,000 cells/ul or 30 million cells total.
  + If our count was 300 using the hemocytometer, 300 \* 10,000 = is 3 million cells/ml (3000 cells/ul).
  + Since we need 30 million cells and we have 3 million cells per ml, we can spin down 10ml of cells and resuspend to a total volume of 750ul with PBS.

1. Aspirate off the PBS from the cells, making sure not to disturb the cell pellet, and resuspend cells in the appropriate volume of PBS. Place cells on wet ice and pre-chill the syringes.

* Cells and Matrigel will be mixed 1:1 for total volume of 100 µL.

1. Using a 1cc syringe, draw up the appropriate volume of Matrigel and mix into the cells suspended in PBS just prior to the start of injections.
   * Keep this cell prep on wet ice to prevent the Matrigel from solidifying prior to injection.
   * Using Matrigel should dramatically improve take rate.
2. Mice are anesthetized with isoflurane in an induction chamber with the isoflurane level at 2-3% until unconsciousness is achieved and then lowered to 1-2% for remainder of procedure.
3. When animals are unconscious, pull them out one at a time and place on a paper towel on a disinfected surface. Quickly, but effectively, cleanse the injection site with betadine.
   * We inject on the posterior, lateral aspect of the lower rib cage on the right side of the mouse.
   * Some method of animal identification should be used at this time.
4. Draw up the appropriate volume of the Matrigel/cell suspension using a 1cc syringe fitted with a 21G needle. Insert the needle through the skin into the subcutaneous space to inject. You should lift the skin with your needle prior to injecting to ensure that you are not in the muscle. The needle should be inserted approximately 5 mm beyond the end of the needle bevel.
5. While removing the syringe, pinch the injection site for 15-30 seconds to maintain tumor placement.
6. Place the animal back in its cage and repeat the process until all animals are injected.
7. Label the cage with the appropriate xenograft number and record the appropriate information in a lab book or computer file.
8. Observe the mice two to three times a week for tumor development and growth. Depending on the tumor line, tumors may take 1 to 12 weeks to appear.