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

**Cell Harvest from Flank Tumor Protocol**

**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.
* Short-term explant cultures can be readily derived from most established xenograft lines, and these cultures can be used for *in vitro* studies or establishing tumors in immunocompromised mice.
* When needed for establishing tumors, these short-term cultures can be transduced with lentiviral vectors. Recipient labs are responsible for performing any transductions with the appropriate institutional approvals in place at their institution.

**Materials for STEM CELL CULTURES:**

* Stem cell media (StemPro NSC SFM kit: ThermoFisher Scientific #A1050901; subsequently referred to as SCM).
  + To make 500ml, use the following kit components plus L-glutamine and Pen-Strep solution as follows and filter sterilize:
    - KnockOut DMEM/F-12 Basal Media - 500ml
    - StemPro NSC SFM Supplement - 10ml
    - FGF Basic Recombinant Human - 10ug
    - EGF Recombinant Human - 10ug
    - Reagents not included in the kit:
      * L-glutamine (Corning #25005CI) 10ml of 200mM solution
      * Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) 5ml
* Laminin (Sigma #L2020-1MG) for coating vessels

**Materials for FBS CELL CULTURES:**

* DMEM medias (Corning #10-013-CV): (1) with FBS supplementation at 2.5% **and** (1) with FBS supplementation at 10%; subsequently referred to as 2.5% and 10% DMEM.
  + To make 500ml, supplement the DMEM as follows and filter sterilize:
    - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
      * 50ml = [Final FBS] 10%
      * 12.5ml = [Final FBS] 2.5%
    - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) – 5ml
      * [Final] = 1% P/S
* 500 ml sterile filters (Nalgene: Thermo Scientific #156-4020)
* Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol) for coating vessels

**Common materials for cell harvest:**

* 500 ml sterile filter with receiver (Nalgene: Thermo Scientific #156-4020)
* 150 mm Tissue Culture Flasks (Corning #430825)
* CO2 source
* Betadine (Carefusion #29906-016)
* Sterile Tissue Culture plate
* Sterile Scalpels
* 1 cc syringe
* Wet ice

**Preparation of coated flasks**

* **STEM CELL CULTURES:**
  + Thaw Laminin at room temperature. Coat flasks by adding 1ul per cm2 of Laminin to 3.6 mL of stem cell media for each 150 mm flask to be coated. Allow the plates to sit at room temperature on a level surface for approx. 2 hours prior to use to allow the Laminin to properly adhere to the plates.
* **FBS CELL CULTURES:**
  + Thaw Matrigel aliquots on wet ice. Make up a stock solution for coating flasks by adding 400 µL of Matrigel to 3.6 mL of 2.5% DMEM for each 150 mm flask to be coated. Coat flasks with Matrigel by adding about 4 mL to each one and then tipping the plates in order to coat the entire culture surface. Allow the plates to sit at room temperature on a level surface for approx. 30 minutes prior to use to allow the Matrigel to properly adhere to the flasks.

**Harvest**

1. Euthanize a mouse with an approx. 2 cm tumor using CO2 asphyxiation.
2. Swab the tumor area with Betadine, cut out the tumor using a sterile scalpel, separate the tumor from the skin, and mince the tumor sample in a sterile culture plate using a sterile scalpel blade.
3. Add approximately 3 mL of 2.5% DMEM (FBS cell culture) or SCM (Stem cell culture) to the plate and continue to disrupt the tumor chunks using a 1 cc syringe.
   1. Tip the culture plate on its side and break up the tumor into smaller chunks by pulling it into the syringe and expelling it back into the plate many times. This will allow for better disruption of the tumor and a more even distribution of the cells on the tissue culture flasks.
   2. This low percentage (2.5%) DMEM media favors growth of glioma cells over murine fibroblasts and should be used with initial plating.
4. Pull up 1 cc of tumor cells/media slurry and place into each coated vessel. If there are still cells/media left over, distribute evenly to each plate. Add an additional 25-35 mL of the appropriate media to each flask.
   1. Additional media can be added after tumor disaggregation depending on the number of plates being seeded. Cells seem to grow best using Corning tissue culture plastic.
5. Maintain cells in an incubator at 37°C and 5% CO2.
6. Check the flasks daily until the cells have adhered to the treated plastic.
   1. Depending on the tumor line, this may take between 1-7 days. Take care to not disturb the cells until they have fully adhered to avoid cell loss.
7. Once cells adhere to the plate, remove the debris from the plate by vigorously shaking the plate and then aspirating the media, debris, and non-adherent cells.
   1. To avoid dislodging cells, refrain from (i) beating the flasks against your hand and (ii) washing with additional media. A sterile Pasteur pipette can be used to remove any stubborn debris or tissue chunks.
8. Replace media with 10% DMEM (FBS culture) or SCM (stem cell culture) once the cells become adherent.
9. Monitor the cells and change media as necessary until they are approx. 80-90% confluent. At this point, they are ready for experimental use.
   1. Make sure that the cells are well fed. If the media turns yellow, they may not recover from the stress or they may change their response, making it difficult to reproduce experimental results.
   2. The Sarkaria lab routinely uses cultured cells for *in vivo* tumor initiation or *in vitro* experiments from the initial culture. We do not recommend passaging these lines as primary cells tend to slow proliferation over multiple passages. Cells should be maintained in culture for (preferably) <14 days and no greater than 30 days.
10. When re-plating the cells for *in vitro* assays, it is not necessary to use coated vessels. For most lines, cells will grow in non-adherent spheres in SCM without a laminin coating. If adherent stem-like cells are warranted, the flask must first be coated with laminin as described above.