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

**Cryo-preserved PDX Cell Culture Instructions**

**Last updated Oct. 30, 2023**

**A few important notes:**

* These lines will not behave like established cell lines. Because of the cellular heterogeneity of these PDX lines, each line will have its own unique growth characteristics, and these characteristics may vary from batch to batch.
* The ability to expand these lines is minimal and varies from line to line. See the [GBM PDX characteristics database](https://www.mayo.edu/research/documents/sarkaria-gbm-pdx-lines/doc-20503362) for more information regarding cell culture attributes. Ideally, cells should be used within the first few passages.
* Transduction with lentiviral vectors is possible in these cell lines but may vary by line and will almost always be more efficient in fresh cell cultures.
* Whether FBS or stem cultures, similar techniques are used to maintain these PDX lines although some different materials will be needed.
* Each PDX line provided will have a unique lineage which is highlighted and explained below.
  + 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
* Users are responsible for confirming the presence of desirable PDX features upon receipt.

**Materials for STEM CELL CULTURES:**

* Stem cell media (StemPro NSC SFM kit: ThermoFisher Scientific #A1050901).
  + 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
* 500 ml sterile filter (Nalgene: Thermo Scientific #156-4020)
* Optional: Laminin (Sigma #L2020-1MG) if adherent cultures are needed.

**Materials for FBS CELL CULTURES:**

* DMEM media (Corning #10-013-CV)
  + To make 500ml, supplement the DMEM as follows and filter sterilize:
    - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150) – 50ml
      * [Final] = 10% FBS
    - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep) – 5ml
      * [Final] = 1% P/S
* 500 ml sterile filter (Nalgene: Thermo Scientific #156-4020)

**Care upon receipt:**

1. Open package and remove cryovial(s). Store at -80°C if using immediately. Liquid nitrogen must be used for long-term storage.

* If you want **stem** cultures to grow adherently, you will need to prepare coated vessels ahead of time. Instructions are shown below. We do not generally coat vessels when we thaw **FBS** cultures.
  + **Coating instructions:** Thaw Laminin at room temperature, add 1ul per cm2 of Laminin in media (150ul laminin in 4 mL for each 150 mm flask of stem cell media, or 75ul in a T75 flask to be coated.) Allow the plates to sit at room temperature on a level surface for 1-2 hours prior to use to allow the Laminin to properly adhere to the plates.

1. Pull cryovial(s) from storage and place on dry ice. Bring to your cell culture area.
2. Place the frozen cryovial(s) in a 37°C water bath just until thawed.
3. In the meantime, fill your tissue culture vessel with the appropriate amount of fresh media. We recommend one cryo-vial in a T75 flask.
4. Transfer total volume of thawed cells to the tissue culture vessel and place in 37°C incubator at 5% CO2.
5. Monitor cultures daily and change media once adhered. Some lines may need up to a week in culture before they start to proliferate. Use cells as soon as possible after thawing. If expansion is required, split cells 1:2 and up to 1:5 using trypsin or TrypLE. Change media at least twice per week, and many lines will only need to be passaged once per week (passage when ~80% confluent). We do not recommend long-term passaging as it may change cell behavior, render cells nontumorigenic, and potentially cause genetic drift.