Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Cryopreservation of Xenograft Tissue

Cryopreservation of xenograft tissues is a key methodology used to re-establish heterotopic xenografts from archived tissue. Because extended tumor passage in vivo likely leads to genetic and epigenetic drift of the tumors away from the original patient tumor characteristics, early passage xenograft material is cryopreserved for each xenograft line.

For xenograft lines that are maintained continuously in vivo for experimental studies, the xenograft lines are passaged only 15 to 20 times before being restored using early passage material. Similarly, lines that are used infrequently can be preserved in liquid nitrogen and then restored on demand for a specific experiment.

The standard procedure with each new xenograft line is to cryopreserve tissue from five passages of the given heterotopic xenograft, which should provide adequate tissues to restore the xenograft line 50 times. If this resource nears depletion for any given line, then a restored early passage xenograft will be expanded and cryopreserved. In this way, cycles of cryopreservation and restoration can be used to maintain xenograft lines as early passage tumors indefinitely.

Materials:

- Mouse bearing tumor measuring 1 to 1.5 cm in greatest dimension (for cryopreservation)
- Recipient mouse (for restoration of cryopreserved tumor tissue; Envigo #Hsd:Athymic Nude-Foxn^{nu})
- 1.8 mL Cryogenic tube (Thermo-Scientific #375418)
- Scalpels
- Sterile culture plates
- Betadine (Carefusion #29906-016)
- 1 cc syringe
- 16 gauge hypodermic needle
- Freezing media:
    - DMEM (Corning #10-013-CV)
    - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep (P/S))
    - DMSO (Fisher Scientific #BP231-1)
    - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
- Cryo 1^{o}C Freezing Container (Thermo Scientific #15-350-50))
- Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
- Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
- Bell jar desiccator for anesthesia
- 150 mL sterile filter (Nalgene; Thermo Scientific #09-740-28E)
- Sterile phosphate-buffered saline (PBS) (Corning #21040CV)

- Wet ice
- Dry ice

Preparation:

1) Identify an appropriate mouse tumor for use, preferably measuring 1 to 1.5 cm in greatest dimension. Label the cryogenic tubes with the lineage information for the tissue that is being preserved prior to euthanizing the mouse.
2) Make freezing media by adding 50 mL of FBS and 15 mL DMSO to 85 mL of Complete DMEM (DMEM containing 10% FBS and 1% Pen/Strep solution). Filter the freezing media using a 150ml sterile filter. The freezing media can be stored at 4˚C for up to 3 months.
3) Prior to euthanizing the animal, label all specimen containers with tumor lineage and passage information.
    - This will be important for future studies to record the data in a file where the passaging, archiving and tumor preservation can be tracked.

Tumor resection and cryopreservation:

4) Euthanize the tumor-bearing mouse by CO_2 and swab the tumor area with Betadine.
5) Cut out the tumor using a sterile scalpel, separate the tumor from the skin and mince the tumor sample in a sterile Petri dish using a sterile scalpel blade.
6) Use a 1 cc syringe to break up the tumor into smaller chunks, pull up about 0.5 cc of tumor into the syringe and then pull up about 0.5 cc of freezing media.
7) Place tumor and freezing media into a prelabeled cryogenic tube (from Step 3). Set tissue aside at room temperature for at least 30 minutes but no more than 60 minutes.
8) Place cryogenic tubes into the freezing container and place the freezing container into a minus 80^{o}C freezer overnight. Transfer the cryopreserved tissue from the minus 80^{o}C freezer into a liquid nitrogen storage tank for long-term storage.
    - The freezing container controls the rate of cooling of the tumor samples.
9) Record the pertinent tumor information on the tissue preserved and the archival location in a file exclusively for tracking xenograft information (e.g. an Excel spreadsheet).

Restoration of a xenograft from cryopreserved material:

Preparation:

10) Locate the tissue in the liquid nitrogen tank and update the tissue log to record its use.
11) Pull the sample from the liquid nitrogen tank and place it on wet ice. Loosen the top of the cap slightly to allow any liquid nitrogen that entered the tube during the archiving process to escape or the tube may explode.
12) Thaw Matrigel aliquot on wet ice.

Flank injection from cryopreserved tissues:

13) Spin down the sample in a centrifuge at 320 RCF for three minutes, aspirate off the freezing media from the specimen, and resuspend the entire contents of the tube in 200 microL of sterile PBS.
14) Using a 1 cc syringe, pull up 200 microL of the tumor into a 1 cc syringe. Insert the syringe into a capped 16-gauge needle and place it back on wet ice.
15) Draw up 200 microL of Matrigel into each syringe/needle and mix with the tumor by rapidly pulling the plunger back and forth. After dispelling the air from the tip of the needle, the total volume should be about 400 microL of a 1:1 solution. Recap the needle and place on wet ice until ready to inject.
16) Inject tumor sample into the mouse as described below.

Anesthetize the mice:

1) Mice are anesthetized with isoflurane in a plastic desiccator. Place the desiccator into an externally vented fume hood. If a hood is not available, the biosafety department should test that laboratory personnel are not being exposed to excessive isoflurane fumes using this method.
2) Place a paper towel in the bottom of the desiccator and add 1-2 mL of anesthetic to the towel.
   - Add additional anesthetic as needed to maintain the required effect.
3) Place an individual mouse in the desiccator.
4) 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. Because the procedure is quite quick, we typically do not confirm depth of anesthesia and we do not warm the animals during anesthesia. We do observe the mice, and if they are unconscious for more than 5 minutes, we will typically group them with other mice to maintain their body temperature.

Inject tumor sample into mouse:

5) Swab the back of the mouse with Betadine or rubbing alcohol over the injection site. Although the operator should wear sterile gown, gloves and mask (typical garb for handling nude mice), an aseptic field with a sterile drape is not necessary.
6) Inject all 200 microL of the tumor/Matrigel mixture into the flank of the mouse.
   - The injection site is typically 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 ensure that you are not in the muscle. The needle should be inserted approximately 5 mm beyond the end of the needle bevel.
7) While removing the syringe, pinch the injection site for 15 to 30 seconds so that the tumor/Matrigel mixture does not leak out of the injection site.
8) Place the animal back in its 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.

17) Observe the animals weekly for visible tumor growth. Using our cryopreservation technique, approximately 90-95% of cryopreserved tumor samples are successfully restored. A restored tumor may take two to three months to start growing.