SOP_MTL-1.3 Viably Frozen Tissue Processing for Implantation

A. Purpose: To provide aseptic techniques and instructions for thawing viably frozen tissue specimens for implantation.

B. Scope: This SOP can be used to process any tissue specimen (of human or mouse origin) that was previously viably frozen for implantation into mice.

C. Definitions:

DMEM: Dulbecco's modified Eagle medium

Restart: Each time tissue from an established PDX model is taken from viably frozen conditions and implanted into mice to produce the next series of transplant generations. For example, PDX tissue thawed from BCM-2147-TG3 would become BCM-2147-R1TG4 upon transplantation

TG: Transplant generation

Transplant generation: The number of times that PDX tissue has been transplanted from mouse to mouse with the purpose of maintaining an actively growing PDX model

D. Materials and Reagents:

Name	Quantity	Cat number	Sterility status for use
1.7 mL Microcentrifuge tubes	1/specimen	NC9818380, Denville	Sterile
15 mL Conical tubes	1/specimen	430790, Corning	Sterile
2 mL Tube	1/specimen	24-283, Olympus	Sterile
25 mL Serological Pipette	2	P7865, Greiner	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Cutting board/petri dish	1/specimen	NA / FB0875712, Fisher scientific	Clean with bleach and 70% EtOH/Sterile
DMEM	50 mL/specimen	10-013-CV, Gibco	Sterile
Glass Pasteur pipets	4/specimen	22-230-482, Fisher scientific	Sterile
Labels	1-10	B-490, Brady	Non-sterile
Liquid nitrogen + thermo-flask container	Enough to cover tubes	11-670-25C, Fisher scientific	Non-sterile
Matrigel	175 µl/specimen	354234, BD	Sterile
Paper towels	Several	10714-002, VWR	Non-sterile
Parafilm	1 strip/specimen	P7793, Millipore sigma	Non-sterile
Pipet-aid	1	4-000-100, Drummond	Sterile
Razor blade/scalpel	1/specimen	55411-050, VWR	Sterile
Regular forceps	1/specimen	RS-5139, Roboz Surgical	Sterile
Securline marker (ethanol resistant)	1	14-905-30, Fisher scientific	Non-sterile

E. References: NA

F. Procedures:

General Considerations:

In order to keep the tissue as sterile as possible, all steps will be done inside the cell culture hood. Use aseptic techniques throughout the procedure. All items going into the cell culture hood should be sprayed with 70% ethanol and wiped down with a paper towel.

1. Fill a small container with ice and place the appropriate number of matrigel aliquots in the ice to thaw.
2. Warm the DMEM in the 37˚C bead bath prior to removing the sample from liquid nitrogen.
3. Place the specimen tube in the 37˚C bead bath.
4. Start processing the tissue as soon as it is thawed.
5. Label a 15 mL tube with all the original information from the viably frozen specimen tube.
6. Transfer the contents of the specimen tube into a 15 mL tube. Check to make sure all tissue fragments are transferred.
7. Fill the 15 mL tube containing the tissue with 37˚C DMEM media.
8. Mix the contents by gently inverting several times.
9. If there are tissue fragments that float, use the forceps to take them out and place them on the cutting board/petri dish. Use the glass Pasteur pipettes to vacuum off the DMEM after the rest of the tissue settles. If necessary, place the tissue pieces that were removed back into the tube for the next wash.
10. Repeat steps 7-9 twice for a total of three washes.
11. Transfer the tissue to the cutting board/petri dish by tilting the tube until all contents are out of the tube. Use the forceps to remove any tissue stuck in the tube.
12. Vacuum off any remaining DMEM media being very careful not to aspirate any tissue fragments.
13. <u>Patient tissue</u>:
    - 13.1. Cancer tissue: If the tissue pieces are too large, cut them into smaller fragments (1-2 mm^3). Select fragments that appear to contain cancerous tissue, attempting to avoid necrotic or fatty areas. Try to take representative small fragments from all the available tissue.
    - 13.2. Normal tissue: Select fragments that are representative of the tissue. If needed cut the pieces to smaller fragments (2-4mm^3). They can be larger than cancer fragments since the tissue is softer.
    - 13.3. Label a 2 mL tube containing 175 µL of matrigel with all the information from the original specimen tube and put 10-12 tissue fragments (or more depending on the number of mice to be transplanted) into the Matrigel.
    - 13.4. Keep the tissue on ice until it is ready to be transplanted.
    - 13.5. If there are any extra fragments from cancer samples, snap freeze one chunk in a 1.7 mL tube for DNA extraction and STR validation. Label the tube with the original tube information and “frozen from restart”.
14. <u>PDX Tissue:</u>

Some PDX models that are difficult to restart might need to be placed in matrigel similar to patient tissue. Consult with the core director to confirm processing conditions.

- 14.1. If the PDX tissue pieces are too large, use the forceps and razor blade to cut the tissue down to 1-2mm^3.

14.2. Place 12-15 pieces into a new 15mL conical tube and label with all the information from the original tube.
14.3. Add 13 mL DMEM to the tissue fragments.
14.4. Wrap the top of the tube with parafilm to prevent leakage. Place the 15 mL conical tube in a 37°C incubator until ready to transplant.
14.5. If there are any extra fragments from the PDX sample, snap freeze one chunk in a 1.7 mL tube for DNA extraction and STR validation. Label the tube with the original tube information and "frozen from restart".
15. Replace any unused matrigel back into the -20°C freezer.
16. Clean up the cell culture hood and place all used supplies in the trash or the appropriate area to be cleaned.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	10.23.2020	All	SOP created
2	02/04.2021	D	Format updated