SOP_MTL-1.4 Tumor Tissue Excision for PDX Maintenance

A. Purpose: The purpose of this SOP is to provide detailed instructions on how to excise and process tumor tissue from maintenance mice.

B. Scope: This SOP is intended to provide detailed instructions for tumor excision from maintenance mice including all equipment and resources necessary to complete the task. Any caveats that may occur while processing tumor tissue are noted.

C. Definitions:

Derivatives: Any specimen type (including transplanted mice) created from an excised tumor

DMEM: Dulbecco's modified Eagle medium

DMSO: Dimethyl sulfoxide

EDTA: Ethylenediaminetetraacetic acid

FBS: Fetal bovine serum

FFPE: Formalin-Fixed Paraffin-Embedded

Formalin: 10% neutral buffered formalin

Freezing media: 50% FBS, 40% DMEM, 10% DMSO

Maintenance mice: Mice transplanted with PDX tissue for the purpose of continuing propagation of the model; mice were not under any study protocol

OpenSpecimen (OS): Inventory database, https://www.openspecimen.org/

Restart: When viably frozen tissue is implanted to mice to regrow a model. TG designations begin with "R_". For example, the first restart of a model from TG4 viably frozen tissue would be designated as R1TG5.

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

Transplant-size fragments: 1-2 mm^3 pieces of tumor tissue

D. Materials and Reagents:

Name	Quantity	Cat. Number	Sterility status for use
0.5 M EDTA, pH 8	50-100 µL/mouse (for blood)	15575-020, ThermoFisher	Non-sterile
1.7 mL Microcentrifuge tubes	2/mouse (for blood)	NC9818380, Denville	Sterile

1000 µL Pipette tips (+pipette)	1-3	1000 µL: 05-403-18, Eppendorf	Sterile
15 mL Conical tubes	1/PDX to harvest	430790, Corning	Sterile
2 mL Cryovials	Variable	W985865, Wheaton	Sterile
23g Hypodermic Needles	6	14-826-6B, Fisher scientific	Sterile
25g Hypodermic Needles	1/mouse (for blood)	14-826AA, Fisher scientific	Sterile
50 mL Conical tubes	1	14-959-49A, Fisher scientific	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Body bag	As needed	BCM Animal Facility	Non-sterile
Clawed forceps	1	RS-5158	Sterile
Cotton tip applicator	1-2/mouse	22-363-168, Fisher scientific	Non-sterile
DMEM	12 mL/tube	10-013-CV, Gibco	Sterile
Euthanasia box	1	NA	Non-sterile
Foam board	1	NA	Non-sterile
Formalin	Enough to cover cassettes	22-050-105, Fisher scientific	Non-sterile
Formalin container	1/specimen	Any container with a sealed lid	Non-sterile
Freezing media	1.25 mL/cryovial	NA	Sterile
Gauze pads	Small stack	22-037-985, Covidien	Non-sterile
Isoflurane	5-10 mL	BCM Mouse Facility	Sterile
Kimtech wipes	2-2	06-666A, KC	Non-sterile
Labels	Variable	B-490, Brady	Non-sterile
Liquid nitrogen + thermo-flask container	Enough to cover tubes	11-670-25C, Fisher scientific	Non-sterile
Maintenance sheets	1/PDX to harvest	NA	Non-sterile
Paper towels	2-3/PDX to harvest	10714-002, VWR	Non-sterile
Petri dish	As needed	25384-088, VWR	Sterile
Razor blades	1/PDX to harvest	NC9148528, Fisher scientific	Sterile
Scissors	1	RS-5880, Roboz surgical	Sterile
Securline marker (ethanol resistant)	1	14-905-30, Fisher scientific	Non-sterile
Slow freezing container	1	Nalgene cryo-freezing, Biocision CoolCell	NA

Square cutting board	1/PDX to harvest	Teflon Sheet 1/4"x12"x12", United States Plastic Corp (cut to size)	Non-sterile
Syringe	1/mouse (for blood)	14-823-434, BD	Sterile
Tissue cassettes	1-2/PDX to harvest	89199-446, VWR	Non-sterile

E. References:

SOP_MTL-1.2 Freezing Media

SOP_MTL-1.5 Terminal Blood Collection from Mice

SOP_MTL-1.11 Harvest Sheets

F. Procedures:

General Considerations:

Use the table below to determine the amount and type of derivatives to be collected according to the transplant generation.

FFPE: Sections can be collected either longitudinally or transversely from the tumor depending on the tumor size so long a representative section is collected. Smaller tumors should have a transverse section taken.

Priorities: If the take rate is low and tumors are small for TG 1-5, the priority (from all mice) is to get one representative FFPE section, one snap frozen tube with 3-5 chunks, and the rest should be viably frozen. Higher transplant generations typically have enough tissue.

TG to be harvested		**Type of specimen and quantity to be collected at each TG**				**Total # of specimens to be collected for a given TG range**		
TG	**FFPE**	**Snap Frozen**	**Viably Frozen**	**Organs**	**Mice to Transplant**	**Snap Frozen**	**Viably Frozen**	**Blood**
1-5	One/mouse # mice=3	Two vials/mouse # mice=3	All remaining tissue	Collect Lung, Liver in FFPE cassette with Tumor	5 (or 3 double-sided if needed)	Up to 6 vials per TG	Bank all remaining tissue up to 81 vials	Bank up to 2 mL total (20 aliquots)
6-10	One/mouse # mice=2	One-two vials/mouse # mice =3 Total vials =4	Check inventory		3	Up to 4 vials per TG	Bank additional tissue to get to 81 vials	Bank additional blood to get to 2 mL total

11-20	One/mouse # mice=1	One vial/mouse # mice=2			2	Up to 2 vials per TG		
Restart 2-6	One/mouse # mice=1	One vial/ mouse # mice =3	Check inventory		2	Up to 3 vials per TG	Check initial inventory and fill in as needed	Check inventory and bank up to 2mL total
Restart 7-20	One/mouse # mice=1	One vial/ mouse # mice =2			2	Up to 2 vials per TG		Check inventory and bank up to 2mL total

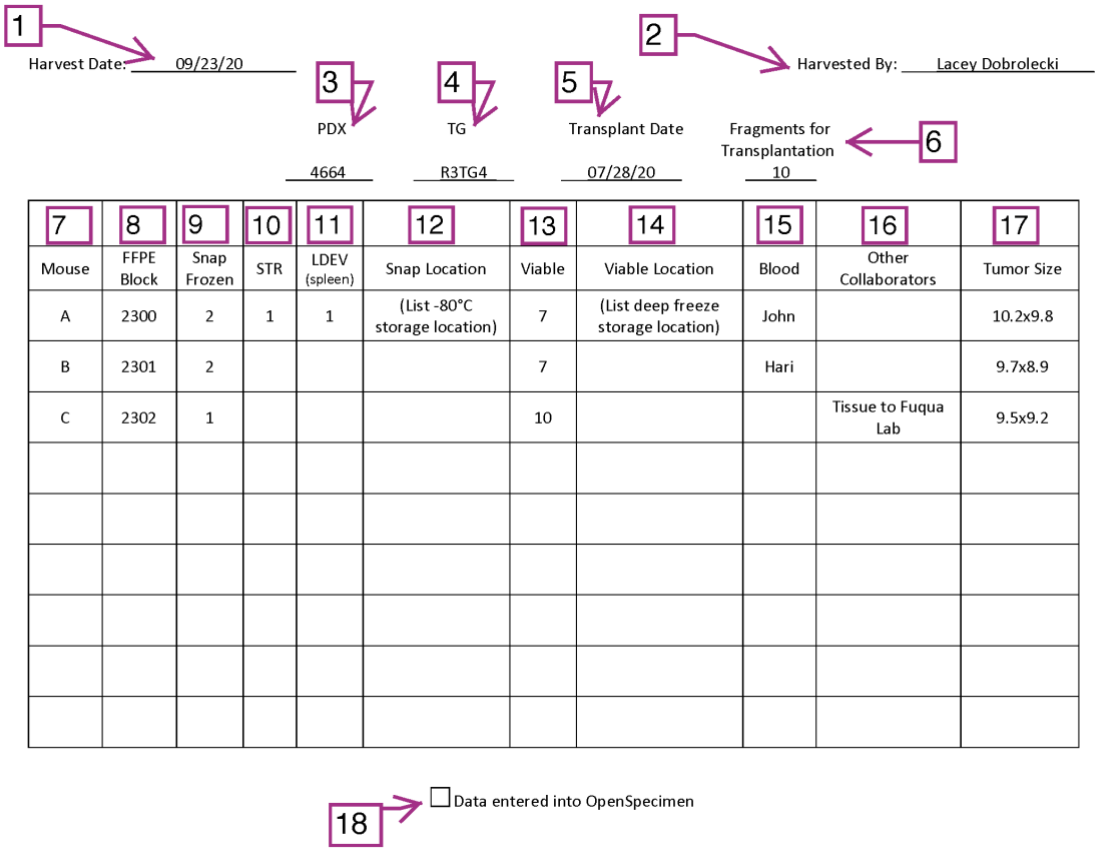
1. Make sure all mice from each individual PDX are labeled appropriately, either by ear tag or colored tail markings. Each technician should only handle one PDX model at a time and should only have supplies for that model in their working area.
2. Get one maintenance sheet from the harvest log binder for each model to be harvested.
3. Fill out the information listed on the sheet. *SOP_MTL-1.11 Harvest Sheets.*
4. Prepare a euthanasia Box:
    - 4.1. Put KIMTECH wipes in a 50 mL conical tube.
    - 4.2. Place the euthanasia box in the chemical hood and pour isoflurane in the 50 mL tube until the wipes are saturated. Pour off any extra isoflurane back into the isoflurane bottle.
    - 4.3. Place the tube in the bottom of the box and make sure no isoflurane leaks out.
5. Prepare workstation by placing an absorbent pad on the benchtop. Gather supplies needed for the harvest.
    - 5.1. Select a foam board with the appropriate number of 23G needles. Place a napkin on the foam board if desired.
    - 5.2. Get razors, white cutting board, cotton swabs, gauze pads, and autoclaved scissors and forceps.
    - 5.3. Spray the white cutting board thoroughly with 70% Ethanol and dry it with a paper towel.
6. Fill a formalin cup with enough formalin to fully the cover the number of cassettes to be collected. Use tape to make an EtOH check box label ("☐EtOH") on the lid of the formalin container.
7. Fill the Thermo Liquid nitrogen container with liquid nitrogen.
8. Label all tubes and tissue cassettes with appropriate PDX model, TG, date, and mouse identifier.
9. If collecting blood, make sure to prepare the syringe and 25G needle, and aliquot EDTA if needed. *SOP_MTL-1.5 Terminal Blood Collection from Mice.*
10. Place a mouse in the euthanasia box. Double check the ear tag/color marking.
11. After the mouse stops breathing, place body onto foam board ventral side up. Secure body onto foam board by restraining limbs using 23G needles.
12. Spray then entire ventral surface with 70% ethanol to wet the hair on the mouse.
13. Use forceps to hold skin at the lower abdomen and make a midline incision from between the #4 nipples to the base of the neck using the scissors. From the lowest incision point, make an inverted Y incision by cutting distally toward each hind limb.

14. Gently separate the skin on each side of the body from the peritoneum using a dry cotton-tipped applicator. Fold each flap back and pin to the board using a needle.
15. If blood is needed, collect it first. *SOP_MTL-1.5 Terminal Blood Collection from Mice.*
16. Use forceps and scissors to carefully cut out the tumor. Trim off excess mouse tissue and fat pad surrounding the tumor and take care not to miss any tumorous tissue while excising. Place excised tumor on square cutting board.
    - 16.1. If the tumor is fused with the skin or body wall, trim those tissues away even if it means leaving some tumor tissue behind.
    - 16.2. If the tumor was ulcerated, leave all ulcerated tissues attached to the skin.
17. Process the tumor tissue and create the different derivatives as needed:
    - 17.1. Remove all visibly necrotic tissue before processing.
    - 17.2. Proceed through the next steps quickly as DNA/RNA/Protein degrade in the tumor once the mouse is dead. In order to have high quality protein for analyses, try to have the snap frozen tissue fragments in liquid nitrogen within one minute of tumor excision.
    - 17.3. If a tumor is large (>0.8 g), place tissue fragments in a petri dish on ice while processing.
    - 17.4. **For FFPE:** Cut a slice from the mid-section of the tumor and place in a green tissue cassette. Place tissue cassette in formalin container. If tumor size allows, the section should be as thick as a nickel. If there is a lot of excess tissue, two sections can be placed in one cassette. If organs need to be collected, add those to the cassette AFTER the snap frozen vials have been processed.
    - 17.5. **For Snap Frozen:** Cut roughly six 3 mm^3 fragments of tumor tissue and place in a cryovial. Each fragment should weigh between 25-50 mg.
    - 17.6. **For Viably Frozen:** Cut transplant-size (1-2 mm^3) fragments of tumor tissue and place 12-15 fragments in a cryovial filled with 1.25 mL freezing media.
    - 17.7. **For Transplant Fragments:** Cut transplant-size fragments of tumor tissue and place in a 15 mL conical tube filled with 10-12 mL of DMEM. The number of fragments is dependent upon the number of mice to be transplanted. A minimum of 10-15 is needed for maintenance transplants. For studies, take 30-40% more fragments than needed.
18. Store the tumor tissue derivatives accordingly:
    - 18.1. **Snap Frozen:** Immediately remove tubes from the liquid nitrogen container, and store in -80ºC freezer boxes. Enter derivatives and their locations on the harvest sheet.
    - 18.2. **Viably Frozen:** Place cryovials in slow-freeze containers and store in the -80ºC freezer. Each Friday, gather all containers, put tubes in liquid nitrogen boxes, and place boxes in a liquid nitrogen tank or the Deep Freeze freezer (-140°C). Enter all derivatives on the harvest sheet.
    - 18.3. **Transplant Fragments:** Place in 4°C refrigerator until ready to transplant. Enter the number of mice to be transplanted on the harvest sheet. Transplants will occur on the day of tissue harvest.
    - 18.4. **FFPE:** Keep cassettes submerged in 10% formalin solution overnight. The next morning, pour the formalin into the proper waste container, blot off remaining formalin (in the chemical hood), and refill the container with 70% EtOH. Mark off the check box ("☑EtOH"). Submit all cassettes to the Pathology Core each Friday.
19. Once the needed tissues are collected, place the mouse body in the bag. Close the bag with tape and place into the -80°C freezer. Each week remove all frozen bags and take to the animal facility for disposal.
20. Log all the derivatives from the harvest sheet in OpenSpecimen.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	02.04.2021	All	SOP created

H. Appendix:



7 Mouse	8 FFPE Block	9 Snap Frozen	10 STR	11 LDEV (spleen)	12 Snap Location	13 Viable	14 Viable Location	15 Blood	16 Other Collaborators	17 Tumor Size
A	2300	2	1	1	(List -80°C storage location)	7	(List deep freeze storage location)	John		10.2x9.8
B	2301	2				7		Hari		9.7x8.9
C	2302	1				10			Tissue to Fuqua Lab	9.5x9.2

H.1 Harvest sheet.