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Implanting PDX Tissue into SCID mice

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1 Works for me dx.doi.org/10.17504/protocols.io.qmpdu5n

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ABSTRACT

Protocol for the establishment of patient derived xenograft from fresh patient tissue to SCID mouse or to passage from one mouse to another

GUIDELINES

Notes:

This protocol has been used for the passaging of head and neck tumors as well as lung and lung/brain tumors

Tissue should be transferred from OR to lab in either a base media or PBS (not formalin or ethanol)

Fast frozen – place tissue in 1.2ml cryovial – label. Place cryovial in LN2, move to -80 freezer for storage

Slow Frozen – If slow freezing, chop entire sample (minus fast frozen tissue), dissociate halfway. Take amount intended for freezing and place in cryovial. Bring volume to 600ul, add 60ul of DMSO. Immediately place in Mr. Frosty Nalgene freezing container (or use controled rate freezer) and place in -80 for 24 hours. Move to LN2 after 24 hours.

Matrigel- Matrigel has to be kept on ice before and while using as it solidifies at room temperature.

Drawing tissue into syringe – Be sure to draw up mixture through needle and syringe. If you draw mixture through syringe and then add needle you may draw up large chunks that will not pass through needle.

Scissors “any” These scissors are for cutting through the mouse skin to allow for excision of tumors. Use scissors that will not be used for excising tumors or chopping tissue. Cutting through the skin dulls the blades quickly.

MATERIALS

NAME	CATALOG #	VENDOR
Phosphate Buffered Saline	28374	Thermo Fisher Scientific
Matrigel, Phenol Red Free, 10ml	CB 40234C	
PDX Media		
1.2ml Eppendorf Tubes		Fisher Scientific
Straight, sharp/sharp	5906	Roboz
Scissors - Curved, sharp/sharp	5908	
18G needle	305196	BD Biosciences
Amphotericin B	15290026	Thermo Fisher Scientific
Penicillin-Streptomycin (10,000 U/mL)	15140148	Thermo Fisher
Corning™ DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate	10013CV	Thermo Fisher Scientific
1 ml syringe	309628	Bd
Ear tags and applicator	10-000-958	Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Corning™ DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate	10013CV	Thermo Fisher Scientific

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NAME	CATALOG #	VENDOR

EQUIPMENT

NAME	CATALOG #	VENDOR
Micro Dissecting Scissors 3.5" Curved Sharp/Sharp, Tungsten Carbide, SureCut	RS-5908SC	

SAFETY WARNINGS

all experiments should be approved by institutional IRB and IACUC

Human to mouse implant- Preparation

1 Place fresh tissue (should be passaged within 48 hours of removal from patient ; stored at 4 degrees C) in 60mm cell culture plate filled with

2-4 ml cold 1X PBS. Trim and clean tissue to remove areas of necrosis, fat, muscle.

1.1 Ensure that there is enough PDX media (used to mix with tissue sample in later steps) to perform injections :

PDX Media Recipe:

13.5ml DMEM (choose preferred brand)

Corning™ DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate
by Thermo Fisher Scientific
Catalog #: 10013CV

1.5ml FBS
75ul of 10,000 U/mL penicillin/streptomycin
150ul of 250 µg/ml Amphotericin B

Mix together in 15ml tube, yields 15ml – recommended to use within 2 weeks

2 While cleaning tissue, set out matrigel on ice to thaw. Do not allow to reach room temp, as it will solidify and be useless.

3 Examine tissue and determine if you have enough to save to prepare fast frozen and/or slow frozen. 1g of total tissue should be enough to passage and save tissue. (See *Patient and PDX Tissue Biobanking*)

Xenograft tissue establishment

4 For the tissue that will be passaged to a new mouse, transfer to a 1.2ml Eppendorf tube filled with 200-400ul of PDX media.

5 Use sharp curved scissors to thoroughly dissociate tissue with constant cutting while in tube

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Micro Dissecting Scissors 3.5" Curved Sharp/Sharp, Tungsten Carbide, SureCut
Tool/PDX equipment

Roboz RS-5908SC

6 Mixture should be well dissociated so that no large chunks remain. This mixture will need to pass through an 18g needle

7 Add additional media based on the amount of patient tissue you began with. Generally samples smaller than 0.5g need 200-350 ul of total media, and samples bigger than 0.5g need 300-400 ul of total media.

8 Once the final volume of media has been added, add matrigel equal to the amount of media in tube (1:1 ratio). Mix thoroughly by pipetting up and down 10x or until mixture appears homogenous. Keep on ice to prevent solution from solidifying.

Anesthesia and Injection

9 Perform injections on anesthesized mice (using isofluorane).
While mouse is being anesthesized, prepare work area

9.1 Draw up tissue-matrigel/media mixture into 1ml syringe with 18g needle already attached.

9.2 Prepare ear tag applicator, ear punch or any other tagging method.

10 Once mouse is anesthesized, place mouse dorsal side up with nose securely in nose cone. Inject the mouse subcutaneously in 1-4 sites, 100-200ul/site depending on the volume in syringe and the number of sites desired.

10.1 Immediately after injecting into each site, pinch skin for 10 seconds – 18g needle leaves a large puncture and the mixture tends to leak.

10.2 Apply ear tag while the mouse is still under anesthesia, mark cage with relevant information

11 Place the mouse backin the cage for recovery before returning cage to colony room (mouse should be awake within 2 minutes of removal from anesthesia.

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11.1 Monitor mouse 1-2x a week for tumor growth

11.2 Euthanize mouse when tumor size is 600mm^3 or when other human end point is reached.

Mouse to mouse xenograft Implant - Preparation

12 Determine and confirm the mouse with tumor ready for passage. Place the mouse in CO_2 chamber and turn the CO_2 tank rate to 1 liter per minute.

12.1 Once the mouse stops breathing, perform secondary method of euthanasia as described in the protocol under use. Setup the BSC for tumor harvest during CO2euthanasia. Clean the working area of BSC with 70% ethanol. Also clean all the materials required for the procedure with 70% ethanol before placing it inside the hood.

Tissue harvest

13 Use a pair of blunt scissors to cut open the midline of the euthanized mouse while making sure the tumor is untouched.

13.1 Excise the tumor by snipping around the base of the tumor with sharp scissors, by light snips around the base of the tumor. Tumor will be held in place by fascia and blood vessels. Cut all around that to collect as clean tumor as possible.

13.2 Place excised tumors into a cell culture dish with PBS. Rinse and clean the tumor further in PBS. Measure the size of the tumor with Vernier Calipers for your records. Place the tumor into a dish with PDX media.

13.3 Move the clean tissue into a 1.5ml tube with 200 µl PDX media. Dissociate it with sharp sterile scissors into fine matter. No solid matter should be remaining in the solution. Add more media if the solution seems too thick to be handled in a syringe.

13.4 Follow the steps above for xenograft establishment, and anesthesia and injection once a fine solution is prepared.

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