

SOP_MTL-1.6 Surgical Procedure- Mammary Fat Pad Transplantation

- A. Purpose:** Provide aseptic technique and general instructions to transplant viable tissue fragments into the #4 mammary fat pad in mice.
- B. Scope:** This SOP can be used to implant viable tissue (of human or mouse origin) into mammary fat pad in mice.
- C. Definitions:** NA
- D. Materials and Reagents:**

Name	Qty.	Cat. number	Sterility status for use
0.1 mg/mL Buprenex	As needed	Ben Taub Vet Supply	Sterile
27g Hypodermic Needles	1 per model	14-826-48, Fisher scientific	Sterile
50 mL Conical tubes	1	14-959-49A, Fisher scientific	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Absorbent under pads	1	S67011, Fisher	Sterile
Absorbent underpads -white spunbound polypropylene facing sheet with blue polypropylene backing sheet	1	56617-006, VWR	Sterile
Alcohol Wipes	As needed	13-680-63, BD	Sterile
Betadine Scrub	1	Ben Taub Vet Supply	Non-sterile
Cauterizer	1	231, Select Medical Products	Sterile
Clawed forceps	1	RS-5158	Sterile
Cotton tip applicator	1-2/mouse	22-363-168, Fisher scientific	Sterile
Tissue specimen	1	NA	Non-sterile
Electric shaver	1	Andis Pro Clip Ion Pet Hair Trimmer, Amazon	Non-sterile
Glass beads sterilizer (Germinator)	1	Roboz Surgical Store DS-401	Non-sterile
Gloves	As needed	11-462-68B, Microflex	Non-sterile
Surgery / Transplant cage cards	1/cage	BCM Mouse Facility	Non-sterile
Isoflurane	5-10 mL	BCM Mouse Facility	Sterile
Instant sealing sterilization pouch (large)	2	01-812-57, Fisher scientific	Sterile
Regular forceps	1	RS-5139, Roboz Surgical	Sterile
Micro dissecting spring scissors (fat pad cutter)	1 per model	RS-5658BT, Roboz Surgical	Sterile

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Micro forceps (tissue placing forceps)	1	Roboz Surgical Store, RS-5069	Sterile
Mouse cages for post-surgery housing	As needed	BCM Mouse Facility	Sterile
Oxygen tank	1	Air gas	NA
Petri dish	As needed	25384-088, VWR	Sterile
Petrolatum ophthalmic sterile ointment	1	Ben Taub Vet Supply	Sterile
Rescue solution	As needed	https://rescuedisinfectants.com	Non-sterile
Rubber surgical pad	1	RS-S40-28, MSC	Sterile
Razor blade/scalpel	1/specimen	55411-050, VWR	Sterile
Scavenging pump system	1	Multiple parts, Kent Scientific	NA
Sterile surgical drape	1	50-118-0337, Fisher scientific	Sterile
Surgery board	1	SurgiSuite, Kent Scientific	Sterile
Surgical scissors	1 per model	RS-5960, Roboz Surgical	Sterile
Surgical gloves	As needed	20-1065N, McKesson (6.5)	Sterile
1 mL Syringe	1	14-823-434, Fisher scientific	Sterile
Tape	1	15-901-10R, Fisher scientific	Non-sterile
Tin foil 4x4 inches	2	(any grocery store)	Sterile
Treatment sheet	1	NA	Non-sterile
Vet bond	1	Ben Taub Vet Supply	Sterile
Warming surface (slide warmer)	1	12858, Ted Pella	NA
Wound clip applicator	1	427630, BD	Sterile
Wound clip remover	1	427637, BD	Sterile
Wound clips	2 per mouse +extra	01-804-5, BD	Sterile
Wrench	1	NA	Non-sterile

E. References:

SOP_MTL-1.7 Surgical Pack Preparation

SOP_MTL-1.8 Estradiol Stock Formulation

SOP_MTL-1.9 Estradiol Drinking Water

F. Procedures:

General Considerations: This SOP describes the transplantation procedure assuming there are two persons working together, one animal “prepper” and one surgeon. The procedure can be done with one individual, but it is recommended that the same set up be utilized so that sterility around the surgical board can be maintained.

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Also, if an individual is working alone, new sterile surgical gloves will need to be donned prior to initiating the transplantation of tissue for each animal.

Follow institutional guidelines for handling immunocompromised animals. All supplies should be sprayed or wiped down with Rescue prior to placing them into the surgical space. All animals should be given pre-operative analgesic medication per institutional guidelines

Part A: Preparation of the non-sterile surgical area (can be done concurrently by the prepper and surgeon in regular gloves)

1. Upon entering the surgical space, spray all working surfaces with Rescue solution and allow the solution to sit for at least one minute. This includes all bench tops, cage warming devices, supplies, spaces around the surgical set-up, etc.
Note: Surgical procedures may be done in a dedicated surgical space or inside a biological safety cabinet. If a surgical suite is used, care must be taken to keep the room free from contamination from immune-competent animals if it is a shared space.
2. Bring the animals from their housing room into the surgical space (VD2).
3. Gather the correct number of autoclaved cages needed for post-surgery housing.
4. Place the cages on the slide warmers located on the bench. The slide warmers should be set to 37-40°C. The cage should be positioned transversely on the warmer (half on and half off).
Note: Alternate warming sources such as heating discs may be used in place of a slide warmer. Follow warming instructions for proper heating time so that the temperature is accurate.
5. Set up the prepping area on the left side of the surgical space by placing a heat source down and covering with an absorbent underpad.
Note: A warming disc, heated surgical board, or warming mat may be used.
6. Place the following supplies in the prepping area: alcohol wipes, sterile petri dishes (one half for the ophthalmic ointment and one half for each PDX being transplanted), a 50 mL conical tube and holder, betadine scrub, cotton swabs, and ophthalmic ointment.
7. Fill the 50 mL tube with enough betadine scrub to cleanse each mouse 3 times (about 5-15mL) and place it into the tube holder.
Note: Disposable betadine swab sticks may also be used.
8. Squeeze out enough ophthalmic ointment into one half of a petri dish to cover both eyes for each mouse. Use a cotton swab for application.
9. The electric shaver should be wiped down with rescue and placed into the prepping area.
Note: Some labs prefer to use Nair to remove the remaining hair after shaving. If that is desired, an additional petri dish, cotton swab, and alcohol wipe (one per mouse) will be needed to remove the Nair.
10. Spray the rubber tubing that is connected to the anesthesia machine with Rescue and arrange it in the surgical space so that the longer tube is on the prepping side and the smaller, flexible tubing with the nose cone is on the surgeon's side.
11. Place the glass bead sterilizer in the surgical space so that it can be easily reached by the surgeon.
12. Put the applicator tube in the vet bond and place it next to the surgical area with a cauterizer.
13. Check the isoflurane and oxygen levels in the anesthesia machine:
 - 13.1. When refilling isoflurane always be sure to use a scavenging pump system. Unscrew the metal lid and fill the reservoir to the top indicator line.

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- 13.2. When replacing the oxygen tank, make sure the oxygen is off and the valve has been flushed. Remove the blue seal from the valve post and place the tank in the holder on the anesthesia machine. Next, unscrew the regulator from the old tank and place it onto the new one. Make sure to line up the pins correctly and tighten.

Part B: Preparation of the Sterile Surgical Area (prepper and surgeon will work together while maintaining sterility)

14. The surgeon can now don surgical gloves but must keep them sterile. If sterility is broken, remove gloves and don a new pair.
15. The prepper will open Surgical Pack #1 (*SOP_MTL-1.7 Surgical Pack Preparation*) and allow the surgeon to remove the items. Remove the sterile drape and lay it out in the middle of the surgical area.
16. Remove the board and blue-backed underpad and wrap the board with the underpad (blue plastic side out). Secure with tape on the back side.
17. Next, the prepper will tape down the nose cone in the center of the surgical board leaving enough space to tape down the mouse.
Note: Most tape will not stick directly to the rubber surgery board.
Note: The surgical board is not considered a sterile surface once surgeries commence since the mouse will be taped to it.
18. The prepper will open Surgical Pack #2 and the surgeon to remove the half sheet surgical drape and lay it to the left side of the surgical board.
19. The surgeon should then remove the supply pouch and open it on the right side of the surgical board. The foil and small square surgical drapes can then be removed and placed on a sterile surface. Organize the rest of the supplies so that they are within easy reach for the surgeon.
20. The surgeon should take the sterile foil pieces and carefully wrap the vet bond and cauterizer. They can then be placed in the sterile surgical area for use.
21. The prepper will spray the first tissue specimen tube into the surgical space, pour it into a petri dish, and place it on the surgeon's side making sure not to touch anything that is sterile.
22. The prepper will turn on the anesthesia machine:
 - 22.1. Open the oxygen flow using the oxygen tank wrench.
 - 22.2. Ensure the oxygen gauge is at 2.5 LPM.
 - 22.3. Turn the isoflurane gauge to 2.5 LPM.
 - 22.4. Check both ports on the tubing to make sure they are in the "open" position.
23. Put the cage of mice into the hood on the far left of the prepping area. Spray with rescue.

Part C: Preparing the Mouse for Surgery (prepper)

24. Shave the area over and around the #4 nipple (from below the #4 nipple up to the rib cage) and place the mouse's nose into the nose cone.
25. Apply betadine scrub in a circular motion going outward from the center of the shaved area using a cotton swab, then remove using an alcohol wipe. Repeat two more times using a new cotton swab and new alcohol wipe each time.
26. Once the mouse is unconscious, gently apply ophthalmic ointment to completely cover each eye. Be sure not to scratch the eye of the mouse.

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27. Transfer the mouse onto the surgery board, do a final toe pinch to ensure the mouse is in the appropriate plane of anesthesia, put the head into the nose cone, and tape down the mouse's limbs.

Part D: Surgical Procedure to Implant the Tissue Fragment (surgeon)

28. At this time the surgeon can begin the transplantation.
29. Place a sterile drape with cutout over the mouse, only exposing the surgical area.
30. Using the regular forceps, pinch and lift up the skin at the #4 nipple.
31. With the blunted side down, use your scissors to make a short (about 1 cm), parasagittal incision, from the #4 nipple towards the head.
32. Hold the edge of skin up on the medial side of the incision with regular forceps and use a cotton swab to separate the skin from the peritoneum.
33. Then pull the lateral side of the incision away from the body and use the same swab to gently peel the #4 mammary fat pad off the skin. It will take a couple of sweeps of the swab to completely separate the fat pad.
34. Once the fat pad is separated and pushed toward the body, pin down the lateral skin flap using a 27g needle placed close to the animal's body.
35. Use the regular forceps to gently hold and pull out the fat pad and perform the clearing technique if indicated (if clearing is not indicated proceed to step 36):
 - 35.1. The goal of this step is to remove the area of the fat pad containing the lymph node and all mammary epithelium
 - 35.2. Temporarily turn off the oxygen during this step. Cauterize medial to the lymph node, making sure to cauterize each vessel to prevent bleeding
 - 35.3. Using the Micro Dissecting Spring Scissors cut each cauterized vessel from the bottom to the top (to ensure there is no bleeding) until the section of the fat pad that contains the lymph node and epithelium is removed.
 - 35.4. Place the cleared fat pad on the prepper's mat without touching the forceps to the mat.
36. Gently pull out the remaining fat pad and push back the peritoneum with the jeweler's forceps or a cotton swab.
37. Locate the remaining vessel in the #4 fat pad and use the tip of the jeweler's forceps to poke a hole just above the vessel and close to the body wall.
38. Take the jeweler's forceps and slightly open the hole into a small pocket.
39. With the jeweler's forceps still in your hands, pick up a fragment of tissue from the petri dish and place it in the small pocket. Make sure that it goes completely into the pocket and doesn't fall out when the forceps are removed.
40. Release the fat pad from the forceps and unpin the skin.
41. Starting at the base of the incision collect the skin on each side using your regular forceps. While still holding the skin, use the clawed forceps to bring the two sides together and lift slightly to prepare the skin for wound clip application. Uncurl the edges of the incision and make sure they form a continuous surface at the top.
42. Holding the two sides together with your regular forceps, use the wound clip applicator to place one wound clip in the center of the incision.
43. Apply vet bond above and below the wound clip to seal then ends of the incision.
Note: Two would clips may be used to secure the incision if desired.

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44. Remove the drape from the mouse

Part E: Post-Surgical Procedures (prepper and surgeon will work together while maintaining sterility)

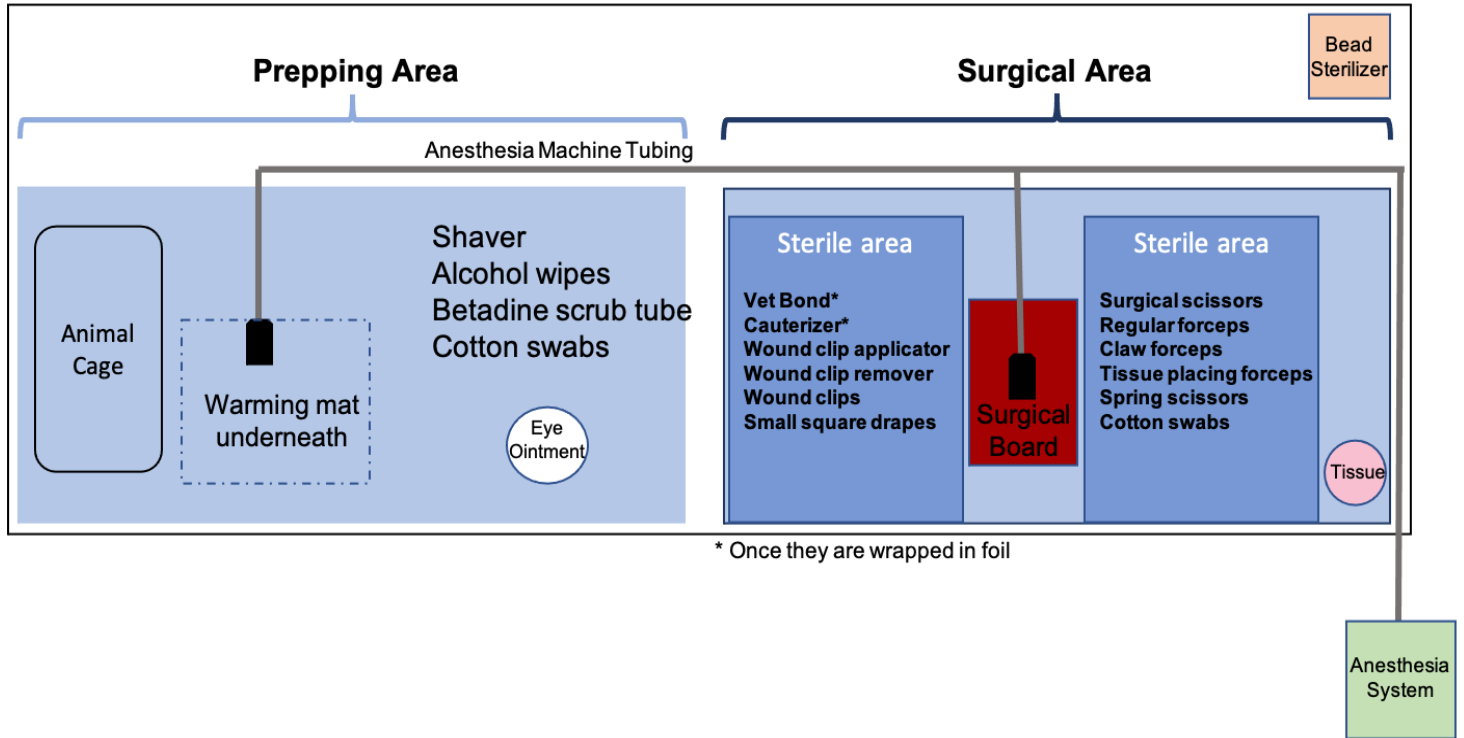
- 45. The prepper will now untape the legs of the mouse and move it over to a clean cage on the slide warmer
 - 45.1. Place the mouse in a prone position and be sure not to touch the incision.
 - 45.2. Be sure there is bedding under the mouse and that the mouse is in a section of the cage over the warmer.
- 46. The surgeon will clean the tools for 10 seconds in the glass beads sterilizer before continuing with the next transplant. Wait for the tools to cool before proceeding.
- 47. Repeat steps 23-46 until all mice are transplanted.
- 48. If more than one PDX model is transplanted in the same session remember to do the following:
 - 48.1. Remove and discard the petri dish with tissue from the previous transplant.
 - 48.2. Discard the 27g needle used to pin back the skin and open a new one.
 - 48.3. Clean tools thoroughly with alcohol wipes and bead sterilizer or have additional sets of tools.
 - 48.4. Place the petri dish with new tissue in the hood after validating and logging the tissue identification.
- 49. Check mice for bleeding and make sure they have resumed normal activities. Animals should be fully awake and alert within 5-10 minutes after the procedure.
- 50. Take cages back to the housing room and add fresh water and food.
- 51. Follow your protocol's post-surgical care plan.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	02.15.2021	All	SOP created

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H. Appendix:




H.1 Schematic representation of surgical space.

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H.2 Schematic representation of surgical incision and tissue placement.

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 A01-BW9-012-02001789
 PI: LEWIS (713 7983296) Prot: AN-2289

Species/ # animals/ animal ID: _____

Room/ cage location: **VD7** **Lewis Lab SX**


Surgical procedure performed: Straight incision single side; tumor tissue into #4 fat pad

Date of Surgery: _____

Pre-emptive analgesics given? Yes No Time: _____

Drug: **BUPRENEX** Dose (mg/kg): **0.6** Frequency: ONCE

Animal recovered (alert & mobile)? Surgeon's Initials: _____ Time: _____



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POSTOPERATIVE MONITORING

	Day of Surgery	Post-op Day 1	Post-op Day 2	Post-op Day 3
BUPRENEX 0.5mg/kg time given				
Incision appearance				
Overall Health				
Checked by: (initials)				

Notes: _____

H.3 Front and back surgery card.


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 A01-BW9-012-02001847
 PI: LEWIS (713 7983296) Prot: AN-2289

XENOGRAFT : _____

TRANSPLANT GENERATION : _____

TRANSPLANT DATE : _____

NUMBER OF MICE : _____

HEALTH ISSUES: _____

H.4 Cage card.