

### **Perfusion (non-survival surgery): (rat or mouse)**

The perfusion will be done inside a fume hood and will utilize a peristaltic pump to deliver fixatives at controlled speed and a hot plate to warm the solutions (ca., 30-37 °C) . Suction beneath the perfusion tray will be used to remove waste fluids. Deep surgical anesthesia with appropriate anesthetic agents will be achieved before the procedure begins.

Before and during the perfusion, the animal will be monitored visually and the withdrawal reflex will be tested frequently. There is no pre-op for this procedure. The deeply anesthetized animal will be taped to the tray with the chest upward. The skin will be reflected from a midline incision and the abdominal cavity will be opened. The chest will be opened by cutting the diaphragm and cutting the ribs along the lateral surface up towards the 2nd rib. The chest flap will be reflected, then the descending aorta will be clamped in the thorax. The left ventricle of the heart will be pierced and a custom perfusion cannula will be inserted into the ventricle and into the aorta until the tip is visible in the aorta. The cannula will be clamped in place with a hemostat, and the flow of perfusate will begin. Immediately, thereafter, the right atrium will be opened. The entire procedure takes approximately 3 minutes from chest opening to the opening of the right atrium.

*(Solutions& Anesthesia used for the perfusion should be included in the description.)*

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Following anesthesia, mid-axial abdominal-thoracic incisions will be made in the animal to expose the heart, and then a needle (attached to a gravity perfusion system) will be inserted into in the left ventricle. The right ventricle will be cut to allow drainage. The animal will then be perfused with phosphate buffered saline (PBS) to flush blood from the circulatory system, and then perfused with 4% paraformaldehyde (PFA) in saline for 30 min using a gravity perfusion system in a chemical fume hood.

### **Bilateral Ovariectomy: (mouse)**

The anaesthetized mouse will be placed on its ventral surface with its tail towards the surgeon, so the back of the mice naturally assumes a slight humped-back posture. The surgical site is aseptically prepared with alternate washing with alcohol and betadine (2-3 times) followed by a final betadine wash. A small midline dorsal skin incision will be made approximately half way between the middle of the back (the hump) and the base of the tail. Scissors will be inserted subcutaneously through the incision and blunt dissection will be performed through the connective tissue to allow access to the muscle. The skin incision will be retracted with forceps, first to one side to remove one ovary and then to the other side to remove the second one. The muscle incisions will be made half to 2/3 of the way down the side of the body. Upon opening of the peritoneal cavity, the ovaries will be pulled out by grasping the freely moveable peri-ovarian fat which is the landmark. The ovary itself must not be touched, or small pieces may become detached and re-implant and carry on normal function. With two pairs of curved forceps the junction between the Fallopian tube and the uterine horn, together with all accompanying blood vessels and fat, will be clamped. The forceps will be drawn apart sharply from each other causing the ovary (enveloped by fat) to tear away (or a cut will be made between the forceps). The horn will be returned into the abdominal cavity. No other attempt at hemostasis is necessary as any bleeding is small and inconsequential. The muscle incision will be closed with a single absorbable suture (Vicryl). The same will be done with the other ovary. Finally the skin will be closed using the clips or by interrupted absorbable sutures (Vicryl). In case of sham surgery, the procedures are the same except that the ovary will be exposed but not removed. If clips used, they will be removed at 10-14 days post-surgery.

### **Ovariectomy: (mouse) (alternative)**

The anesthetized mice will be prepared aseptically by alternate washing with alcohol and betadine for three times, with a final betadine paint. A lateral retroperitoneal incision will be made midway between the costal margin and iliac crest, parallel to the spine. The muscle and fascia will be gently retracted, exposing the fallopian tube that is followed anteriorly to the ovary. The ovarian artery and vein will be ligated, then the ovary will be removed. The area will be irrigated with saline, then the muscle will be repaired with absorbable suture (Vicryl) and the skin is closed with *wound staples or by interrupted sutures using Vicryl*. The wound will be infiltrated with 0.25% bupivacaine for post-operative comfort.

**SPLENECTOMY: (mouse)**

1. Anesthetize the mouse.
2. Shave area on right side of mouse. The surgical site is aseptically prepared with alternated washing with Chlorhexidine or alcohol and betadine (2-3 times) with a final betadine paint.
3. With a clean scissors, make a left-sided skin incision, -2.5 cm long, midway between the last rib and the hip joint.
4. Loosen any connective tissue under the skin using the blunt end of the forceps. The spleen is easily seen through the abdominal wall attached to the greater curvature of the stomach by mesentery.
5. Make a 1- to 2-cm incision in the peritoneal wall and gently pull the spleen onto the exterior surface of the abdominal wall. A small artery, not always visible, is attached to the hilum of the spleen, closest to the stomach.
6. Tie off the artery with a 4-0 chromic gut suture by looping the suture through the mesentery. Make a single knot at the tip of the spleen. The efferent venule is attached at the other end of the spleen. It may be necessary to tie this vessel if excessive bleeding occurs when the spleen is removed.
7. Cut away the mesentery and connective tissue, and remove the spleen.
8. Close the peritoneal wall with one or two separate sutures using Vicryl. The skin is closed with 2-3 autoclips or interrupted absorbable suture material (Vicryl).
9. Remove any blood from the skin by wiping with a sterile gauze section.
10. Place the animal on its side 15 to 22 cm from a desk lamp. Allow the animal to rest until the anesthesia wears off (30 to 60 min), making sure that the airway is not obstructed.

### **Parabiosis: (mouse)**

Parabiosis will be performed using age and sex matched mice. At any sign of illness in either of the pair, the pair will be euthanized. Pairs will be housed singly and mushy food will be kept in the cages to allow adequate feeding. Mushy food will be included to insure that the animals receive proper nutrition and hydration.

1. The mice will be appropriately anesthetized.
2. The skin will be shaved and aseptically prepared with alternate washing of alcohol and betadine solution.
3. A ~ 2 inch incision in the skin along the apposing flanks of the two animals that will be joined will be made using sterile/clean scissors. The incision will be of sufficient length to ensure that the two animals cannot become twisted in opposite orientations after joining.
4. Sutures (4-0) will be placed in the scapular and flank regions to bring the body walls of the two animals into direct physical contact.
5. The skin flaps will be joined with surgical clips, which are placed at regular intervals to ensure that no separation occurs.
6. The clips will be removed after 10-14 days post-surgery.

### **Separation of parabiotic pairs (categorized as multiple survival surgeries): (mouse)**

- 1) Selected pairs of joined mice will be anesthetized.
- 2) As much fur as possible will be clipped from the incision site. The flanks of the mice will be cleaned with betadine solution and an incision along the joined flanks will be made to separate the mice.
- 3) The skin incisions will be closed with wound clips.
- 4) Mice will be kept warm and will have fully recovered from anesthesia before being returned to a mouse holding room.
- 5) Animals will be monitored daily after surgery for weight loss, posture and mobility. Monitoring will be recorded in written logs.
- 6) Animals that show excessive weight loss (>20%) or immobility will be given saline by i.p. injection and euthanized within 24hrs if no improvement occurs.
- 7) Once the incision has healed (~1-2 weeks), the clips will be removed.
- 8) A written record of these observations will be maintained for each mouse.
- 9) Wound clips will be removed ~10 days post surgery.
- 10) At various times later, the mice will be euthanized for analysis of tissues.

### **Superovulation: (mouse)**

To obtain embryos for microinjection or fusion with embryonic stem cells, super-ovulated donor females (CD-1, FVB, B6) are mated to stud males.

Super-ovulation of a fertile female mouse is initiated by an initial IP injection of 5 IU of pregnant mares serum (PMS) in 0.1ml sterile saline using a 27 G tuberculin syringe, followed by a second IP injection of 5 IU of human chorionic gonadotropin (hCG) in 0.1ml sterile saline 46~48 hours later.

### **Isolation of embryos: (mouse)**

To collect mouse embryos, pregnant females are euthanized by cervical dislocation. The abdomen is cleaned with 70% ethanol and the body cavity opened by vertical and transverse cuts through the skin and body wall. The proximal region of the oviduct and a portion of the attached uterine horn is removed from each side and transferred to a 100 mm petri dish and embryos are collected in embryo culture media. To harvest older embryos, the entire uterine horn will be removed and the surgeon will flush (preimplantation) or dissect the embryos/fetus (post implantation) from the uterus.

### **Embryo transfer into foster mothers: (mouse)**

A micro-capillary is prepared and heat sterilized with a small flame. It is then loaded with manipulated embryos in culture medium and demarcated by air bubbles, which will serve as markers during the transfer process. Pseudo-pregnant female 0.5 to 2.5 day *post coitum*, (pseudo-pregnant females are prepared by mating with vasectomized males) are anesthetized. The achievement of an appropriate surgical level of anesthesia is determined and monitored by toe pinch. Eyes are coated with ophthalmic ointment to prevent drying during surgery. The dorsum of anesthetized females is wiped with 70% ethanol. After surgical site preparation a small incision is made in the skin at the midline at the level of the last rib. Any broken hair can be removed easily with forceps under the microscope and prevent them from getting into the body cavity. We do not shave the animal at the site of incision. From our experience, if we shave the animal, we have observed a lot of loose hair fragments at the site of incision with the dissection microscope. It is extremely difficult, if possible at all, to completely remove all the hair fragments, and they often get into the body cavity.

The skin incision will be positioned over the ovary (pinkish red) or fat pad (white), which are visible through the thin dorsal body wall, and a second small incision is made over the ovary. A blunt forceps is used to grab the fat pad and then gently pull the ovary out of the body cavity. The microcapillary containing the embryos is inserted into the oviduct via the infundibulum. For intrauterine transfer, a 27-gauge needle will be used to introduce a small hole in the uterus proximal to the oviduct. The microcapillary is then inserted into the small hole to deliver the embryos. The fat pad, ovary, oviduct and uterus are then gently pushed back into the body cavity and the skin closed with wound clips. After embryo transfer is completed recipient females are wrapped in an autoclaved tissue and then returned to their cage for recovery (see section g for detail of post-operative care). During the recovery period animals are kept warm by a heat pad and monitored for signs of pain and discomfort. Wound clips will be removed 7 days after operation.

Following surgery, buprenorphine at a dosage of 0.05 mg/kg of body weight will be mixed with 1 ml of saline and then administrated subcutaneously between the shoulder blades as analgesics as well as to prevent dehydration of the post surgery animals. The animals will then wrapped with sterilized tissue and put in a cage pre-warmed with a heat pad until they wake up. The weight, alert/ responsive, activity, eating, drinking, bleeding and open wound are monitored everyday for 7 days. Animals will be provided with additional buprenorphine as described above if they show any signs and symptoms of discomfort such as pilo-erection, lethargy, lack of motion and hunched posture.