Survival Surgery for the Production of Genetically Modified Mice

IACUC Standard Procedure
Effective Date: October 2019

UCSF Office of Research
Institutional Animal Care and Use Program

The following policies must be followed for all procedures:

- UCSF IACUC Guidelines for Rodent Anesthesia
- UCSF IACUC Guidelines for Rodent Surgery
- Analgesia should be provided at the time of surgery per the LARC Veterinarians' Anesthesia and Analgesia Recommendations for UCSF Laboratory Animals (January 2018)

The protocol must identify:

- Anesthesia and analgesia used for each surgical procedure
- Surgical Wound Closure method(s)

Objectives:

- To describe the surgical procedures required to generate genetically modified mice.

Description of procedure:

Please note: the procedures described take considerable practice and experience in order to obtain optimal results. Please consider using a core, such as the LARC Mouse Model Generation core that specializes in offering these services.

General: These techniques are essential to obtain offspring from genetically manipulated embryos and constitutes a necessary step for the development of genetically modified models. Embryos from donor mice are transferred into oviducts of pseudopregnant recipient mice; vasectomized male mice are required to generate pseudopregnant recipient females.

Always prepare your surgical site before conducting any approved surgical manipulation. For more guidance on how to properly prepare the surgical side please read UCSF IACUC Guidelines for Rodent Surgery

Production of sterile males: Vasectomy

Note: Vasectomized male mice may be purchased commercially through JAX or Charles River, or produced in-house.

Abdominal approach: Perform a longitudinal skin incision in the medial line of the abdomen, about 1 cm above the penis followed by a 5-10 mm longitudinal incision in the linea alba. Exteriorize the testes by grasping the fat pad that lies adjacent to the bladder. After tidentification of the vas deferens, ligate with suture in two spots about 0.5 cm apart, and then sever between the ligatures. The remaining ends of vas deferens are placed back into the abdomen. The process is then repeated on the other side. The incision is closed in two layers using absorbable suture on the linea alba, and suture or wound clips on the skin layer. Skin suture or wound clips should be removed in 10-14 days.

Scrotal approach: Alternatively the vas deferens may be accessed through the scrotal sac. Push both testes down into the scrotal sacs by gently applying pressure to the abdomen. Make a 10-mm skin incision through the skin along the midline of the scrotal sac. Make a 5-mm incision in the testes membrane close to the left side of the midline wall. Using forceps, pull the vas deferens out while holding the testis in place. Hold the vas deferens with one pair of forceps and cauterize it with the red-hot tips of a second pair of forceps or cut with fine scissors such that the portion (~1 cm) of the vas deferens in the loop is removed. Repeat on the other testis. Suture or clip the skin together with wound clips.

Preparation of donor females: Superovulation & collection of embryos

Females are superovulated with an IP injection of PMSG, followed 42 – 48 hours later with an IP injection of hCG. After hCG injection, pair females with a singly-housed, intact stud males and allow to mate overnight. The next morning, remove females from the males and check for vaginal plugs. If a plug is present, female is euthanized within four days to obtain appropriate stage embryos. If a plug is not noted at that time, female may be rested for two weeks, and then the procedure is repeated.

Production of pseudopregnant recipient females: Uterine or oviduct transfer of embryos

House vasectomized males with recipient females at least the day before embryo microinjection is scheduled to produce pseudopregnant recipient females. Zygotes are transferred to the recipient female's oviduct; blastocysts are transferred into the uterus. The surgical approach is similar for both procedures. Make flank incision and locate the ovarian fat pad, ovary, oviduct and proximal uterus. The ovary will lie rostral to the kidney and can be identified by the large fat pad attached to it. Grasp the fat pad, exteriorize the reproductive tract. Zygotes or blastocysts are delivered to the appropriate part of the reproductive tract using pipette (for oviduct) or needle (for uterus). The incision is closed in two layers. A separate incision may be used for the contralateral oviduct/uterine horn. Wound clips or suture may be left in place until the litter has been born and weaned (total of approximately 6 weeks).

Literature search words required:

Literature search performed for refinement of this Standard Procedure in July 2019.

Key Words	Search Site/Source	Years Covered
Mouse/rodent Vasectomy; Mouse Embryo Transfer; Production of Genetically Modified/Transgenic mice	Pubmed, Google Scholar	Full database up to 2019

Agents: This procedure requires anesthetics and analgesics, PMSG, and hCG. All agents administered to animals should be listed in the "Agents" section of RIO.

Adverse Effects to be considered: Infection, hemorrhage, dehiscence

References:

Bermejo-Alvarez, P., Park, K. E., Telugu, B. P. Utero-tubal Embryo Transfer and Vasectomy in the Mouse Model. *J. Vis. Exp.* (84), e51214, doi:10.3791/51214 (2014).

Miller, Amy L., et al. "A Comparison of Abdominal and Scrotal Approach Methods of Vasectomy and the Influence of Analgesic Treatment in Laboratory Mice." *Laboratory Animals*, vol. 46, no. 4, Oct. 2012, pp. 304–310, doi:10.1258/la.2012.012078.

Tian XL., Wang Q.K. (2006) Generation of Transgenic Mice for Cardiovascular Research. In: Wang Q.K. (eds) Cardiovascular Disease. Methods in Molecular Medicine, vol 129. Humana Press