Standard Procedures for Genotyping Mice and Rats

PURPOSE

The proper identification of genetically engineered animals is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often the genotype is determined by analysis of DNA extracted from tissues of pre-weaned rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from ear punches, hair or fecal samples, oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives.

Obtaining tissue from a mouse or rat for DNA analysis via tail snip and biopsy is a safe, effective and humane procedure that causes minimal or transient pain and distress when performed properly. DNA prepared is suitable for analysis by either Southern Blot or PCR. The ideal time to collect tail tissue is 10 to 15 days of age. At this age, the tail tissue is soft and the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice to be identified prior to weaning which can save valuable space in the animal facilities. Tail snips can be performed on animals greater than 15 days of age but will produce a lower yield of DNA. The same procedures are utilized as for the animals greater than 10 days of age.

Toe clipping, on the other hand, may be used both as a method of identification for animals and to obtain biopsy tissue for genotyping. However, this can only be used when no other identification method is feasible. As this is a painful procedure, this must be justified in the protocol and must be performed in the most painless and humane way consistent with current veterinary practice and standards.

PROCEDURES

Tail Snip and Tail Biopsy

- Tail snip is obtained by removal of a fleshy tip or a small amount of tail tissue while tail biopsy involves the amputation of the tail between bony vertebral segments

1) Manually restrain the mouse/rat.
2) Confirm its identity.
3) Requirement for anesthesia/ analgesia is determined by the age of the animal:
   a.) Tail snips in animals < 17 days of age, **tail tip sample not exceeding 5mm** is obtained**. Anesthetics or analgesics are encouraged but not required.**
   b.) Tail snips in animals 18-21 days of age, **tail tip sample not exceeding 5mm** is obtained using a **local anaesthetic** agent such as Bupivacaine. Immerse the tail in the Bupivacaine for 10 sec. before snip. Systemic analgesic agents such as **Meloxicam or Buprenorphine are encouraged** but not required.

c.) Tail biopsy (>21 days old) must be performed with **general anesthesia** (i.e. Isoflurane**) followed by 24 hours of post-procedure analgesic such as Meloxicam or Buprenorphine.**

4. Wipe tail with 70% ethanol and allow to dry. Then disinfect the work surface on which the tail is placed between mice/rats.
5. Wipe scissors with sterile water followed by 70% ethanol between animals. The use of sterile water is critical as it help reduce contamination between samples especially for PCR analysis. Please note that disposable scalpel blades are not designed to be used on multiple animals.
6. Assure hemostasis by using a sterile gauze and applying digital pressure, silver nitrate, tissue adhesive or cautery to control bleeding.
7. After ensuring hemostasis, then return the animal to its cage.
8. Repeat tail biopsies (>21 days old) require general anesthesia and must be justified in the protocol. The use of post-procedural analgesia should be considered. When general anesthesia is used, the animal must be observed until it regains consciousness.
9. If you anticipate the possibility of needing an additional sample from an animal at a later date, cut the original sample in half and preserve the extra piece at -20^{o}C or -80^{o}C

Rodent Age	**Anesthesia/Analgesia Requirement**
Less than 17 days	Anesthetics/analgesics encouraged but not required
18-21 days	Local anaesthetics: required Systemic analgesics: encouraged but not required
More than 21 days	Tail biopsy: General anesthetics
NOTE: Tail snip samples must not exceed 5mm Must always control bleeding	

Ear Pinnae

Ear tissue can be harvested either by ear punching of a circle of tissue or ear snipping of the edge of the pinna. The procedure should not cause bleeding if done properly. If bleeding does occur, ensure the bleeding has stopped before returning the animal to its cage.

Ear Punch: Ear punching (2 mm diameter) taken from the middle of the pinna is the preferred sampling site. Care should be taken to not accidentally lose track of the small

piece of tissue following the punch. This method does not require anesthesia, but should be performed on mice close to weaning age or older to ensure that the pinnae are large enough for the punch size.

Ear Snip: Small portion (2-3 mm) of the edge of the pinna is cut off with sharp scissors to obtain tissue. This can be done on mice or rats once the ears have developed (>8 days of age) and does not require anesthesia.

Buccal Swabs Saliva

Salivary samples to harvest epithelial cells from the mouth can be performed on rodents once they are a few days old; this method does not require anesthesia. Individual sterile mini-cotton swabs (rubbed against both inner cheeks per swab) should be used to sample cells. Care should be taken within the mouths of animals to ensure gentle swabbing.

Fecal Pellets

Samples of feces (n=3 pellets) can be collected directly from the animal at the time of defecation, or from the cage floor of individually housed animals. Epithelial cells shed in the feces are the target tissue type for processing and analysis.

Hair

Tufts of hair (n=2 tufts per rodent) are plucked from the animal using tweezers or hemostats to obtain samples. Samples can be collected at the neck line between the shoulder blades. Animals should not have exposed patches of skin following sampling, as only small tufts are needed. Care should be taken to avoid contamination with fomites and with hair from cage-mates of the animal to be assessed.

Toe clipping

Toe clipping is acceptable only in mice and must only be carried out by a competent personnel.

Mice up to 7 days of age may be toe clipped for identification purposes without anesthesia. Mice 8-28 day of age, which require general anesthesia (e.g. isoflurane), may be toe clipped ONLY if the toe tissue is also used for genetic analysis.

It must be preceded by disinfecting the digit with iodine or 70% alcohol. Sharp, sterilised/ cleaned sharp scissors must be used and only the tip of the toe (distal phalanx) can be clipped. Excision of the first digit of either front paw is prohibited**.** The procedure must only be carried out on one toe per foot and only on one occasion.

After the procedure, bleeding must be monitored and if there's any, this must be stopped by applying gentle pressure before putting back into the cage.

References

1. Guide for the Care and Use of Laboratory Animals, National Research Council, 8th Edition, pp 120-123.
2. Hankenson FC, Garzel LM, Fischer DD, Nolan B, and Hankenson KD. 2008. Evaluation of tail biopsy collection in laboratory mice (Mus musculus): vertebral ossification, DNA quantity, and acute behavioral responses. JAALAS 47(6): 10-18.
3. NIH "Guidelines for Toe Clipping of Rodents". May 2010. Available at http://oacu.od.nih.gov/ARAC/