BROWN

Institutional Animal Care and Use Committee (IACUC)

Rodent Genotyping Policy

Date of IACUC Review and Approval: February 3, 2017

Revised: June 1, 2018

I. Purpose: The Brown University IACUC has adopted the following guidelines to provide information and guidance to the research community regarding genotyping techniques in rodents. Whichever method is used must be indicated in the protocol and approved by the IACUC. For training in any of these methods, please contact veterinary services.

II. Genotypic Monitoring:

The "*The Guide for The Care and Use of Laboratory Animals* (the *Guide*)" provides guidance as to "best practice" with respect to genotypic monitoring and screening of Genetically Modified Animals (GMAs). Specifically, the following are indicated for inbred strains:

- It is important to periodically monitor genetic authenticity of the line.
- Appropriate management systems should be designed to minimize genetic contamination resulting from mutation and mismatching.
- Each GMA line represents a unique resource and thus care should be taken to preserve the line through standard colony management programs. Cryopreservation of lines should be considered as a safeguard against the loss of lines, and as a protection against genetic drift over time. Cryopreservation of lines should also be a part of each lab's disaster planning efforts to protect against the loss of valuable animal resources (see p. 35 of the *Guide*).
- Carefully designed breeding strategies and accurate genotype assessment should be ensured to minimize the generation of animals with unwanted phenotypes.

III. General:

- Only the least amount of tissue necessary to perform genotyping should be removed.
- It is important to realize that all genotyped animal must be uniquely identified to allow the results to be matched to the animal. If animal identification is being performed through the removal of a piece of tissue (e.g., ear punching, toe clipping), that sample of removed tissue should be used for genotyping purposes.
- Methods that do not permanently alter the animal or produce slight momentary pain should be prioritized, when scientifically applicable.
- If multiple animals are to be genotyped in a single session, instruments should be disinfected (e.g., wiped with chlorhexidine or 70% ethanol) between animals to

prevent DNA contamination. Alternatively, hot bead sterilizers or newly sterilized equipment for each animal can be used.
- Scissors should be sharpened or replaced at appropriate intervals (based on use). Blades should be discarded after each session (discarded at least each day).

IV. Tail biopsy:

- Sharp scissors, a razor blade, or a scalpel can be used to cut the tip of the tail perpendicular to the long axis to obtain a sample.
- **Limit the amount of tail to be amputated to 2-5 mm**; 2 mm would be preferable and will minimize cutting bone. If re-sampling for repeat genotyping from the same mouse, no more than 5mm cumulative of the distal tail should be harvested. In this situation, other tissue sources (listed below) are strongly recommended.
- Biopsy of tail tissue can be performed without general anesthesia in mice prior to weaning age, because this is prior to completion of mineralization and is therefore associated with minimal pain. However, it is recommended to use ice-cold ethanol for topical anesthesia in mice 7-15 days old.
- General anesthesia and scientific justification are required when tail biopsy is performed on animals older than 21 days of age.
- Hemostasis must be achieved following the biopsy. If less than 2 mm is taken, hemostasis can usually be achieved by direct manual pressure with clean paper towel or gauze on the end of the tail. If direct pressure does not stop the bleeding, the use of hemostatic agents (e.g., styptic powder (Kwik-Stop®)) is recommended and should be readily available as a precautionary measure. Animals may not be left with actively bleeding collection sites.
- If general anesthesia has been administered, the mouse must be observed until it regains consciousness.

V. Toe clipping:

- Sharp scissors can be used to remove the distal phalanx in neonatal rodents.
- The aim is to remove only the complete distal phalanx, if possible (please see fig 1 below).
- This method can only be performed in rats 5-7 days old and mice 7-10 days old.
- The primary use of this procedure is for identification purposes, however, the sample should serve a dual-purpose if genotyping is to also be performed.
- Front toes should never be clipped if animals may subsequently be used in grip testing.
- This method does not require anesthesia.

VI. Ear punching:

- A sharp commercial punch device can be used to remove a 2 mm diameter piece of tissue from the middle of the pinna.
- This method can be performed on animals 14 days old or older.

- Care should be taken to not accidentally lose track of the small piece of tissue following the punch.
- This method does not require anesthesia.
- Ear punching should be performed on mice close to weaning age or older to ensure that the pinnae are large enough for the punch size.

VII. Hair:

- Tufts of hair (2 tufts per mouse) can be plucked from the animal using tweezers or hemostats.
- 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.
- This method does not require anesthesia.
- Care should be taken to avoid contamination with fomites and with hair from cage mates of the animal to be assessed.

VIII. Fecal pellets:

- Samples of feces (3 pellets) can be collected directly from the animal at the time of defecation, or from the cage floor of individually housed animals within 24 hours of defecation.
- Epithelial cells shed in the feces are the target tissue type for processing and analysis.
- This method does not require anesthesia.

IX. Buccal swabs/saliva:

- Salivary samples to harvest epithelial cells from the mouth can be performed on rodents once they are a few days old.
- 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 and prevent biting/breakage of the swab.
- This method does not require anesthesia.

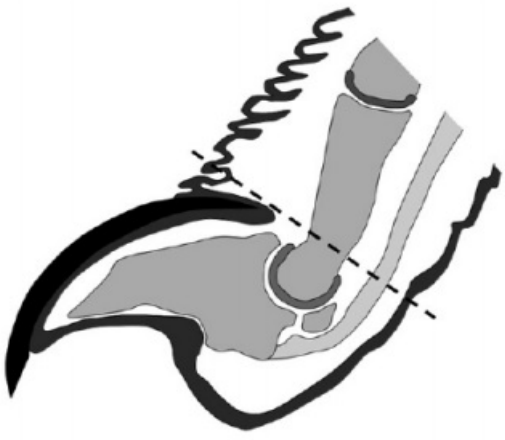

Fig 1: Proper technique for toe clipping requires removal of the entire most distal toe bone (3rd phalanx).

X. References:

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