This SOP replaces:	Date: 11.11.2015 Version: B
Reason for Change:	AWO comment
Next revision:	22.01.2024
Related SOPs:	None
Indication of Use:	Mark individual mice and/or acquire samples for genotyping
Aim of SOP:	To provide detailed instructions on the methods used to biopsy and/or mark individual mice using ear punching, tail clipping and toe clipping. Biopsies are required for the genotyping of mice which may be done in house, or externally in which case the biopsies will be packaged and sent to the researchers.
Distribution:	1. Original: Server
Attachments:	

Generated at: 22.01.2021	Checked and approved at: 22.01.2021
by: Thorsten Buch	By: Dr. Vanhecke

Responsible Persons: Animal technicians, persons on permit, Modul 1 required

Method: Ear, toe or tail biopsy, tattoo.

Principle of Method: Restraint of mouse, removal of tissue from the target area with the appropriate tool, storage of the tissue.

Materials to be used:

- Ear punch
- Fine scissors (make sure they are sharp)
- Tweezers
- Tissues
- 70-80% ethanol
- Water
- Eppendorf tubes
- Green paste, Ketchum Manufacturing Inc, Canada
- 30 G needles

Safety:

Steps should be taken to avoid contamination of tissue samples for genotyping (see procedure description). Special care should be taken in husbandries where biopsies taking must be conducted in the hood under sterile conditions.

Article 5 of TVV Marking of small rodents (Article 120 TSchV)

1 Invasive methods such as tattoos, microchips, ear notches or amputation of toe tips may be used for marking small rodents intended for breeding.

2 For marking small rodents not intended for breeding, the use of invasive methods must be justified in the context of the specific experiment.

3 Marking with ear tags is not permitted.

4 If marking is indispensable for genotyping, the marking and biopsy must be combined

Toe biopsies must not be conducted on animals older than 12 days.

Method Description:

When to biopsy mice: The animal technician will be informed as to which mice should be biopsied either via iRATS or other communication.

Biopsy collection and labelling: In all three cases described below, once obtained, biopsies are placed into individual Eppendorf vials, labelled with the appropriate cage number and the biopsy ID number, which will usually be in chronological order.

Cleaning equipment between biopsies: As it is the goal of the researcher to distinguish individuals by their individual genetic makeup, DNA contamination must be avoided. Any residual organic matter on the biopsy equipment could confound the results. After each biopsy is performed the tools (ear punch, scissors and tweezers) should be cleaned first with water, and once dry, with 70-80% ethanol. If any samples touch the work surface, the same cleaning procedure should be used on the surface.

Ear biopsy: At the time of weaning (approximately 21 days of age depending on the strain of mouse) the mouse is about the minimum size for the procedure and this is also a convenient point in time to conduct the biopsies. The mouse is restrained and the ear punch is used to remove a circular (or half circular) piece of tissue from the mouse's ear(s). The samples are collected using tweezers and dropped into an Eppendorf vial. The biopsy ID number should correspond to specific locations of holes in the mouse's ear (see chart below).

Toe biopsy: Toe biopsies offer an easier alternative to ear biopsies both in terms of the procedure itself and subsequent identification of individual mice. Maximal 2 toe biopsies per animal are to be taken when pups are between 4 -12 days of age. Pups usually remain very still when lifted gently by the skin on their back of their necks, and then the relevant tips of their toe(s) may be cut off with fine scissors causing minimal distress. The toe tip(s) are collected using tweezers and transferred into an Eppendorf vial. The biopsy ID number should correspond to the specific toes which have been biopsied (see chart below).

Tail biopsy: Tail biopsies do not serve to mark individual mice and are therefore usually conducted in addition to ear/toe biopsies. They have to be applied-for individually with a reason at the veterinary office (Gilg Simone <simone.gilg@veta.zh.ch>). They are used when the method of genotyping requires a greater amount of DNA. The mouse is restrained such that the tail is secured between the technician's little finger and ring finger and using fine scissors, a 3-5 mm piece of the tip of its tail is cut off directly into an open Eppendorf vial using fine scissors. If bleeding occurs, the tip of the tail should be held with a tissue and pressure applied before the mouse is returned to its cage.

Toe tattoo (only when part of permit): Hold the newborn as explained for toe clipping, then clean paw with 70% ethanol. Next, immerse 30G needle in animal totoo ink (green paste, Ketchum Manufacturing Inc, Canada). Insert needle with ink into superficial layer of skin and advance 1-1.5 mm. Leave there 2-3 s, then remove. One may use different toes to identify different individuals. Place newborn in foster cage. This method is performed when single newborns or little litters (up to 3) have to be placed with foster dams.

Factors influencing outcome:
Age of the mouse is critical for both practical and legal reasons.

Documentation:
A digital record will be updated with biopsy IDs as soon as they have been taken. iRATS will also be updated with the relevant ID number for each mouse.

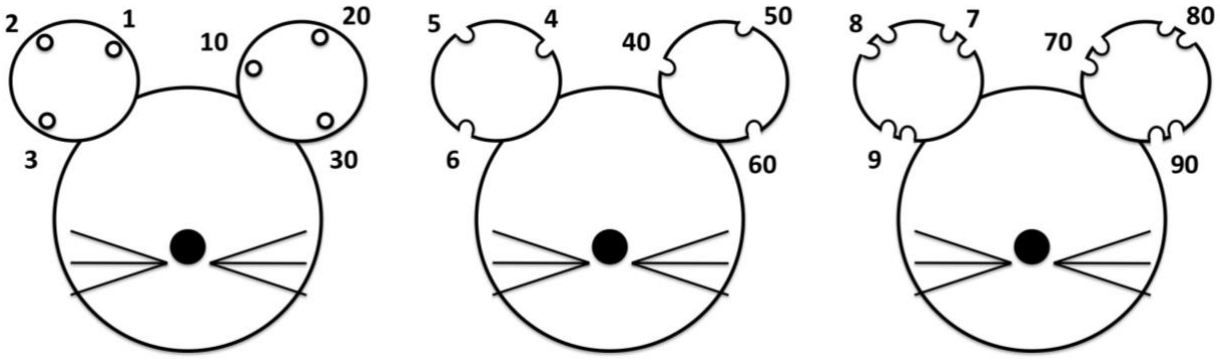
Problem management:
Biopsy mistakes can occur. If the edge of a mouse's ear is accidently breached with the ear punch or the correct toe tip is cut off the wrong foot, the ID number changes. In such cases the technician should ensure that the mistake is rectified by making the ID unique. For example, if a mouse is accidently marked as number 2, one of them can be changed into 92 by cutting the leftmost digit off the left front paw of the mouse.

Another situation is that ears of young mice are still growing and in some cases the hole that was made 2 months earlier has migrated, or even breached the edge of the ear becoming a different number. This should be monitored.

Sample storage:
Once all biopsies have been taken, they are placed in a plastic zip lock bag with a card containing the details of the biopsies within the bag. The project name, biopsy IDs and date are also written on the front of the bag for ease of identification. A record of which mice have been biopsied and their specific ID is recorded. The vials are then placed in a -20°C freezer where they will remain until genotyping.

Literature:

- Assistant Laboratory Animal Technician Manual, American Association for Laboratory Animal Science, 2009, page 74-75
- Castelhano-Carlos MJ, Sousa N, Ohl F, Baumans V. Identification Methods in Newborn C57BL/6 Mice: A Developmental and Behavioral Evaluation. Laboratory Animals 2010; DOI: 10.1258/1a.2009.009044.
- Schaefer D, Asner I, Seifert B, Bürki K, Cinelli P. Analysis of Physiological and Behavioral Parameters in Mice after Toe Clipping as Newborns. Laboratory Animals 2010; 44:7-13.
- Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 2011, page 75
- Paluch LR, Lieggi CC, Dumont M, Monette S, Riedel ER, Lipman NS. Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. J Am Assoc Lab Anim Sci. 2014 Mar;53(2):132-40. PubMed PMID: 24602538; PubMed Central PMCID: PMC3966268.
- Castelhano-Carlos MJ, Sousa N, Ohl F, Baumans V. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. Lab Anim. 2010 Apr;44(2):88-103. doi: 10.1258/la.2009.009044. Epub 2009 Oct 23. PubMed PMID: 19854756.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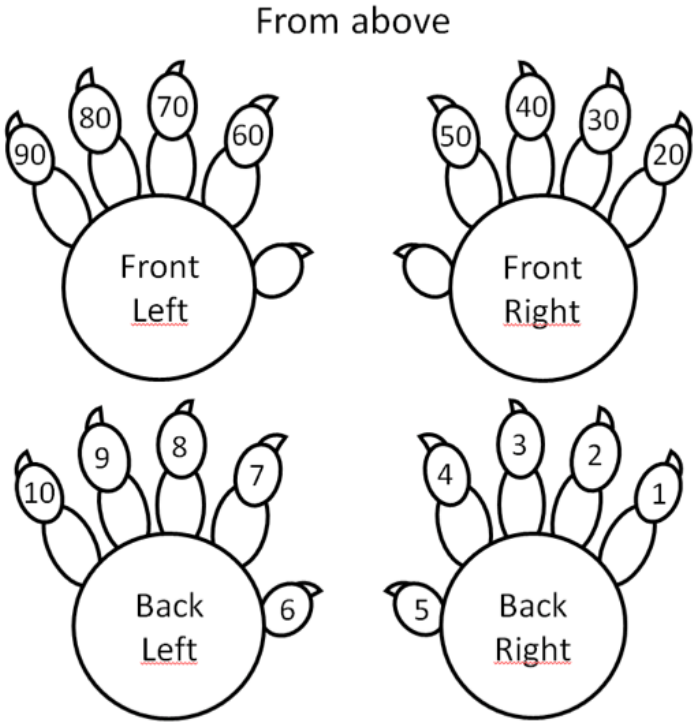
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