

Standard operation procedure: ID marking and biopsies/samples

SOP nbr: 10-01

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Valid to: 01.11.2025

Created by: Frøydis Kilmer, Nicoline Kongsten,  
Mikael Vestberg

Revised by: Helene Tandberg

Approved by: Espen Engh

## ID MARKING AND BIOPSIES/SAMPLES

### 1.0 PURPOSE

- 1.1 The animals are ID-marked in order to be able to distinguish between them.
- 1.2 Taking biopsies/samples provides material for genotyping with a view to selecting animals for experiments and breeding.
- 1.3 To minimise the number of animals housed unnecessarily.

### 2.0 DIVISION OF RESPONSIBILITY

- 2.1 The users are responsible for setting up requests for ID marking and biopsies.
- 2.2 Staff at the Section for Comparative Medicine (KPM) are responsible for ID marking and taking biopsies/samples.
- 2.3 The users carry out genotyping and register the results in Science Linker (SL).

### 3.0 PROCEDURE

#### Setting up a request for ID marking and biopsies

- 3.1 When setting up a «breeding request», the user decides whether the animals are to be ID marked by ear marks (“Tag”) or whether they need biopsies for genotyping (“Take sample”).
- 3.2 Ear marking and biopsies are performed after the animals have been separated from the breeding cage. A «Tag» or a «Tag + Sample» request are automatically generated when the litter is separated, if the user has chosen this.
- 3.3 In the case of purely KO (knock out) or WT (wild type) breeding, genotyping the first litter(s) is recommended in order to make sure that the correct breeding has been set up. If the user does not require any further biopsies, he/she informs KPM by generating an “Other” request in SL with a short description under “Description” (specify if ear marking is still required).
- 3.4 The user generates a «Tag» request for purchased animals, if these are to be ear marked.
- 3.5 Breeding cages already includes tag/sampling in the cage price. When tagging/sampling of ordered animals or if in need of retags, we will take a sum listed on our [website](#).
- 3.6 All forms of ID marking must be approved in the FOTS application and be agreed in advance with KPM.



### General information

- 3.7 A good capture technique is required before biopsies can be carried out.
- 3.8 See appendix 1 for the ear marking system.
- 3.9 It is preferable to wait until five days after the separation from the breeding cage before carrying out ear marking or biopsies. The Tag request must be performed before the subsequent 14 days have elapsed.
- 3.10 Begin by registering a «Work list» in SL: mark the Tag request that you want to perform, right click on it, choose “Request” and then «Create tag/sample work list».
- 3.11 A «Work list» can be created for several litters from the same line and project.
- 3.12 If the «Work list» is only marked «Tags», the animals are to be ear marked and the user does not need samples. If it is marked «Tag + sample», the animals are to be ear marked and the user also needs biopsies/samples for genotyping.
- 3.13 MDU and KPMc/conventional: animals from different racks in the same room may be ear marked without cleaning and disinfecting the LAF bench and equipment.
- 3.14 The Barrier: the LAF bench and equipment must be thoroughly cleaned and disinfected between carrying out ear marking and biopsies on animals from different racks.

### Ear marking with biopsies

- 3.15 The name for this kind of request is «Tag + Sample».
- 3.16 Use clean gloves when preparing the ziplock bag and Eppendorf tube. Mark the bag with the «Work list» number, project number and the name of the user.
- 3.17 Fetch the cage and check against the «Work list» to make sure that you have fetched the correct cage.
- 3.18 Place the biopsy samples in 1 ml. Eppendorf tubes. Using a black felt-tip pen, mark the tubes with a number from I to X after the «Sample number» on the «Work list».
- 3.19 List the males in the litter first with for example number 1, 2, 3 and 4. Place the biopsy/sample from male # 1 in the tube marked # 1, which corresponds to M1.
- 3.20 List the females in the litter after the males. Mark the females with for example number 1, 2, 3 and 4. Place the biopsy/sample from female # 1 in the tube marked # 5, which corresponds to F1.
- 3.21 Make sure that you clean off the lubricant on the biopsy forceps with soapy water and 70% ethanol before using it. Rinse off or dry excess ethanol.
- 3.22 Have a clean 500-cage with an empty food basket and extra paper at hand for the temporary housing of the earmarked animals. Use the food basket from the empty cage when catching the mouse. Wipe away urine between each mouse.
- 3.23 Make sure the tubes are placed in the right order. Check the biopsy sample before you put the mouse in the extra cage. Insert the sample(s) in the right tube and close the lid tightly.
- 3.24 The biopsy forceps and the tweezers must be cleaned in mild soapy water and autoclaved water between each mouse. Wipe up excess water.
- 3.25 When you have finished taking the samples, place all the Eppendorf tubes in the ziplock bag and close it tightly. Register the earmarking of each individual on the cage card.

- 3.26 Complete the finished «Work list» in SL. Right click on a random «Tag» request, choose «Complete tag/sample work list» and then choose the right work list. (Erase any animals that no longer exist). Print out the “Completed work list”. Send an email to the user group via SL.
- 3.27 An earmark will automatically be generated in SL for the animals. Adjust for any deviations in earmarking (for example if the animals have been marked 1, 2, 4 and 5.)
- 3.28 Empty the soapy water down the sink. Clean the LAF bench, rinsing bowls, biopsy forceps and tweezers with soapy water and disinfect them with 70% ethanol. Lubricate the biopsy forceps with a suitable lubricant. Disinfect pens and felt tips with 70% ethanol and place them in the pen holder. Sweep the floor.
- 3.29 Enter the biopsies in the ring binder at the printer corner and place the biopsies in a labelled container in the freezer.

#### **Earmarking without taking biopsies**


- 3.30 The name for this kind of request is «Tag».
- 3.31 Fetch the cage and check against the «Work list» that you have chosen the correct cage.
- 3.32 Make sure that you clean off the lubricant on the biopsy forceps with soapy water and 70% ethanol before using it. Rinse off or dry excess ethanol.
- 3.33 Have a clean 500-cage with an empty food basket and extra paper at hand for the temporary housing of the earmarked animals. Use the food basket from the empty cage when catching the mouse. Wipe away urine between each mouse.
- 3.34 Enter the ear mark of each individual on the cage card.
- 3.35 Complete the finished «Work list» in SL. Right click on a random «Tag» request, choose «Complete tag/sample work list» and then choose the right work list. (Erase any animals that no longer exist).
- 3.36 An earmark will automatically be generated in SL for the animals. Adjust for any deviations in earmarking (for example if the animals have been marked 1, 2, 4 and 5.)
- 3.37 Empty the soapy water down the sink. Clean the LAF bench, rinsing bowls, biopsy forceps and tweezers with soapy water and disinfect them with 70% ethanol. Lubricate the biopsy forceps with a suitable lubricant. Disinfect pens and felt tips with 70% ethanol and place them in the pen holder. Sweep the floor.

#### **Retag:**

- 3.38 Retag takes place in the same way as "earmarking with biopsies", see Appendix 2 "Are you in need of Retag" to see the new number for the animal. Make sure you always have an overview of which mice you have re-tagged and to whose number.
- 3.39 After earmarking, one must go into science links and manually change the numbers of the animals. This is done by selecting the animals in question, right-clicking and selecting "animal records (advanced)", select ok when pop-up appears, change to the correct number in "Nbr". Note down in "notes" what previous number the animal had and what date the retagging was done.

## 4.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)

- 4.1 All members of staff must have received sufficient training to ensure that they use the correct clothing and protective equipment.
- 4.2 All members of staff must have received sufficient training in the proper handling of animals.
- 4.3 The work must be carried out on a LAF bench in order to minimise exposure to allergens and agents and to limit the spread of these to the surroundings.
- 4.4 All those handling chemicals must have received sufficient training and have access to the correct protective equipment in order to ensure that the chemicals are used in the correct manner.
- 4.5 All staff must be familiar with Eco Archive and the safety sheets for the chemicals to which they may be exposed.

Ethanol	CAS no.	Pictogram	Hazard statements	Precautionary statements
Ethanol	64-17-5		H225: Highly flammable liquid and vapour. H319: Causes severe eye irritation.	P210: Keep away from heat sources, hot surfaces, sparks, open flames and other sources of ignition. Smoking forbidden. P280: Use protective gloves/clothing/eye protection/face shield. P305+P351+P338: IF IN CONTACT WITH THE EYES: Rinse carefully with water for several minutes. Remove contact lenses if used and easy to do. Continue rinsing. P370+P378: In case of fire, extinguish using foam, carbon dioxide, dry powder or water spray. P403+P235: Store in a cool, well-ventilated place. P501: Dispose of contents/container in accordance with national regulations.

## 5.0 EQUIPMENT AND MAINTENANCE

- 5.1 LAF bench
- 5.2 Overview of the marking system
- 5.3 A 500-cage with extra paper and an empty food basket
- 5.4 Biopsy forceps

5.5 Tweezers

5.6 A bowl/bottle containing soapy water

5.7 A bowl/bottle containing autoclaved water

5.8 Ziplock bags

5.9 Eppendorf tubes

5.10 Black felt-tip pen

5.11 SL

5.12 Work list

5.13 Paper towels

5.14 Soapy water

5.15 70% ethanol

5.16 Lubricant for the biopsy forceps. The forceps should be delivered to the mechanical workshop for service when necessary.

## 6.0 HISTORY OF EDITING

6.1 Written 22.07.2014 by Mikael Vestberg

6.2 06.10.2015: major amendments (Nicoline Kongsten Tømt and Frøydis Kilmer)

6.3 09.09.2021: amended to comply with the new database SL (Frøydis Kilmer)

6.4 30.10.2023: Added information regarding retag, and revised the SOP. (Helene Tandberg)

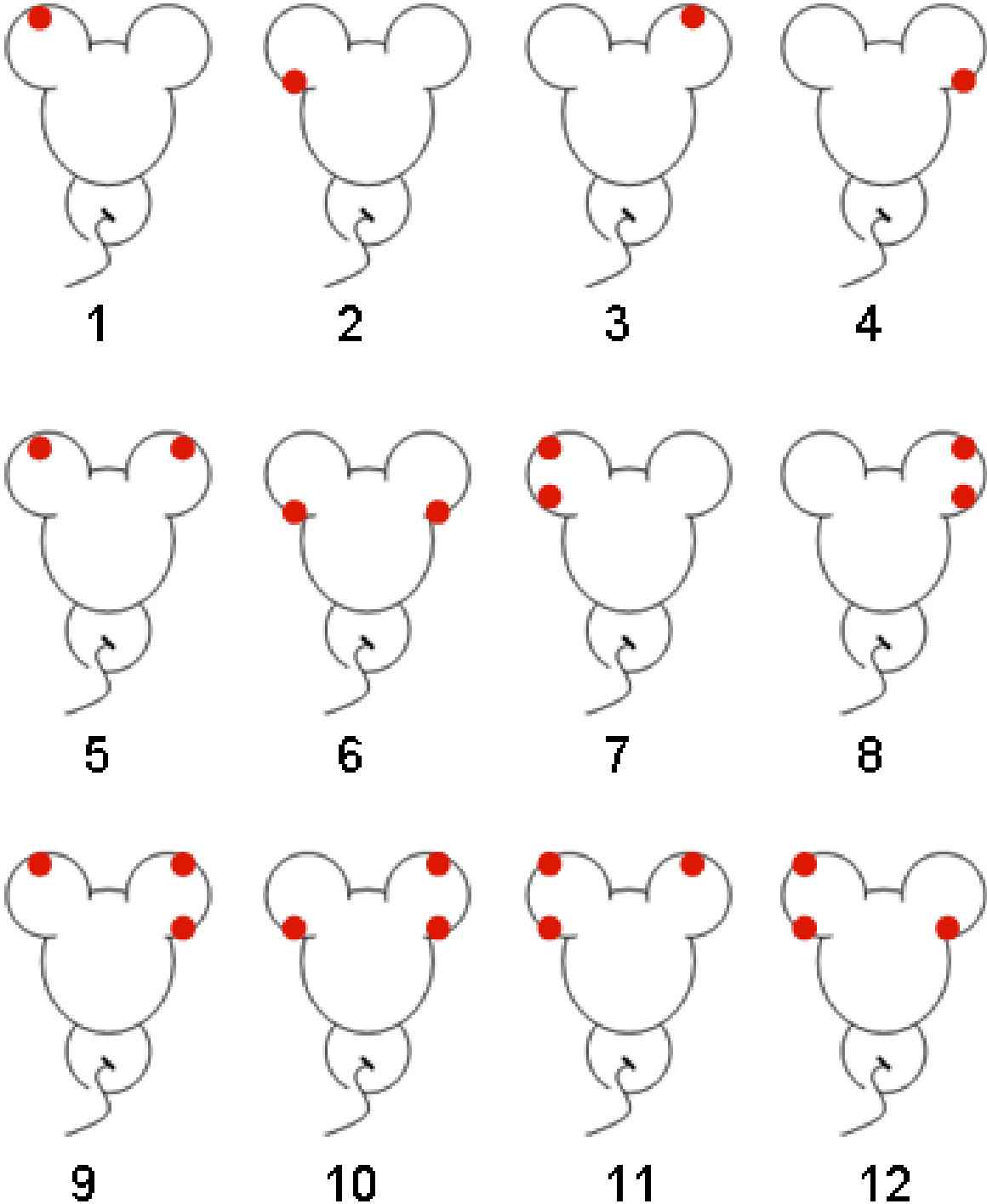
## 7.0 REFERENCES

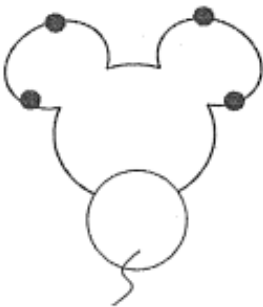
7.1 Protocol for taking biopsies, written by Harald Carlsen 13.06.2013

Appendix 1

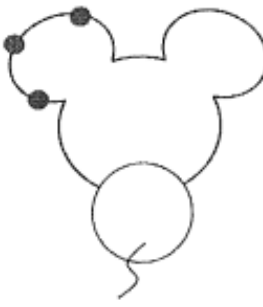
# Ear marking system

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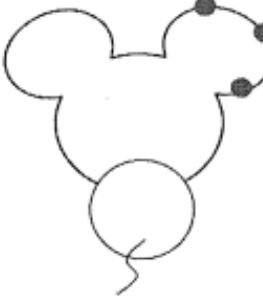




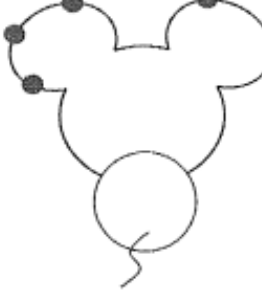
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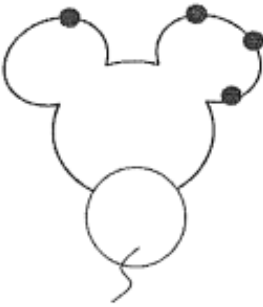
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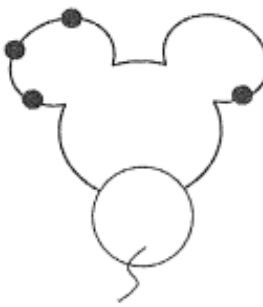
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16



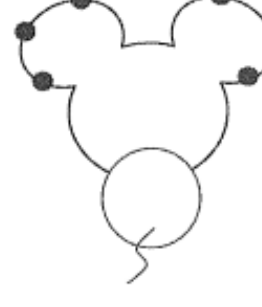
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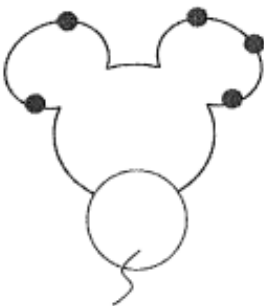
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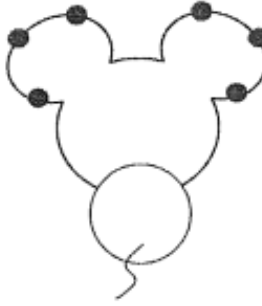
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Attachment 2:

## Are you in need of retag?

#1 → #7

#2 → #6

#3 → #5

#4 → #8

#5 → #9

#6 → #12

#7 → #11

#8 → #10

#9 → #13