Standard Operation Procedure (SOP)

Management and Maintenance of Mouse Breeding Colonies

MEDIZINISCHE UNIVERSITÄT INNSBRUCK
Tierhauseinrichtungen

Applies to:	Laboraotry Animal facilities of the Medical University Innsbruck				
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1. Purpose

In order to help researchers to manage and maintain colonies of breeding mice to ensure the best possible care and welfare of breeding mice as well as their offspring, this Standard Operating Procedure (SOP) accompanies the recommendations of the Medical University of Innsbruck (MUI) Animal Welfare Body on Cage Density.

The standard cage type in the animal facilities of the Medical University of Innsbruck is the cage type “II-long”.

The Medical University of Innsbruck Animal Welfare Body Recommendations on Cage Density in a cage type “II-long” is:

- One litter per cage
- Maximum of 5 mice in a post-weaning cage
- Single housing has to be reasoned

2. Breeding Schemes

2.1. Paired/monogamous

1 Female (F) : 1 Male (M)

It is one of the most commonly used methods.

The male and the female can be:

- Left together permanently.
  - Previous older litter must be weaned before the next litter is born.
  - Euthanasia of the new litter prior to weaning of the older litter is not permitted.
- Separated from each other:
  - Before the female gives birth (“on/off” breeding).
  - After the female gave birth – this takes advantage of post-partum estrus.

Because the male may kill the pups: the male and female must not be recombined until after the litter has been weaned.

Monogamous breedings are often used to maintain pedigree (backcrossing or intercrossing) and they are useful with regards to limiting surplus animals by utilizing “on/off” breeding.

Males from pair cages can be used in rotation mating when large numbers of similarly aged animals are required.

2.2. Trio

2F:1M

One of the females remains in the cage. If the pups of the strain are weaned with 18-21 days, this female can stay with the male continuously.

The second female is removed prior to either female giving birth.

2.3. General notes

In some cases, multiple adults with multiple litters in one cage is permitted: for example, poor breeder strains where the average litter size per female is less than the average litter size of unaffected strains (e.g. the overall number of pups from two females is equal to, or less than the average litter size from one unaffected strain; please see Table 1)

Alternate breeding strategies, such as adding a companion/auntie/nanny female (usually a surplus female, or retired breeder female) to singly housed pregnant females, may also be employed.

Alternate breeding schemes require the permission from a MUI veterinarian.

3. Breeder ages

Breeding mice should be mated and retired at appropriate ages. The maximum age for breeders must not exceed the natural reproductive lifespan for the strain.

Generally, breeders are set up between 8-12 weeks of age and retired between 6-12 months of age. Breeding females should be retired after 5-7 litters.

Strain characteristics, including breeding performance, phenotypes or behavior, may influence the age at which breeders are set up and retired.

4. Weaning

Age at weaning will vary by strain. Most mice should be weaned between 18 and 28 days of age.

Weanlings should be separated by gender and housed at the appropriate density (maximum 5 mice in post-weaning cages of cage type “II-long”).

Early (< 18 days) and late (> 28 days) weaning requires permission from a MUI veterinarian.

In case of weaning after more than 21 days, attention must be paid to ensure that two litters are not born into one cage. This can be done by removing the male prior to the previous litter being born, to avoid post partum estrus.

Sexual Maturity	4 – 7 weeks (Ave. 6 weeks)
Breeding Maturity	8.5 – 12 weeks
Reproductive Lifespan	Approx. 8 - 10 months
Gestation	18 – 21 days
Litter size	2 – 12+
Weaning Age	18 – 28 (Ave. 21 days)

Tabelle 1 Summary of mice breeding/weaning information.

5. Cage density

Cages are considered overcrowded if:

- A new litter is born before the older litter from the same female or cage (cage type “II-long”) has been weaned.
- Females from a trio are not separated prior to any of the females giving birth and if still housed with the male. Exceptions must be justified and approved by a MUI veterinarian.
- A litter remains with the female beyond 28 days of age, as most litters can be weaned between 19 – 21 days.

6. Identification (ID) of mice

In most cases it is important to be able to identify individual animals and the method should ensure a permanent and indisputable identification (ID) of an individual, without negatively affecting animal welfare.

Animals used in breeding colonies should have unique IDs, which allow them to be tracked easily. This is vital in cases of disease outbreak or unexpected phenotypes, where tracing back to the original cage can prevent an entire colony from having to be euthanized.

7. Record keeping

7.1. Cage records

Minimum requirements for information:

7.1.1. Breeding cages:

- Principal Investigator (PI) name
- Investigator contact information (phone number and E-Mail)
- number of the animal license (GZ)
- Breeding cage ID (ZK)
- date of mating set-up (Date in)
- dam/sire information (strain, date of birth, IDs)
- parturition and weaning dates
- litter information (date born, number born, number and sex of weaned pups)

7.1.2. Post-weaning cages

- PI name
- Investigator contact information (phone number and E-Mail)
- number of the animal license (GZ)
- Cage ID (Käfig-Nr)
- strain, sex, date born, parents or breeding cage number they originate from
- IDs and number of animals per cage
- if genetically modified: transgene or mutation, and whether positive for the transgene or mutation
- any experimental procedures those mice have been involved (e.g. blood sampling)

7.2. Lab records:

To track the number of offspring produced and the number of animals used/produced lab records must be kept and reported upon request.

Lab records should include the reason and date of euthanasia or death of any offspring, whether mice were recycled into the breeding colony, or transferred to a research protocol, or to a different investigator.

8. Breeding of Genetically Altered Mice

Most commonly inbred mice are used. During inbreeding alleles are permanently lost, but none are introduced, which leads to homozygosity and isogenicity. For most purposes they can be regarded as genetically identical.

Spontaneous mutations occur (mouse spontaneous mutation rate is 0.9 per gamete per sexual generation). A portion of these spontaneous mutations is effectively eliminated by inbreeding, another portion may become progressively fixed in the homozygous state, leading to genetic drift. This is why inbreeding (brother x sister mating) should never be relaxed. (Inbreeding does not prevent mutations, but contributes to the loss of new mutant alleles.)(1)

Please refer to the table below (TABLE 31.3) for common Mating Schemes and Genetic Monitoring Strategies (2).

Genetic quality control and monitoring that should be done:

Verification of normal characteristics for the strain (coat color, body size and shape, reproductive performance, behavior)

Purchase animals from reliable vendors

Replace breeding colony with animals from the same vendor after 10 generations or refresh the background with a backcross every 5 generations

Test colonies with a small set of informative microsatellite markers or SNPs to confirm integrity

Please refer to the 2019 FELASA Working Group Report in Genetic quality assurance and genetic monitoring of laboratory mice and rats(3).

TABLE 31.3 Suggested Mating Schemes and Genetic Monitoring Strategies for Various Types of Animals Where Ensuring That Homozygosity and Genetic Uniformity Is Retained

Genetically homogenous				
		Options for genetic quality control testing		
Strain type	Mating scheme	Which animals to test	Strain background assays	Allele-specific assays
Inbred	Sister × Brother (sib mating)	Breeders	Homozygous for strain/substrain-specific SNPs	Can test for strain/substrain-specific alleles
Congenic or isogenic	Hom × Hom	Breeders	Homozygous for strain/substrain-specific SNPs	Homozygous for mutant allele
	Hom × Het (or reciprocal)	Progeny, including presumptive breeders		Homozygous or heterozygous for mutant allele
	Het × Het	Progeny, including presumptive breeders		Homozygous, heterozygous or wild-type for mutant allele
	Wild-type from colony (or inbred) × Heterozygous or hemizygous carrier (or reciprocal)	Progeny, including presumptive breeders		Heterozygous/hemizygous or wild-type for mutant allele
F1 hybrid	Inbred 1 × Inbred 2	Breeders	As for inbreds	As for inbreds
Established recombinant inbred (RI) lines	Sister × Brother (sib mating)	Breeders	Homozygous for line-specific SNPs	

Abbildung 1 Table 31.1 Mating Schemes and Genetic Monitoring Strategies

9. Strain Nomenclature

Strain nomenclature for mice is standardized across the international research community. While complex, it is necessary.

Proper strain nomenclature allows you to specify:

- Background strain (e.g. pure, hybrid, mixed, unknown)
- Strain development/origins
- Any mutations present (the type (knock-out, knock-in, targeted, etc.) the gene affected, and the transgene allele)
- This information can then be used to identify the strain and appropriate controls

Guidelines for nomenclature of mouse and rat strains: http://www.informatics.jax.org/mgihome/nomen/strains.shtml

How can I confirm the genetic background of my mice: https://www.jax.org/news-and-insights/jax-blog/2013/march/how-can-i-confirm-the-genetic-background-of-my-mice

Jax Nomenclature Tutorial: https://www2.jax.org/nomenclature-tutorial/

Jax Nomenclature Quick Guide: https://jackson.jax.org/rs/444-BUH-304/images/Guide_Mouse_Nomenclature.pdf

Where two strains are being intercrossed for the first time, and the resultant phenotype is unknown, or may affect their health and welfare, specific colony management must be implemented.

Note, **this may requires project authorization**: https://ec.europa.eu/environment/chemicals/lab_animals/pdf/posters/DG_ENVI_A1_poster_laboratory_animal_Vertical_190524_PRINT_HD_GAA.pdf

10. References

(1) Guénet JL, Benavides F, Panthier JJ, Montagutelli X. Genetics of the mouse. New York: Springer; 2014. 428 p.

(2) Laboratory animal medicine. Third edition ed. Amsterdam: Elsevier/Academic Press; 2015. 1708 p.

(3) Benavides F, Rulicke T, Prins JB, Bussell J, Scavizzi F, Cinelli P, et al. Genetic quality assurance and genetic monitoring of laboratory mice and rats: FELASA Working Group Report. Lab Anim. 2020;54(2):135-48.