This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Experimentation Ethics Committee (AEEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document 018: Mouse Breeding Standards V1.0

Background

While, mouse breeding is a standard practice, there are standards that must be followed to minimise any risk of impact on animal wellbeing to the animals breeding and to reduce the risk of excessive production of animals.

As per the Code there are specific requirements for managing, maintaining and keeping records regarding breeding:

3.2.2 When animals are specifically bred for scientific purposes, the breeding program must be managed in accordance with current best practice to ensure the wellbeing of the colony, herd or flock, and all animals involved, including:

> *(i) maintaining, monitoring and reviewing adequate records. To allow an assessment of reproductive performance, records should include data relevant to fertility, fecundity, morbidity and mortality*
>
> *(ii) ensuring that specified requirements for genetic constitution and health status are met and certified*
>
> *(iii) ensuring that breeding of excess animals is avoided or minimised (see Clause 1.27), including assessment of the details and reason for culling of animals and, when relevant, accurate and timely genotyping.*

Further information about breeding animals, including genetically modified animals, is provided in Clauses 2.4.26–2.4.27, 2.5.15 [i] and 3.3.24.

Definitions

The Code: Australian code for the care and use of animals for scientific purposes 8th Edition 2013

Colony managers: persons appointed to determine the appropriate number of breeders set up to deliver the required number offspring for experimental use and for maintenance of the strain (future breeders).

Dystocia: Difficult birth, may have various causes. Classified as a mouse that is found in active labour with pups already born and a delay over many hours for further births.

Whittening: The exposure of female mice to male pheromones, most often through the use of soiled bedding, to trigger oestrus. Often used prior to timed mating.

General Information and Considerations

Breeding Management

All breeding must be covered by an animal ethics protocol and not proceed until approval is granted in writing. Mice strains may be bred under a researcher's own approved animal ethics protocol or, if appropriate, under the Animal Facility's core breeding protocol after approval is given by the breeding protocol Primary Investigator.

Where a research group wants to manage its own strain breeding, the numbers of mice utilised for breeding and animals unable to be used due to incorrect genotype or sex must all be accounted for in the group's approved animal ethics protocol.

Reduction and Refinement

Animals must only be bred for research purposes where there is no alternative option and the option of complete replacement has been ruled out. Consideration must be given to the principles of reduction and refinement when managing breeding colonies.

Colony managers must determine the appropriate number of breeders set up to deliver the required number of offspring for experimental uses and for maintenance of the strain (future breeders).

There is a number of resources available to assist with developing appropriate breeding strategies to minimise wastage and ensure the required numbers for research purposes are achieved. Assistance from experienced animal care staff or managers may also be sought.

Where a strain is not intended for use for over six months, the strain should be frozen down as either sperm or embryo freezing and reanimated if/when it is needed again. It is not acceptable to have a strain on 'maintain only' which results in the culling of pups and unneeded offspring, for periods over nine months unless there is specific justification to do so (such as complex interbreeding or back-crossing or strains that cannot be cryopreserved).

It is recommended that where a research group manages their own breeding colonies a colony manager is assigned to manage the breeding strategy and to control breeding numbers. Where a large number of strains requires management, more than one colony manager may be required. The colony manager should be clearly identified on relevant documentation in each facility as appropriate (i.e. handover forms, agreements with researchers etc.)

Selection of Control Animals and Strain Management

The use of control animals that represent true controls of the strain being produced in terms of genetic background, nutrition and environment is highly recommended. In many instances, this is best provided by breeding strains as heterozygous x heterozygous animals where the wild type offspring which are produced as littermates of the experimental animals can be used for control animals. There are a number of other factors that may need to be taken into account so it is recommended the research group investigate the best option for their particular work.

On occasion, these breeding strategies may not be beneficial, particularly if only homozygous animals are valuable for research purposes. Maintaining homozygous x homozygous crosses may be suitable, but consideration must be given to whether the phenotype of such animals will affect animal welfare or reproductive success. If it is safe to use homozygous cross animals, the background strain may need to be used to produce the altered strain as control animals.

Genetic Quality Control

All strains are subject to genetic drift, the accumulation of mutations and chromosomal rearrangements over time. To minimise genetic drift of any altered strain, and to preserve reproducibility of data produced using mouse lines generated on inbred background strains, it is recommended to refresh the strain either by continuously maintaining breeding to the reference inbred strain or incorporating the original background strain into the pedigree breeding every five generations. If the strains is of an unknown mixed background, backcrossing to the reference background strain or the original background strain is necessary. This can be undertaken for ten generations to achieve 99.9% genetic purity. If the strain is suitable for cryopreservation, stock can be frozen through embryo freezing or sperm freezing and the colony refreshed using this frozen stock. Strains can be monitored for accurate genetic composition by undertaking genetic screening of mice. The maintenance of closed colonies, that is breeding of a strain by only mating animals generated within the same strain for long periods, is not best practice and is to be avoided.

For lines maintained as homozygous with respect to the gene of interest, where the genetics are offspring are expected to be the same genotype, it is recommended that breeders be genotyped to ensure there has been no misidentification or error with breeder set up. For lines with variable genetic offspring it is recommended mice are genotyped for experimental purposes so their genetic composition is known.

Standard Breeding Practices

Pre-Pairing Health Checks

Male and female mice must be examined for underlying health concerns, prior to being paired for breeding. Animals with potentially congenital conditions (e.g. head tilt, shortened tails, malocclusion, anopthalmia) should be excluded from breeding. In C57BL/6 related strains it is recommended to check females for imperforate vaginas (i.e. where a hymen without an opening completely obstructs the vagina) or septal malformations as these can impact the female's ability to become pregnant, maintain pregnancy and may increase the risk of dystocia.

Pairing Age

Mice can be paired from 6 weeks of age. Pairing of animals at a younger age must be avoided to minimise the risks of dystocia, fighting and poor mothering. Aged mice are unlikely to breed successfully and any animals over six month of age should be assessed fully by animal care staff for suitability for breeding. This would include the weight of the animal and its general condition.

Cage Management

Male mice can be left paired with a female permanently, thus taking advantage of post-partum oestrus. If there is any concern of paternal cannibalism or aggression then the male should be removed prior to parturition to allow the best opportunity for successful birth and raising of pups. If removed, the male should not be replaced into the cage until the pups are weaned. Aggression and cannibalism may vary depending on the mouse strains used and this should be taken into account in cage management.

Harem Breeding

Two females may be paired with a single male in an accepted harem strategy. This can include rotating a male between two females in the same cage or between two cages each with a maximum of two females in each cage. Where rotation of one male occurs between multiple cages, the male mouse can be placed with a maximum of two females per week. Any proposal to expose a male to more than two females per week requires specific ethics approval and consideration of any potential impact on the male in particular.

Fostering

Where a female mouse dies, or requires euthanasia, and there are dependent offspring who are valuable animals, cross fostering to another female may be a suitable option. Cross fostering is generally performed with an animal of different colour (or other suitable identifiable difference) with a good breeding history and is most successful done at an early age of pups. The foster mother's litter should not differ by more than four days in age (preferably two to three days) from the litter to be fostered. The foster mother's litter may require reduction to enable the female to take on the additional pups. Close monitoring of the fostered litter must be undertaken for the first five days post fostering to ensure success.

Litter Reduction

Reduction of litter size or culling of mice prior to weaning should only be used where there is no other option to manage colony size. It may be employed where rodents produce significant numbers of offspring (in particular outbred strains) or where a particular sex or phenotype is known to be not required. On occasion, some phenotypes may be detrimental to animal health and therefore early culling may be recommended as mitigation of expected welfare issues, this must be detailed in an approved animal ethics protocol. Culling of pre-wean pups must adhere to accepted euthanasia practices.

Weaning

Offspring are weaned between 19 to 28 days of age. Where animals are kept with their parents after 21 days of age, the cage must be monitored for the birth of the following litter and an assessment made about the welfare of each litter and the risks associated with weaning and/or keeping both litters in the breeder cage. Weaning animals prior to 21 days must only be

undertaken when there is confidence on the weight and condition of the pups and/or where there is a clear need for early weaning (e.g. the mother is unwell).

Breeding Strategies

Breeding strategies must, where possible, aim to produce the required number and genotype of offspring with the least amount of breeding. Estimation of numbers of mice that cannot be utilised (i.e. incorrect genotype, gender that is not suitable for experiments) must be included in ethics protocols. It can be helpful to provide estimated numbers together with a proportion of total numbers bred and expected usage.

Breeder Replacement and Selection

Breeder females must be replaced once they have had their sixth litter as per best practice. This may need to be sooner if the strain is subject to early death or susceptible to other diseases.

Animals that have been utilised for experimental purposes must not be utilised for breeding without specific ethics approval to do so as this is considered re-use. This does not include tissue collection for genotyping or single one off blood collection.

Acclimatisation prior to Breeding

Acclimatisation periods should meet the ANU Guideline for Acclimatisation of Rodents.

Acceptable Advanced Breeding Practices

Timed Mating

Timed mating is considered a standard practice where a male and female is paired on a known day in order to develop a pregnancy of a ‘known’ gestation length. In this case the female is checked for a seminal plug in the urethra to determine she was successfully mated. If a plug is identified, the male and female should be separated. If there is no plug and no indication of successful mating the male may be left in the cage for further days with daily plug checks. This process does not guarantee pregnancy. A timed mating procedure must be followed to ensure accuracy and that the methodology is appropriately followed.

Timed mated animals that are needed past E10 (i.e. 10 days post recorded mating) can be weighed before and after the timed mating to verify weight gain that is consistent with pregnancy. Palpation can be undertaken but must be done cautiously and by trained individuals to determine if foetuses are present in the abdomen. These methods do not guarantee pregnancy.

Whittening

The use of Whittened females is considered standard practice. This is where male bedding from mice of the same hierarchy (and therefore health status) is used to ‘prime’ females 3 days prior to pairing to increase the chance of successful mating and pregnancy.

Backcrossing

Backcrossing of a genetically modified allele to a new background strain to create a congenic strain, or a strain mixed or unknown genetic background, or when regular backcrossing hasn’t

been undertaken will take ten generations to complete to 99.9% genetic purity. Before ten generations are completed the strain can be used but are considered an incipient (incomplete) congenic strain. When backcrossing animals it is important to limit the number of animals produced at each generation if they are not able to be used for experimentation until the backcross is complete. This is performed by removing the male after successful breeding of one or two litters per generation.

Speed congenics may be utilised to fast track the backcrossing of a strain. It involves the pairing of mice for limited generations, ideally at the N2 and N3 crosses, performing genome-wide SNP scanning and/or targeted genotyping to select animals to accelerate the rate of backcrossing to achieve a higher purity by the 5th generation.

Further information on backcrossing strategies is available from facility managers.

Dystocia

Animals that are found to be in active labour must be noted and monitored quietly and without significant disturbance during the day. They may need to be euthanased at the end of the working day if no progress in labour has been evident. The use of oxytocin or other labour assistance is not advised unless a specific dystocia SOP is included in the approved animal ethics protocol and all staff responsible for the care of animals are appropriately trained in the related procedures.

The manual manipulation of ‘stuck pups’ is not advised and must only be undertaken by highly trained and experienced animal care staff under the direction of a veterinarian or by a veterinarian.

Monitoring, Intervention and Reporting

Monitoring for Unsuccessful Breeding

Mice must be monitored regularly for success or failure of breeding. Breeder pairs that have not produced a litter in more than 50 days are considered unproductive and must not be kept past 90 days of pairing unless there is specific justification to do so. Stud or vasectomised males must have records of mating performance and if performing below expected standards should not be kept.

Research staff and animal care staff must be monitoring this and manage production of strains and replacement breeders appropriately. It is the research group’s responsibility to take action with unproductive breeders and ensure replacement is undertaken unless the group has specifically tasked breeder management to animal care staff, with an agreed breeding plan in place.

Mice that are showing any indication of being unwell or have any condition that may affect their welfare and are unsuccessful breeders must not be maintained. It is a shared responsibility between animal care staff and researchers to regularly review breeder pairs for success and ensure that any welfare issues that may exist are managed appropriately.

Minimum Requirements

- All breeding must be covered by an approved animal ethics protocol.
- Animals must only be bred if there is no replacement alternative to the use of animals.
- The principles of reduction and refinement must be considered in all planned breeding strategies.
- Breeding strategies must consider the production of mice that are of the most appropriate genotype (experimental and control) and must follow best practice to ensure high quality research outcomes.
- A single male may be paired with no more than two females in a harem strategy.
- Breeders must be replaced after producing a maximum of six litters.
- Timed matings, Whittening and pregnancy palpation after E10 are all standard advanced breeding practices but must be included in an approved animal ethics protocol.
- Breeder pairs must be monitored for breeding success and action taken to manage unproductive breeders.
- Record keeping must ensure that reproductive performance, genetic composition and morbidity and mortality can be tracked for each strain.

References and Resources

Jackson Laboratories Colony Planning Guide https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-and-husbandry-support/colony-planning (accessed 17th June 2020)

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ANU. Procedure for Managing & Reporting Unexpected Adverse Events https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms (accessed 22nd June 2020)

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