



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

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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)

NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013 (Section 4.4.3) <https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes>

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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