



Frequently Asked Questions About Breeding mice

- 1. What age can mice be first mated?*
Mice are first mated at 6-8 weeks of age and it is best to mate females before 12 weeks of age.
- 2. What age are breeders retired (male and female)?*
A breeding pair is retired after they have produced 6 litters (about 8-9 months of age). Male breeders can continue to be used in other breeding pairs up to 12 months of age. Our standard practice is to cull both males and females after the sixth litter unless instructed otherwise.
- 3. Why can't mice be kept breeding for longer?*
These guidelines have been submitted to and approved by the Animal Ethics committee. These guidelines are based on the fact that production from genetically modified strains decreases after 6 litters. Litters are less frequent and small in size, pre-weaning mortality and deaths from dystocia (trouble giving birth) increases. These are guidelines, so under special circumstances the breeding life of pairs can be extended.
- 4. What is the length of gestation?*
19- 21 days depending on the background strain, litter size and lactational status of the female.
- 5. How old are mice when they are weaned?*
19- 21 days old.
- 6. What is the average litter size?*
Generally 5-6 but the average litter size depends on the background strain of the line.
- 7. How many mice die before weaning?*
The overall average pre-weaning mortality at ABR is approximately 5-6%. This does not include mice that are culled as runts. The pre-weaning mortality may vary significantly over time for any one line. It is not unusual to have "destroyed litters" in lines that are on an inbred background and with small numbers of breeding pairs this can cause significant variation in pre-weaning mortality. Only trends over large numbers of breeding pairs and litters should be considered significant. A history of at least 6 months (and preferably 12 months) should be assessed to determine if losses constitute a trend.
- 8. How and when is tissue collected for genotyping?*
Tissue collection for PCR analysis can be collected at 2 weeks of age if mice are identified using tail tattoos. If researchers need to use ear marking as the means of identification tissue collection will be performed at 3 weeks of age. Tissue is collected by cutting 2-3 mm from the tip of the tail. If FACS is to be used for genotyping, blood is collected later at 5-6 weeks of age.
- 9. Why can't older males be group housed?*
Male mice can only be group housed up to about 4 weeks of age. Group housing of older males will often result in fighting that can cause injury and occasional death. While it is true that some strains are more aggressive than others, the policy at ABR is to only group house young males.
- 10. Why can't I cull or issue all stock and just leave breeders?*
ABR staff will advise strongly against this practice. Genetically modified mice on inbred backgrounds can breed quite erratically. If an existing pair stops breeding it may take some time before replacement pairs become available. Keeping stock and reserving breeders means replacement breeders can be set up without delay.
- 11. Why do I need to keep reserve breeders?*
Keeping reserve breeders is good practice to ensure on-going production from a line. Existing breeders may need to be culled or may suddenly stop breeding (as above).

12. *Why can't I maintain a line with just one pair?* Maintaining a line with just one pair leaves the line in a vulnerable state. The death of one mouse in the pair or cessation of breeding could result in loss of the line.
13. *How many cages are needed to maintain a line?*
It is recommended that at least 2 pairs of breeders and one cage of replacement breeder females and one or more cages of replacement breeder males of an appropriate age and genotype be kept at all times. In addition there may be cages for the next round of replacement breeders or stock waiting on genotype results. Realistically a maintenance line generally consists of 6-10 cages.
14. *How do you maintain a line on a mixed background?*
Genetic drift will occur in all lines so that the mixed background will change over time. One way to minimize the genetic drift is to backcross to an F1 hybrid made from the founding strains of the line. For example if a line was made on a 129/ B6 background a 129/ B16F1 can be used.
15. *Why do females in harems need to be separated when pregnant?*
If two females have litters in the same cage, the small pups are often trampled. It is also difficult to know the parentage of pups from two different litters if they have similar birth dates.
16. *How many mice fit in a cage?*
A maximum stocking density of 5 adult mice is allowed or 1 pair plus a pre-weaning litter. To minimize fighting, older male mice are often better housed at 3/ cage.
17. *Which is better harem breeding or permanently mated pairs?*
Harems allow for a rapid expansion in the first generation of a new line and allow for one male of desired genotype to be mated with multiple females. However as females are removed from the harem when pregnant and are therefore not exposed to the male for post-partum oestrous, permanently mated pairs generally produce most pups in a given space in continuous breeding. Permanently mated pairs are also less work for technicians.
18. *Are researchers contacted before sick mice are culled?*
Unless specified in the Line Instructions that the researcher requires the mouse for research or wants to investigate a phenotype, ABR staff will cull sick mice without advising the researcher. ABR staff are well-trained and follow the cull criteria approved by the AEC for the breeding protocol. If researchers are choosing replacement breeders they will be contacted after the sick mouse has been culled.
19. *Are researchers contacted before breeders are replaced?*
This depends on the Breeding Choice instructions entered in the Lines section of StuartWeb. If researchers want to specify which individual mice are used in future breeding pairs they will be notified before replacement breeders are mated. For routine breeding the choice of replacement breeders is generally best left to the breeding co-ordinator as they can check the health of mice before mating.
20. *Can technicians automatically issue all stock of a certain genotype/ sex?*
You can ask that all stock of a certain genotype/ or sex be issued at a specified age. This must exclude mice set aside for replacement breeding.
21. *How do I communicate with ABR about breeding?*
Routine breeding instructions are communicated through the *Lines* interface on StuartWeb. This provides a set of routine *Line Instructions* to the animal technicians that include tissue collection, phenotype information, culling at weaning, stock culling, and breeding instructions. Technicians are prompted whenever changes are made to the Line Instructions. Specific instructions that are out of the ordinary to routine colony maintenance such as those regarding individual animals can be issued using the *Communications* feature of StuartWeb.

22. *How long does importing take?*
Importing from Australian sources generally takes 2-4 weeks as long as the relevant mice are available at the donor institute. Importing internationally generally takes 6-8 weeks however delays often occur due to a scarcity of mice at the donor institute, delays in obtaining export permits or relevant health certificates.
23. *How long do clean lines have to stay in quarantine?*
Mouse lines with a clean health screen from the donor facility usually stay in quarantine for approximately 6-7wks. Samples are collected from the mice 30 days post arrival and it can take several weeks for results to arrive. Mice that are directed into the 'dirty quarantine room' will stay until rederived. This generally takes 4-6 months.
24. *Can mice be used while in quarantine?*
Mice can't be used while under Biosecurity quarantine without receiving specific permission from Biosecurity Australia. Mice not under Australian Quarantine (such as national or local imports) can be used for research and bred while in quarantine.
25. *What is rederivation?*
Rederivation is performed at ABR using embryo transfer or sperm collection and IVF. In both cases embryos are transferred surgically into clean pseudopregnant recipient females. When the pups are born they receive the pathogen free flora of their birth mother rather than pathogens of their genetic mother. Note a small number of pathogens can be transmitted via the embryo and will not be always removed on rederivation (eg. mouse hepatitis virus) so post- rederivation health screening is conducted.
26. *How long does rederivation take?*
Rederivation generally takes 4-6 months if all goes well. Some lines breed poorly or superovulate poorly making them difficult to rederive. In such cases rederivation can be prolonged.
27. *Do all lines need to be rederived?*
If lines are imported with a clean health screen from the donor institute and the health screen performed at ABR confirms that they are free of pathogens, the line will not need to be rederived. ABR can also hold small colonies of dirty mice in quarantine to allow a researcher to conduct initial experiments before deciding whether the line is worth rederiving. This pre-derivation holding is usually limited to a 6-month period and is subject to space availability.
28. *Can technicians enter genotype results on Stuart?*
It is the researchers responsibility to enter genotype results onto Stuart. If GMG is performing the genotyping the results can be automatically uploaded onto Stuart from GMG.
29. *Can researchers age mice at ABR?*
Aging of mice at ABR is usually possible but is space dependant. The quarantine area is the place where space is most constrained and aging studies may be limited. Researchers must obtain permission from the AEC to age mice to greater than 12 months of age. Mice will then be held at ABR under the experimental aging protocol.
30. *How long does it take to backcross a line onto a different inbred strain?*
Backcrossing ten generations onto a different inbred strain takes about 2-2.5 years. Using speed congenics this time can be reduced to 6-7 backcrosses and takes about 18 months.
31. *How long does it take to produce a double homozygous line by crossing to individual lines?*
This takes 2-3 generations depending on whether double homozygous mice can readily be generated from the double heterozygous cross. Usually there will be only 1 in 16 pups with the correct genotypes from this cross and both a male and female are needed. It is sometimes faster to use an additional step of double heterozygous x single homozygous, fixing one homozygous loci and then intercrossing two mice that are heterozygous for one loci and homozygous for the second loci. The time taken is usually between 6-9 months but can take longer if pairs breed poorly.

32. *How many breeders are needed to produce a standing order of x mice/ week?*
ABR staff can assist in estimating the number of female breeders needed as they are familiar with the breeding performance of individual lines. The formula used is as follows: $\text{No. pups/week} = \frac{(\text{No. breeding females} \times \text{av. litter size})}{(\text{Interlitter interval})}$. If a single sex of mice is required multiply this value by two.
33. *How do I change the breeding plans for a line?*
Update the information on the Line Instructions interface on StuartWEB. Note- if the plan involves introducing a new gene into the line this constitutes a new line and you need to fill out a Line Information form on StuartWEB.
34. *How can I find out if my imported line will need rederivation?*
The ABR imports co-ordinator can advise if the mice will be received into the clean quarantine room for re-screening. If a "clean" line screens positive for pathogens excluded from the ABR, you will be contacted by our rederivation co-ordinator to discuss rederivation. Note: About 5% of mice arriving with clean health screens from donor facilities require rederivation.
35. *Which inbred strains are available at ABR?*
The standard inbred strains available at ABR can be seen on the ABR website:
<http://www.abr.org.au/animals/inbred-mice>
36. *Does ABR offer embryo or sperm freezing?*
ABR offers both embryo and sperm freezing. As sperm freezing is quicker, cheaper and uses fewer animals this is the preferred method unless the line is on a mixed background.
37. *Can I import from overseas frozen embryos or sperm instead of importing live mice?*
ABR has import permits for frozen embryos and sperm. Unfortunately there are strict pre-freezing health screening requirements for Hantavirus that some donor facilities may not meet. The ABR Import Co-ordinator will check if the donor health screen will meet requirements prior to proceeding with the import.
38. *Is it cheaper to import live mice or frozen embryos from overseas?*
If the line requires rederivation it will be both quicker and cheaper to obtain clean offspring by importing frozen embryos. The import of live mice from clean facilities remains cheaper if the line does not require rederivation.