



Australian BioResources Rederivation Requirements

Rederivation is used to eliminate pathogens from mice to establish clean, healthy breeding colonies. ABR has 3 core breeding areas and rederivation requirements for each are listed below. Researchers can choose where mice will be bred at ABR.

Maximum Barrier – All mice to be housed in the Maximum Barrier must be rederived on arrival, irrespective of the health screen status. Compulsory rederivation ensures mice will be free of all pathogens on the ABR exclusions list.

Standard Barrier Level 1 – Imported mice can enter this barrier without rederivation as long as the health screen demonstrates they are not carrying pathogens on the ABR exclusion list.

Standard Barrier Level 2 – Imported mice can enter this barrier without rederivation as long as the health screen demonstrates they are negative for all pathogens on the ABR exclusion list except for *P. pneumotropica* and *K. oxytoca*. These pathogens are accepted in this barrier.

Any imported mice that do not meet the health screen requirements for Standard Barrier Levels 1 and 2 must be rederived.

Please note that some ABR partner institutes have specific requirements about the health status of mice entering their facilities (eg. presence of *P. pneumotropica*). So it is important to check the requirements of the destination experimental facility before making a decision on the desired ABR barrier for breeding.

Rederivation can be performed by using either IVF techniques or standard rederivation practices.

IVF Rederivation

This is the fastest and least expensive option as well as the one likely to yield the most pups. It requires the sacrifice of one sexually mature genetically modified (GM) male of at least 10 weeks of age from which sperm is collected and frozen. In vitro fertilisation (IVF) is performed in the Maximum Barrier using sperm thawed from the GM male and oocyte donors from a standard inbred strain. Please note that if the imported GM male was homozygous, using the IVF method will result in all offspring being heterozygous.

List of inbred mice available for IVF:

B6.129S7-Rag1^{tm1Mom}/JAusb
B6.SJL-PtprcaPepcb/BoyJAusb
BALB/cJAusb*
C57BL/6JAusb
FVB/NJAusb

*Reduced pup numbers expected (~5) due to strain's low ovulation rates

Import requirements: 2 or 3 genetically modified males, no older than 25 weeks.

Have the genotype protocol in place before the mice are sent to save time. Work will not begin until the imported males have had their genotypes confirmed.

Time frame from confirmation of genotypes is approximately 5 months.

Depending on sperm quality, there should be between 10-35 pups born.

Average Time Line

Week 0 - Confirmation of genotype.

Week 1 – Male sacrificed, sperm collected and frozen.

Week 2 – IVF and embryo transfers performed.

Week 5 – pups born on the clean side.

Week 7 – pups identified in Stuart and tail tattooed. Tissue is collected and sent for genotyping.

Week 9 – pups weaned.

Week 11 – Bacterial samples sent for health screening.

Week 10-12 – Breeding pairs can be set up*

Week 15 – Bloods sent for health screening.

Week 17 – Released from rederivation holding area assuming clean health reports.

* There are several restrictions applied to breeding in the rederivation holding area; no cross lines can be set up, pairs must be made using males and females from within the colony or with mice from the standard inbred strain used. The rederivation holding area cannot hold large colonies, there is a maximum of 4 breeding pairs/line.

Important Notes:

- Female oocyte donors must come from standard inbred strains housed in the Maximum Barrier. We are unable to perform IVF using GM females from quarantine due to time restrictions in collecting the oocytes and pathogen contamination of the clean area. If female GM mice are required for rederivation the standard method will need to be used.
- Males **MUST** be genotyped before work can begin to insure the correct mice have been imported.

Standard Rederivation

This is generally much slower and yields fewer pups than IVF. This option is best used for lines that are sex-linked or when it is preferable not to mate mice onto the background strain. This may be because the background strain of the import line is not available for IVF or not held at ABR, it is on a mixed background, or the line is homozygous for multiple genetic modifications.

Import requirements: 2 or 3 males and 2 or 3 females for breeding pairs. The imported mice will need to have their genotypes confirmed before the rederivation can take place.

Breeding pairs will be set up on arrival at ABR. Males will be kept from the first litters to be used as studs. Once mature stud males are ready, 4-5 week old females will be super ovulated through hormone treatment and mated to the stud males. Viable embryos

collected after mating will be transferred into recipient mothers housed in the Maximum Barrier.

Time frame varies depending on the breeding performance of the colony. It could take 6-12 months to complete.

It may take more than one rederivation session to gain sufficient mice to start the clean colony. Average number of pups is 7-15.

Process

Breeding to obtain 8 week old studs



4 – 5 weeks old females available



Rederivation session 1 – embryos transferred into recipients.



Rederivation session 2 – embryos transferred into recipients. (if required)



Pups born in the Maximum Barrier.



Colony released from rederivation holding area 12 weeks after all pups born.

Please note that that there are too many variables to include a comprehensive time line for standard rederivation. Clean pups will be identified at 2 weeks of age with tissue sent for genotyping. Time line from when pups are born flows at the same time intervals as for IVF rederivation, the same breeding restrictions on the clean side also apply.

Important Notes:

- Females are best used at 4-5 weeks of age, before the onset of puberty interferes with the hormone treatment for super ovulation. Using older females will require more sessions and animals and may eventually occur an extra charge if 5 rederivation sessions are reached without sufficient mice born in the Maximum Barrier.

Health Screening:

After rederivation, the recipient females and the rederived progeny undergo health screening to ensure that all pathogens have been removed. Final samples for this health screening are taken after the progeny mice reach mice reach 10 weeks of age, with results taking at least another week or two to receive. Mice are expected to be released from the rederivation holding area around 12 weeks of age.

Mice cannot be moved/issued from the rederivation holding area until all health screen results have been released. Until this time, ABR will not guarantee that the mice are clear of all pathogens.