



## **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. Mice recovered from frozen material or generated in the MEGA service are also allowed in the Maximum Barrier.

**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. This is limited to animals imported from the Maximum Barrier at the Jackson Laboratory and the production area at ARC only.

**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. The IVF method is used in the first instance and the Standard method is only used at the discretion of ABR if the IVF should fail more than once. There is an extra cost involved if Standard rederivation is used for rederiving difficult lines.

Described below is the 2 different ways rederivation can be achieved through IVF and the import requirements for each.

### **IVF Rederivation using WT females from the background strain**

Depending on the background strain required, this could be the fastest way to rederive a line. It does not require the expansion of a colony by breeding and female donors are generally readily available.

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It requires the sacrifice of one sexually mature genetically modified (GM) male from which sperm is collected and frozen. The male needs to be at least 10 weeks old. ABR will supply the female donors if the strain is on the list below. Please note that if the imported GM male was homozygous, using WT females will result in all offspring being heterozygous.

**List of inbred mice available:**

B6.129S7-Rag1<sup>tm1Mom</sup>/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:** 2-3 genetically modified males.

**IVF Rederivation using GM females**

Using GM females as the oocyte donors can cause delays in the IVF however there are also advantages such as a line remaining homozygous.

It requires the sacrifice of one sexually mature genetically modified (GM) male from which sperm is collected and frozen. The male needs to be at least 10 weeks old. It will also require the sacrifice of 3-5 females for oocyte donors. ABR recommends another 2 females are imported for breeding to ensure the line is safe while the rederivation is in progress.

For the hormones used to induce ovulation to work, female donors need to be 4-5 or 9-15 weeks old. If the females imported are older than this, the colony will need to undergo breeding to produce younger females, this will delay the rederivation of the colony.

**Import requirements:** 2-3 genetically modified males. 5-7 genetically modified females.

**Other procedures of note:**

A rederivation service request must be submitted through StuartWeb before any work will commence.

Imported mice should be genotyped before the rederivation is attempted to ensure the correct mice have been received. It is also recommended that a genotyping protocol is in place before the mice arrive to save time. If the genotyping is not performed and the pups produced from the rederivation are not as expected due to the incorrect mice being imported, the researcher is still required to pay for the service.

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## **Important Time Points**

Time frame to completion from confirmation of imported genotypes is approximately 5 months when no breeding of the mice beforehand is required.

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

### **Average Time Line**

Week 0 - Confirmation of imported genotypes.

Week 1 – Male sacrificed, sperm collected and frozen.

Week 2 – IVF and embryo transfers performed.

Week 5 – pups born on the clean side.

Week 7-8 – pups identified in Stuart. 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.

Please also note that there may be delays between collecting sperm and performing the IVF if there is a high demand for the service.

## **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.