ANIMAL SERVICES AND GENOTYPING: A QUICK REFERENCE GUIDE
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Web References
Musterer:  https://musterer.apf.edu.au/
JCSMR Intranet APF:  http://intranet.jcsmr.anu.edu.au/administration/apf-animal-services
New Starter/Training Requirements

It is an ANU requirement that staff and students working with animals at any of the biomedical areas associated with the ANU complete the following courses prior to gaining access to animals, databases and animal facilities.

Ethics Approval

Staff member/Student must be listed on the protocols that they will be working under. Details on how to amend/add a person to existing protocols can be found on the ANU Website at https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-training-and-forms

If you are a new Primary Investigator (PI) and are required to lodge an ethics application you can liaise with the Research Office via animal.ethics@anu.edu.au

ANU General Courses Required

The following courses are mandatory and are prerequisites for training in other techniques. Registration is via HORUS:

- WHHR01 Biological safety
- WHHR18 Gene Technology Practices

ANU Courses – Animal Specific

ANML03 Animal Ethics and Awareness (Seminar and online quiz)

All staff working directly with animals or listed on an animal ethics protocol, even if not handling animals directly, must attend the Animal Ethics and Awareness Training. This course is offered a number of times throughout the year. Individuals may begin work before attending this course but must register, attend and complete the associated quiz requirements within a reasonable timeframe of starting at the University.

Practical

ANML02 Animal Care and Handling* (Theory and practical)

New staff or students that have previously not completed animal handling must undertake the APF Animal Care and Handling Course. This course is run over 3 days and includes theory and practical components. Attendees are marked as Competent or Not Yet Competent. If marked Not Yet Competent, they may be requested to undertake supervised work within their laboratory to enhance their skills before returning for reassessment or further training.

* If you have considerable animal handling experience you may only require refresher training or assessment of skills. You may be able to register only for ANML09: Skills update/assessment and we will contact you to arrange a session. You can work with our training team to determine which course is appropriate for you by emailing training.apf@anu.edu.au
ANU Courses – Technique Specific
The APF provides monthly training and assessment sessions in the following techniques. It is a requirement that you are assessed as competent in the techniques you will be required to do as listed on your protocol. Session details and dates are available on HORUS.

ANML02 Introduction to Mouse Care and Handling
ANML04 Introduction to Rat Care and Handling
ANML05 Sharps Safety with Animals
ANML06 Intra peritoneal injection - Mouse
ANML08 IV Injection - Mouse
ANML20 Retro Orbital Sinus blood collection - Mouse
ANML22 Introduction to Animal Anaesthesia
ANML33 Oral Gavage - Mouse
ANML34 Intra Muscular Injection Mouse
ANML35 Subcutaneous injection - Mouse
ANML36 Intradermal Injection - Mouse
ANML37 Cardiac Bleed Under Anaesthesia

The APF training team may be able to offer training in other techniques upon request. Please contact them at training.apf@anu.edu.au

Musterer Training – APF Mouse Database
The APF has an in-house database that tracks mice, breeding, phenotypes and genotype results. This system is accessible through the web.

Training for the use in this system may be provided by your local area/lab group but if it is not, you can contact the training team at training.apf@anu.edu.au for specific training. You can register for this training by the HORUS code ANML11 Introduction to Musterer.

Training can also include the following at your request:

- Ordering and receipt of animals only
- Looking up/managing strains, ordering and receipt of animals
- Receiving and understanding Musterer Invoices

Some research groups may provide this training within their group but if this is not available the APF is happy to assist with this training.
Area Inductions
Each area of the University requires specific inductions to be completed for your safety and the integrity of the research undertaken in each area.

For APF area inductions you may require any of the following or a combination thereof:

<table>
<thead>
<tr>
<th>Area</th>
<th>Reason for Access/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hugh Ennor Building Administration Only</td>
<td>Meetings Animal Pick Up</td>
</tr>
<tr>
<td>Hugh Ennor Building Animal Facility Level 2</td>
<td>Access to Experimental Animal Rooms Level 2</td>
</tr>
<tr>
<td>Hugh Ennor Building Animal Facility Level 3</td>
<td>Not granted to researchers. Only available to APF Staff</td>
</tr>
<tr>
<td>Hugh Ennor Building Laboratory Area Level 1</td>
<td>Sample drop off for Genotyping, regular contact with Genotyping group</td>
</tr>
<tr>
<td>Hugh Ennor Building Full Access</td>
<td>Not granted to researchers, only available to APF Staff</td>
</tr>
<tr>
<td>JCSMR Containment Suites Pick Up/Drop Off Only</td>
<td>Access to holding room to collect animals Delivery of dirty cages to dirty wash up area</td>
</tr>
<tr>
<td>JCSMR Containment Suites Full Access</td>
<td>Use of Containment Suites Facilities</td>
</tr>
<tr>
<td>JCSMR Satellite Facilities Full Access</td>
<td>Use of Containment Suites, Behavioural Suites or other appropriate APF Managed Areas</td>
</tr>
</tbody>
</table>

Please note that the APF do not manage all animal facilities within JCSMR. Therefore you may need to arrange training and/or inductions with your lab managers for certain areas.

All JCSMR staff and students are expected to attend a JCSMR Building Safety Induction managed by the JCSMR WHS Team.

Animal Ordering and Moving Mice

Animal Orders and move mouse requests
Animal orders are placed through the musterer ordering system [http://sales.apf.edu.au](http://sales.apf.edu.au)

Move mouse requests are placed via [https://musterer.apf.edu.au](https://musterer.apf.edu.au)

Orders/move mouse requests must be placed in the system a minimum of 48 hours prior to collection/delivery.

Orders for delivery are processed as follows:

- HEB Level 3 to HEB Level 2 - **Tuesday mornings**
- HEB to JCSMR Behavioural and Containment Suites - **Wednesday**
- HEB to JCSMR Holding room: Monday - **Thursday**

Training animals

Orders for the following weeks' training mice must be made by 12pm Thursday.
Training animals will be provided in accordance with the ANU Ethics Policy published on the website at: https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-training-and-forms

Requesting Culling of Animals
Animals may be marked for culling until 5pm Friday for processing on the following Monday/Tuesday.

Animals may be saved from culling until Sunday afternoon.

Any last minute cull/save requests should be sent in a ‘high importance’ email to animal.ops.apf@anu.edu.au

APF Available Strains – Quick Reference
The APF has a number of “Multi-User” strains available for purchase including:

- **ASD 513 C57BL/6NCrl** Currently being refreshed, recommended genetically controlled B6 strain
- **ASD517 C57Bl/6J.Anu** ANU substrain, not refreshed for over 40 generations
- **ASD429 Swiss Outbred Colony** Limited availability
- **ASD 514 Rag1** Limited availability, please place large orders well in advance
- **ASD516 Balb/CAnNCrl** Limited availability, please place large orders well in advance
- **ASD619 C57BL/6:ptprca/NCrl** Currently being refreshed, not recommended due to mutation
- **ASD620 C57BL/6:ptprca/Jax** Will not be provided long term, alternative supplier ARC

Room/Hood Bookings
The APF has an Outlook Calendar booking system for the following rooms.

Bookings are made by inviting the room to a meeting

- Building 117, HEB, Room 2.105, Pathology Lab, Dirty side
- Building 117, HEB, Room 2.90, Procedures Lab, Clean side
- Building 117, HEB, Room 2.77, Irradiator Bookings
- Building 117, HEB, Room 2.72, Cytotoxic Hood Bookings
Hierarchy

Due to the varied needs of JCSMR researchers the APF operates a number of different hierarchies to maintain mice with different health status.

**Level 3** Hugh Ennor Building is the ‘cleanest’ hierarchy where breeding and maintenance of strains occurs. No researcher access if allowed in this area except by special request and escort.

**Level 2** Hugh Ennor Building is an experimental area. There are experimental rooms which operate with a high barrier and where the animals must only be handled within a hood. There are also limited rooms where the animals may be handled on the bench or provided with non-irradiated food. These rooms are located on the South Corridor of Level 2.

JCSMR has a number of smaller animal areas. Individuals accessing the small holding rooms, APF managed holding rooms and the behavioural suites may enter the Hugh Ennor Building the following day after a shower and full change of ‘street’ clothes.

Those entering the JCSMR Containment Suites rooms are not to access the Hugh Ennor Building animal rooms for a full two nights (close to 48 hours) due to the higher risk these animals pose to the breeding colony.

The basic hierarchy is identified below, a more detailed hierarchy will be shown to you at induction. For any clarifications please contact animal.ops.apf@anu.edu.au
Importing Strains

The APF offers a specialised service in the import and export of strains. There are a number of pathways animals can enter APF facilities including:

- Import to Quarantine and rederivation (the most common method)
- Import to Quarantine, limited release and rederivation (for pre-approved vendors only including Jackson Laboratories and Charles River ‘Maximum Barrier’ facilities)
- Direct import and release (open to domestic imports only)
- Import to Containment Facilities

The pathway will be determined by the health status of the animals and your experimental needs. Our goal is to provide you with experimental animals as quickly as possible without compromising the excellent health status of the ANU mouse colonies. We aim to rederive all imported strains regardless of their origin to maintain the best and most consistent health status possible across the facility.

All animals imported to JCSMR or APF Animal Facilities must be managed by the APF Shipments Team to ensure the risks of import of animals are appropriately managed.

The process involves the following general steps

1. Contact animal shipping
2. APF will provide the documentation to you to request a shipment
3. Complete in full the required documentation
4. The APF will assess your import, decide on a process and keep you updated on the progress of your shipment.

Importation and rederivation can be a lengthy process in many occasions due to the compliance and paperwork requirements, the involvement of other facilities and researchers and the sensitivity of mice with regards to breeding success and the rederivation process. The appropriate pathway and options for your strain(s) can be discussed with the team by contacting animal.shipments@anu.edu.au and completing the required enquiry documentation.

The completion of forms to their full extent is a requirement before importation or exportation can be undertaken, this includes the detail of phenotype information, genotyping assay requirements and NLRD, Ethics protocol details and full strain nomenclature. Your assistance in completing these forms in full will assist us in expediting your request.

Satellite Facilities

*Animal Orders delivered to JCSMR:*

Animals can be ordered through the APF ordering system and delivered to the JCSMR Holding room on L1 for you to collect each day from Mon – Thu. Delivery is usually made by 10am.

Animals ordered to be delivered to the Holding Room MUST be collected by 2pm that day.

Animals can also be ordered to be delivered straight to the Behavioural Suites and/or Containment Suites on Wednesdays only. These animals will be placed in the room designated on the order form by the APF Technician.
Animal Orders collected from HEB:
Animals ordered and required earlier than 10am on Mon – Thu; or at any time on Fri can be collected from the HEB at the time agreed with the APF staff.

Collections from the HEB MUST be made at the time designated.

Animals MUST be transported as per the AECC SOP for the Transport of Animals.

Animals not picked up by the required time may be moved to a lower hierarchy or culled if the research group cannot be contacted in a suitable timeframe.

Used cages and tubs:
Transport cages and tubs used to transport animals from the HEB to JCSMR MUST be returned to the HEB for processing – NOT the JCSMR Satellite Facility or Behavioural Suites.

Cages taken from the Behavioural Suites to any labs and used MUST be returned to the dirty room of the Containment Suites for processing.

No dirty cages or equipment can be dropped off after NOON on Fridays or before 9am on Mondays.

General:
Animals should be ordered allowing sufficient time for them to sit and acclimate to their new surrounding prior to use.

It is the responsibility of the person ordering the animals to ensure there is sufficient room to house the animals in the designated room.

Genotyping

Where to drop off samples for Genotyping:
With access to the L1 lab area, samples can be placed directly in the freezer in the main genotyping lab. Without access to the genotyping area, samples can be placed in the fridge/freezer at the HEB reception and an email should be sent to genotyping.support@anu.edu.au, informing the team that samples have been dropped off at the reception. For all samples please fill out the corresponding sample log attached to the freezer.

Why is there a large difference in the price charge for ear punch digestion compared to tissue digestion?
Large amounts of sample or organ requires genotyping staff to physically cut up each sample which is time consuming for a small team that is required to genotype mice bred for research. If the samples are ear punches or cut up into small sizes and tissue volume is the same as two ear punches and delivered on a 96 well plate (which can be obtained from genotyping), then we will charge $2 instead of $5.

Any samples not provided in 96 well plates or with too much tissue for normal processing will be charged $5 for digestion. This is because the genotyping team is required to transfer all sample to 96 well plates and adjust the amount of tissue/DNA.

When will I get my results from Genotyping?
Genotyping tries to deliver results within the Client Service Charter (http://www.apf.edu.au/about-us/quality-and-accreditation/client-services-charter) timeframes. These timeframes are set from the time of digestion of ear punches. This occurs every Monday and Thursday.
Results will be entered into Musterer. If a researcher wants to receive an email notifying them of updated results for mice on their project they can contact APF IT (it@apf@anu.edu.au) and ask to be added to the email list.

*What happens when NR or ? is entered in Musterer instead of a genotyping result?*

? means that genotyping automatically gets repeated on the original DNA sample.

NR means that two attempts of genotyping on the original DNA has failed. If genotyping is required on this mouse, then a task request to animal services for a new ear punch needs to be submitted. This is the responsibility of the researchers.

*Why is charging for re-genotyping mice at the discretion of the facility?*

Decision to charge is dependent upon the circumstance. For example if doing an experiment the mice are found not to be the expected genotype, we are happy to provide this for free for a few samples together with the next running of the next same assay. However if there are a large number of samples and/or they are required with a short turnaround time, we need to charge for this.

*Why is the future breeding strategy for imported mice important for assay design?*

This is because it can affect the way that the genotyping team designs the assays. Sometimes when different mouse strains are crossed, the designed assay might stop working due to DNA being excised or the assay may no longer be able to determine the difference between transgenes or other modifications.

*Mice requiring genotyping on mapping plates:*

Mice requiring genotyping on mapping plates will only be genotyped after requesting this via task request. These plates are normally only digested and stored.

*What is a fixed genotype?*

The definition of a fixed genotype is

- If all breeders and the first offspring for a specific strain and generation have identical genotype for one or more genotyping assays this is termed as fixed. Fixed genotypes are not included in the two genotyping free assays in the cage charges.