	Flinders University **Safe Work Method Statement** **Mice – Breeding and Colony Maintenance 18/06/19**	
		**College of Medicine and Public Health Animal Facility**

SWMS Number	RA Number	RA Score	
SWMS- 8.2	RA 8.2	Medium	
Contact Person	**SWMS prepared by**	**AWC Approval Date**	**Review Date**
Roxanne Collingwood	Roxanne Collingwood	18/06/2019	June 2021

Contents

The SWMS **Mice – Breeding and Colony Maintenance** contains the following sections:

Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.*
- *Animal Welfare Act 1985.*
- *Animal Welfare Regulations 2012*
- *Gene Technology Act 2000* (the Act)
- *Gene Technology Regulations 2001*
- *Work Health and Safety Regulations 2012*

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee (via the Animal Ethics Review Sub- Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc
  http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

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- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Safe Work Method Statement

Refer to Risk assessments, Safe Work method Statements for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

RA 1.0 Mouse Sexing, Handling and Restraint
SWMS 1.0 Mouse Sexing, Handling and Restraint
SWMS 7.0 Compliance - Emergency Contingency
RA 7.0 Compliance - Emergency Contingency
SWMS 7.1Compliance -Transportation
RA 7.1 Compliance -Transportation

Personal Protective Equipment Required

- **Gloves – to be worn throughout entire procedure**
- **Gown**
- **Mask**
- **Hair Net**
- **Shoe Covers**

Hazards and Controls

- **Animal bites- training, demonstrate competency, adhere to SWMS.**
- **Animal Scratches- training, demonstrate competency, adhere to SWMS.**
- **Animal Allergies- wear PPE when handling or handling dirty cages to stop the potential development.**
- **Manual handling- IVC cages weigh approximately 10kg, attend manual handling training, adhere to SWMS.**

Before Work Commences

Ensure that you are aware of the locations of the following:

- **Spill Kit**
- **Fire Extinguisher**
- **Eye Wash**
- **Exits**
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Risk Assessment and SDS (Safety Data Sheet) - Ensure that you have read and understood for all the substances being used.

Equipment

- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training before using any equipment**

General Information

- **All procedures are to be performed by trained competent staff.**
- **Training is available from senior animal house staff or Animal Welfare Officer.**
- **Evidence of training is available in the "Training Needs Analysis".**

Environmental Conditions

Temperature	18 – 24ºC
Humidity	40-70%
Light Cycle	12hr light: 12hr dark
Light intensity	350 lux (max)
Room Ventilation	10-20 air changes/ hr

General Mouse Biological Data

Life span	1-3years
Average Weights	Newborn: 1g
Adult Male	25-40g
Adult female	25-40g
Oestrus frequency	4-5days
Duration of oestrus	10 hrs
Gestation period	19 – 21days
Ave litter Size	6 – 12
Weaning Age	21 days
Sexual maturity	40 – 60 days
Breeding Life Span	8 months
Body temperature	37.1-37.4 C
Heart rate	328-780per min
Respiration rate	90 – 220 per min
Daily Food Consumption	3 – 6g
Daily Water Consumption	4-7 mls

Room Entry and Exit

1. Mop the square in the room entrance with F10sc.
2. Stand in the square, and put on mask, hair net, and gown.
3. Put one shoe cover on, step over the square, then put on the other shoe cover.
4. Put on gloves. If they develop a hole at any time whilst in the room, replace the glove.
5. To Exit the room, mop the square with F10sc, remove one shoe cover, then step into the square and remove the other shoe cover (NOTE: Shoe covers can be reused if re-entering the room).
6. Remove and label gown if required to re-enter the room. Discard cap, mask, and gloves.

Time Mating Procedure

NOTE: Plug day is Day 0 dpc (days post coitus). The presence of a vaginal plug does not guarantee that the mating will result in a pregnancy. Pregnancy can be determined by gently palpating the female's abdomen from Day 10.

***** Do not use females with vaginal abnormalities, as mating may occur but the female may have difficulties giving birth.**

Procedure Summary:

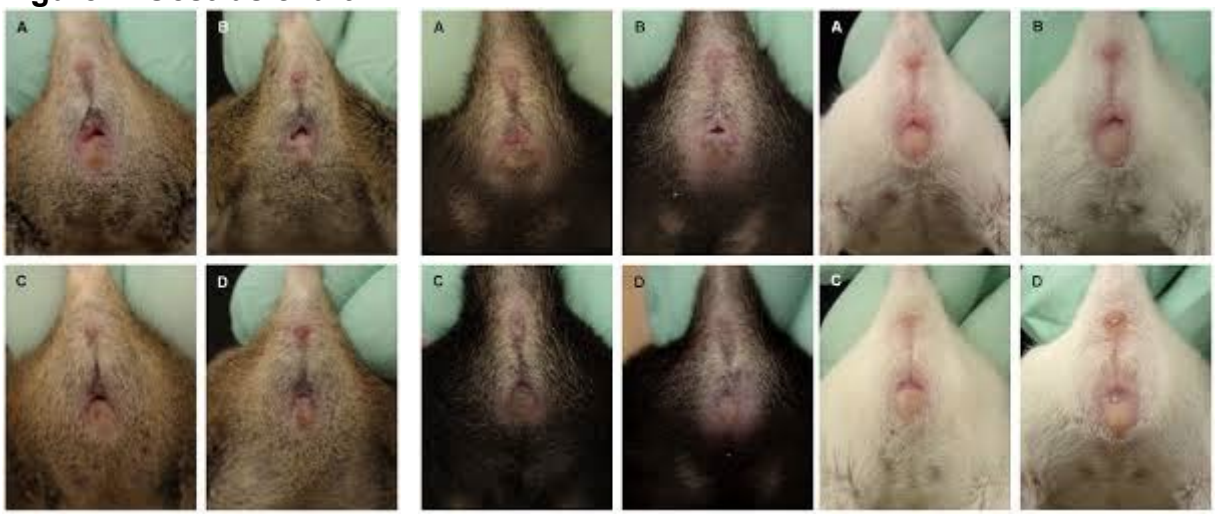
1. Day 0, place male in Time mating cage with the divider in place.
2. Place 1 or 2 females on opposite side of divider to the male.
3. Record what males and females are in the cage on the laminated cage cards for time mating.
4. Day 2, remove divider and allow animals to mate.
5. Day 3, check females for plugs, and record the presence of plugs and date on the time mating record sheet.
6. Place females back into their own cages.
7. Day 14-16, check females for pregnancy confirmation.

Time Mating Mice (oestrus cycle is 4-5 days):

- Females should be aged 8 -10 weeks of age; but mice can be used from 6 weeks depending on the strain and size of the animal.
- Oestrus can be induced by exposing sexually mature female mice to either soiled male bedding, or housed in a "time mating" cage with a male for 3 days. A time mating cage allows males and females to be housed separately in the same cage, and the mesh divider can be then removed for mating.

- The number of positives and strain required will determine the number of matings required to be set up. Where possible, use females that are in oestrus, as evident by red swollen vaginas (*see Figure 1*), as this will minimise the number of females required.
    - Set up 1 male to 2 females if 1-2 positives are required.
    - Set up 7 studs with 2 females per male if 3 positives are required.
    - Set up 7 studs with 3 females per male if 4 positives are required.
    - Set up 14 studs with 2 females, etc.
- Check for plugs the following morning.

Figure 1. Oestrus chart



A. Pro-oestrus- Receptive to mating
B. Oestrus- Receptive to mating
C. Met-oestrus
D. Di-oestrus

- **Copulation or Vaginal Plugs** - Are formed from coagulated seminal fluid, and will remain in the vagina for 8-12 hours.
    1. Remove one female at a time, placing her on top of the cage or table (it is easier to check them on top of the cage as they will hold on to the bars).
    2. Hold the base of the tail with one hand, and gently lift to expose the vagina. Gently insert the tip of the forceps or probe, feeling and observing for any obstruction. The plug maybe clearly visible on the surface, or deep in the vagina (*see Figure 2*).
    3. Check each female in the stud box, placing positives and negatives into separate boxes. Record the date and a cross or a tick (depending whether it was positive or negative), on the stud chart.
    4. Record either ✓ or ✗ on the stud cage card depending whether the male has mated or not. Replace any studs which have failed to mate as evident by 4 consecutive crosses.

- Plugged females must be placed into a separate cage. Record on the cage card the researcher(s) name, ethics approval, number of positive (plugs), the mating date, and the plug date.
- If the required numbers of plugs are not found, set up matings the following night. Inform the Researcher of the outcome of the mating, and the number of plugs found on each day.
- Negatives females may be culled or kept for 2 weeks (observing for any pregnancies), then reused.
- **If time-mated females are required beyond day E10, mice can be gently palpated to determine if they are pregnant. If animals are not pregnant, the researcher must be contacted prior to issue. Pregnancy can be difficult to determine in older or obese animals.**

Figure 2: Mouse vaginal plug

Setting Up Breeder Pairs

NOTE: Refer to pace note for strain breeding information and requirements.

1. Breeders are mated at 8 - 10 weeks of age, with 1 male to 1 female per cage, and kept for a period up to 12 months.
2. Terminate breeders if they fail to produce pups, consistently produce runty pups, or don't wean a viable litter for 2 months. Cross the breeder box number off pedigree chart, and record the cull in the Breeder Records document.
3. Save Reserve Breeders from the 3rd litter if possible, as this allows you to asses which breeders consistently produce large litters and equal ratios of males:females.
4. When the litter is ready to be weaned, house brother and sister offspring separately. The cage must have food and water.
5. Record the number of males or females on the Reserve Breeder cage cards, strain, Parent's breeder Id number, date of birth, and ethics number on the cage card.

6. Join reserve breeders when they are 8 - 10 wks old. Combine 1 male:1 female, record mating date, new box number (next consecutive number), parent's box number, and strain.
7. Insert the new number on to the pedigree chart, and connect with a line to its parent's box number.
8. Before pedigrees are terminated, ensure that new breeders have been saved 8 weeks prior to termination, to continue the line.
9. Remove the old males from the parent box only after the new breeders have been paired and produced their first litter. The female can be culled when the last of the litters have been weaned.

Cleaning IVC ,Open Top Cages and Colony Maintenance

1. All supplies and consumables must be autoclaved
2. IVC Cages are to be cleaned each fortnightly, unless the cage is excessively dirty or wet and may require to be cleaned weekly. Open top cages must be cleaned weekly.
3. Add sufficient corn cob bedding (IVC) or Fibre Cycle to thinly cover the base of the cage, one handful of shredded autoclaved paper towel, and 1 handful of Food.
4. Remove the cage from the rack by supporting the base of the cage, and releasing the locking lever with your right hand. Place cage on the cage change station.
5. Release the red latches on the cage to remove the lid
6. Identify and label any mice that are pregnant, as these must be checked daily, and the births recorded on the cage card and on the weaning whiteboard. Pups do not need to be counted when they are under 4 days old, as handling new born pups may result in mothers eating them. Euthanize any pregnant females that are experiencing birthing difficulties, and record on the "Animal Health Care Form"
7. Count and record the number of pups for any new born litters (record number on the cage card, and the appropriate Breeding Record Sheet).
8. Wean all litters that will be 21 days of age on the Monday or Thursday of that week (check the weaning whiteboard for appropriate dates). If pups are small, check to see if they are within the correct weight range for that particular strain.
9. Record the number of male and females weaned, plus any pre-weaning deaths on the breeder cage card, and the Breeding Record Sheet for corresponding breeder box number.
10. Sex and separate weaners into either the male or female stock boxes (up to 5 per box). Complete cage card including researcher, ethics approval, department, sex, number, strain, weaning date, and parent details.
11. Ensure all cages have food on the bottom of their cages as well as in the food hopper, and all have water bottles and the weaners can reach the sipper tubes, and that the sipper tubes are operating properly and not blocked.

12. Place the clean IVC base on the cage change station and transfer the mice. Top up food if necessary. Dust the metal lid with a damp cloth to remove any accumulated dust. Transfer the metal lid to the clean cage, replace the plastic lid, and secure with the red clips.
13. Repeat the above process until all of the breeding boxes for that strain have been cleaned.
14. If any animals require culling due to illness, euthanize immediately and record on the “Health Report”

Feeding

- “Gordon’s Autoclavable Premium Rat and Mouse Pellets” must be used for all cages in this room, unless a researcher has specified a specific diet. If a researcher has specified a specific diet, then the cage or cages must be identified with a treatment card detailing the specific feed to be used.
- Feed must be sterile, and topped up on the cleaning day, or as required.
- Some food is to be placed in the bottom of the cage, as well as the hopper, when cleaning.
- The feed bin must be kept stocked at all times.

Water Bottles

- Water bottles are to be checked daily. All are to be emptied, scrubbed, and refilled on cleaning days. Fresh water is to be available to the animals each day.
- For the IVC cages that are cleaned fortnightly, all of the bottles are to be topped up on a Friday of a non-clean week.
- Water bottles in cages are to be checked and changed as required daily. If the water level does not change, check the sipper tube as it may be blocked.
- Ensure that all sipper tubes are operating properly by shaking them (ball bearings should move freely). Sipper tubes should be checked whenever the bottles are filled.

Floor and Walls

- To be swept daily to remove any food on the floor, reducing the chance of slipping.
- Swept and mopped with F10sc (8ml/1Litre water) on the day of cleaning.
- Walls and racks are to be wiped over at this time.

SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Position	Name	Contact Details
Manager Animal Facility	Roxanne Collingwood	8204 4380 roxanne.collingwood@flinders.edu.au
Animal Welfare Officer	Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References

http://www.nhmrc.gov.au

http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/home-1

http://www.adelaide.edu.au/ANZCCART/

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.