RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Rederivation of Germ-free mice		
SOP Category: Gnotobiotic	**CMR SOP #: 4.03**	**Page: 1 of 4**
Effective Date: 7/13/23	**Approval: LaTisha Moody, DVM, DACLAM**	
Revisions:4/14/2023, 6/1/23		

SCOPE:
This document describes the procedures to be followed when re-deriving rodents into axenic animals using hysterectomy. This SOP applies to all Gnotobiotic Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to render mouse models as germ free (GF) or axenic to allow researchers to study the effects of the microbiome.

Terminology:

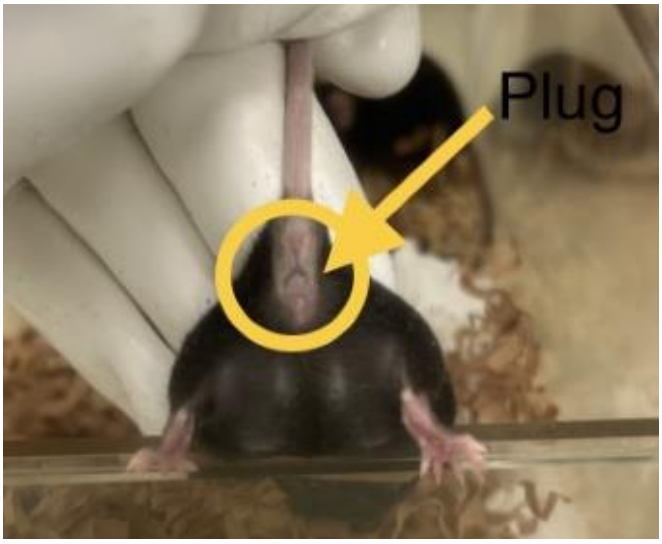
1. **Axenic (germfree)** - an animal free of all foreign microorganisms including bacteria, viruses, fungi and protozoa.

2. **Microbiome –** A microbiome is defined as the community of microorganisms that can usually be found living together in any given habitat. It is the collection of all microbes, such as bacteria, fungi, viruses, and their genes, that naturally live on our bodies and inside us. The human body is home to about 100 trillion bacteria and other microbes, collectively known as your microbiome.

PROCEDURES:

A. Foster & Donor Mice Setup

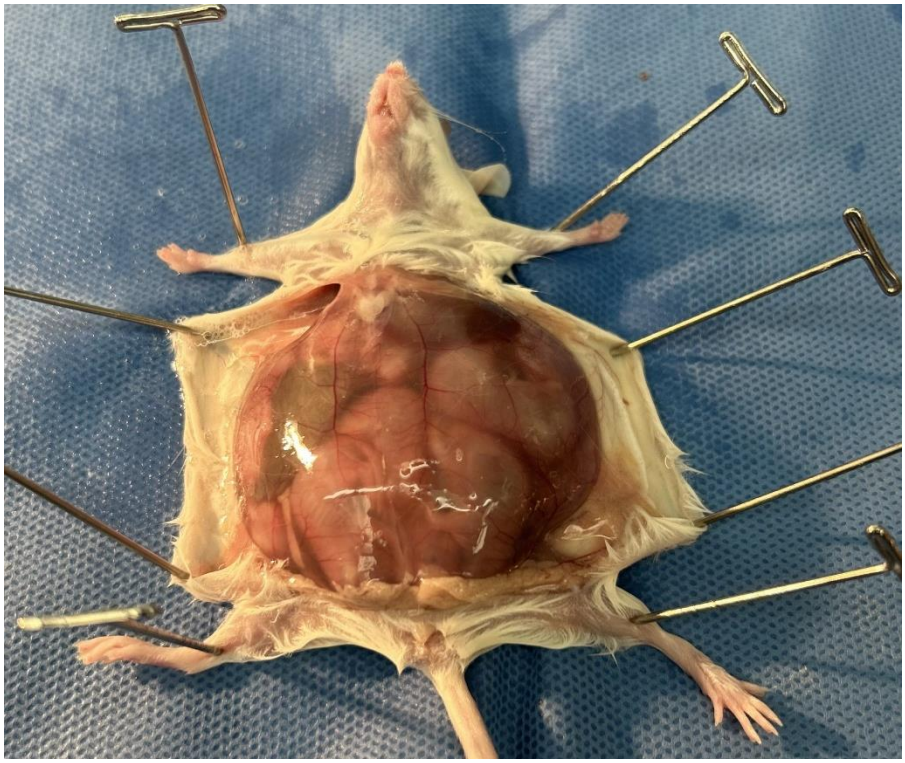
1. Set up mating's of the foster mice before going home.
2. The next day check the visible plug, preferably first thing in the morning or at least before 10 am.



3. If you see the plug in the foster female separate her from the male, set up mating of the donor mice before going home.
The next day check the plug on the donor females. If you see it, then the female must be separated from the male. It will count as 0.5 days of pregnancy.

4. At day 17.5 of pregnancy make sure the donor female is visibly pregnant and administer 0.03 ml subcutaneous injection of MedroxyPROGESTERone (150 mg/ml) using a sterile tuberculin syringe. This hormone will help maintain the pregnancy until the scheduled rederivation.

5. Perform Hysterectomy on day 20.5



B. Hysterectomy for Mice to be Housed in Sentry Sealed Positive Pressure (SPP) Allentown cages.

Sterile supplies needed:

- Freshly prepared Clidox-S® 1:3:1
- Sterile disposable drapes
- Sterile surgical gloves
- Autoclavable pouches for surgical instruments
- 2 tissue forceps
- 2 thumb forceps
- 1 dressing forceps
- 3 operating scissors
- 1 tenotomy scissor
- 1 hemostat or bulldog clamp
- Stainless steel strainer
- Heat lamp
- Soft gauze
- Two 250 ml beaker
- Push pins
- Foam board

1. Perform this procedure early in the morning.
2. Open a sterile drape on the platform under the Biosafety cabinet (BSC).

3. Turn the heating lamp on the left side of the BSC.
4. Designate the right side of the BSC the “dirty” side and the left side as “clean”.
5. The BSC should contain sterile instruments, 2 beakers filled with Clidox-S® 1:3:1 and foam Board.
6. The recipient GF foster litter is housed in the clean SSP cage which is surface sterilized inside the BSC.
7. Place foam board under sterile drape on the right side of BSC. Outside of the BSC, the assistant will euthanize the donor mouse by cervical dislocation without anesthesia and *quickly* soak the body in Clidox-S®
8. After the donor mom is soaked in sterilant, place on top of drape and pin limbs to the foamboard with drape in between foam board and mouse.
9. With sterile gloves, the abdomen of the donor female mouse is cut with scissors and skin is reflected using the “pinch and tear” technique. Pin the dermis to the foam board.
10. Use the spray bottle to wet the exposed abdominal wall with clidox.
11. Using a new set of sterile instruments, open the abdomen with scissors and forceps taking care not to cut into the uterus.
12. Locate the ovaries and oviduct and sever the oviduct with sharp scissors. Handling the uterus gently with the forceps by handling the uterine horns by the oviducts and ligaments.
13. The gravid uterus is exposed by dissecting oviducts and ligaments free and reflected caudally.
14. Clamp the uterine horns a few millimeters (~1/8’) proximal to the cervix using Forceps.
15. Cut the uterine horns between the clamped forceps and the cervix (ensure no part of cervix remains attached).
16. The gravid uterus is removed and immediately immersed in warm germicidal dip at room temperature (Clidox 1:3:1) along with forceps along with clamp.
17. The primary operator will carefully cut open uterus and remove pups quickly to revive under heat lamp.
18. Separate the placenta from pups using blunt dissection. Place pups under heat lamp.
19. Quickly revive pups by rubbing gently with soft gauze sponges to stimulate and clean them. Pups should become pink and active within a few minutes.
20. Ideally, place up to 4 to 6 donor pups with each foster litter. If the foster litter is too large the pups can be sacrificed by decapitation.
21. Check the mice in mid-to-late afternoon.
22. Feces samples will be collected during a designated time (1-2 weeks after C-section) to submit for culture and possible PCR testing.
23. Once litter has been proven to be germfree after 3 negative results, the cage may be moved into the main GF colony and housed in SPP cages or isolator.
24. GF animals may be made available upon request by investigators.