

## FLSC Standard Operating Procedure for Sample Collection for Vaginal Cytology

### Purpose

Understanding the mouse estrous cycle is critical for those investigations involving reproduction and fertility or to note sexual bias in studies. Many mice exhibit puberty/fertility changes that can affect the maintenance of genetically manipulated mice. The following procedures are intended to assist the researcher in the identification of the estrous cycle in mice.

### Cytological Determination of Estrous Cycle

Some stages of estrous cycle are very short (e.g., metestrus), it is important to collect the material at the same time each day. If the objective of the study is to use cycling mice in different phases or to test the effects of drugs on cycling, at least two consecutive baseline cycles should be recorded prior to manipulation. The following is a rapid noninvasive method to obtain material for analyzing the stage of estrous cycle in mice.

### Materials

Pipette tips – 200 microliters	Sterile ddH <sub>2</sub> O at room temperature
Latex bulbs	Clean glass microscope slides

1. Place a latex bulb on the end of a sterile 200 µl tip and draw up approximately 100 µl of sterile ddH<sub>2</sub>O.
2. Lift the mouse out of the cage and place on the wire bar cage top with her tail towards you.
3. Firmly grasp the tail and elevate the hindquarters of the mouse. The mouse will now have only the front paws grasping the lid. If the mouse urinates, wait until urination stops. Should there be urine left at the entrance to the vaginal canal, you can rinse the opening with a small squirt of ddH<sub>2</sub>O using a separate tip.
4. Place the end of the ddH<sub>2</sub>O-filled tip at the opening of the vaginal canal taking care to not penetrate the orifice as vaginal (and cervical) stimulation can induce pseudopregnancy.
5. Gently depress the bulb to expel a quarter to half of the volume of water (~25-50 µl) at the opening of vaginal canal. The liquid will spontaneously aspirate into the canal without tip insertion. Slowly release the pressure exerted on the bulb. The fluid will withdraw back into the tip. Avoid releasing pressure too quickly to prevent aspiration of fluid into the bulb. A filtered tip may be useful for this purpose.
6. Repeat the previous step 4-5 times using the same tip, bulb, and fluid to obtain a sufficient number of cells in a single sample.
7. Place the fluid on glass slide, and allow the smear to completely dry at room temperature. Once dry, these estrous smears can be stained immediately or stored and stained at a later date
8. Each mouse will require a new pipette tip.

This procedure requires more restraint of the mouse and is considered a stressful procedure. Mice when stressed can have disrupted estrous cycles. Vaginal and cervical stimulation can induce pseudopregnancy. Reports suggest mice are less susceptible to this effect nonetheless care should be taken to minimize the degree of stimulation in repeated analyses. Repeated swabs of the vaginal mucosa can cause damage if not performed gently, with proper restraint, and the correctly sized cotton swabs. This method is included but is not preferred over the noninvasive method above.

### Materials

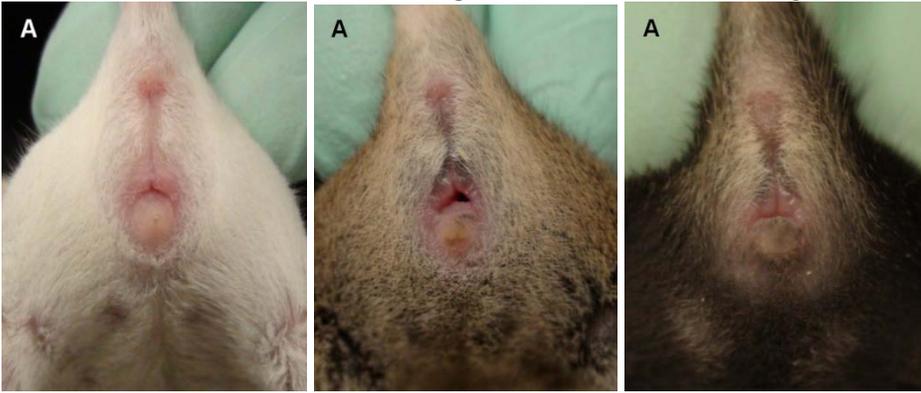
Cotton tipped applicators 2mm diameter tip	Clean glass microscope slides
Sterile saline	

1. Wet a cotton tipped applicator with ambient temperature physiological saline.
2. Insert the tip of the applicator into the vagina of the restrained mouse.
3. Gently turn and roll the tip against the vaginal wall and then removed.
4. Cells are transferred to a dry glass slide by rolling the swab across the slide.
5. The slide is stained according to the selected staining protocol.

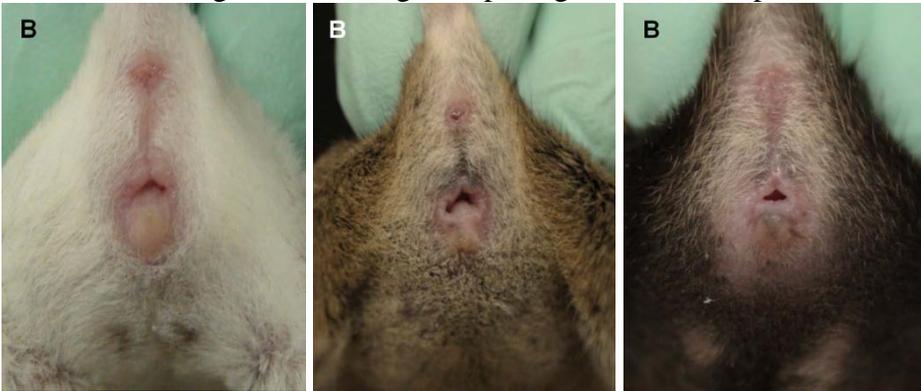
## Visual Determination of Estrous Cycle

The visual detection method is best to identify the stages of proestrus and estrus. It can be difficult to distinguish the other stages using this method alone. If all stages must be identified accurately, the vaginal cytology method is recommended. When evaluating the estrous cycle using the visual method, it is important to perform the visual inspection in the same area with respect to room lighting. The workstation should always face the same direction. The light source is also important to consider as it can change the perceived color of vaginal tissues and make evaluation difficult. Portable lights can be used; however LED lights should be avoided because they have a purple hue that makes visual detection more difficult. To evaluate the stage of the estrous cycle by visual observation, each mouse must be manually restrained by the tail with the forepaws resting on a cage lid. The vaginal opening of each female is evaluated based on the following criteria:

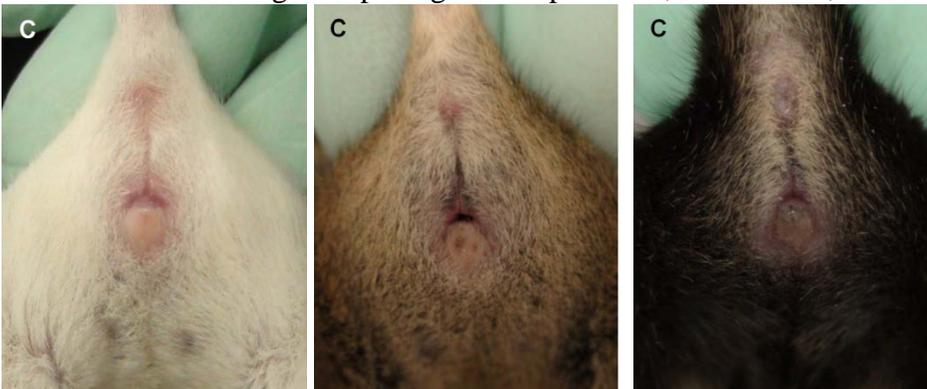
1. Proestrus - The vaginal opening is characterized by swollen, moist, pink tissue. The opening is wide and there are often wrinkles or striations along the dorsal and ventral edges.



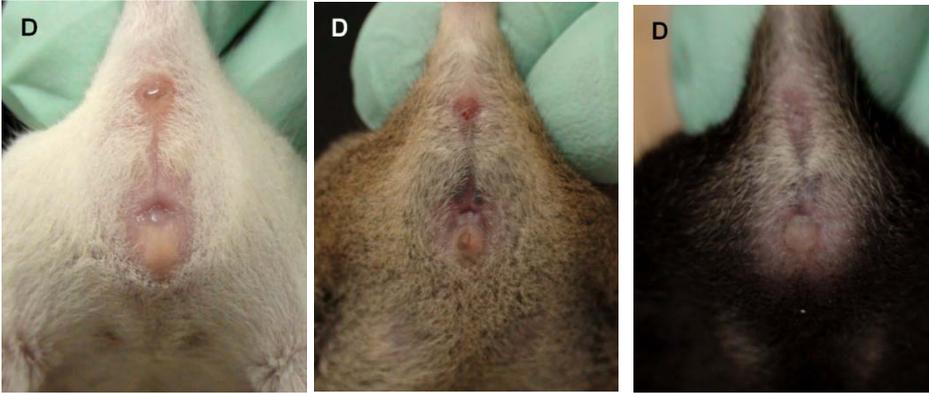
2. Estrus - During estrus the vaginal opening becomes less pink, less moist, and less swollen.



3. Metestrus - The vaginal opening is not open wide, not swollen, and white cellular debris may be visible.



4. Diestrus - In diestrus, the vaginal opening is small and closed with no tissue swelling.



## References

1. Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification, Ashleigh C. McLean, Nicolas Valenzuela, Stephen Fai, Steffany A.L. Bennett, *J. Vis. Exp.* (67), e4389, doi:10.3791/4389 (2012).
2. Mouse Estrous Cycle Identification Tool and Images, Shannon L. Byers, Michael V. Wiles, Sadie L. Dunn, Robert A. Taft, Published: April 13, 2012 DOI: 10.1371/journal.pone.0035538

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