**Procedure Type**: Breeding and Genotyping
**Procedure Title**: Mouse Breeding or Rat Breeding
Under Breeding Procedure Part II Tab

**Background:**
The following are noninvasive genotyping methods that may be included in the rodent breeding procedures in eProtocol. Techniques will comply with ACUC Guidelines for "Antemortem Tissue Collection for Genotyping". Please update the pain/distress category of the procedure to reflect any genotyping (category D if tail clipping rodents over 21 days of age using anesthesia, category C if using other genotyping methods)

**Genotyping**

□ Ear notch (Note: should not be performed before 2 weeks of age)

i. Manually restrain the mouse, allowing access to the ears.

ii. Using a special ear punch tool that has been chemically disinfected, place the ear puncher on the appropriate position on the pinnae of the ear and engage the punch quickly and firmly to ensure a clean cut. The punch will yield a sample of tissue approximately 1-2 mm in diameter.

iii. Place tissue sample into specimen vial.

iv. Return the mouse to its cage. Be sure to remove any blood from the ears of pups if any, before returning to the dam to discourage cannibalism.

v. Note: Disinfect punch between animals.

□ Tail clip

i. If tail clip is performed on mice over 21 days of age, anesthesia is required and must be described in the Anesthetic Regimen Tab

ii. Manually restrain the mouse.

iii. Moisten site with alcohol.

iv. Using autoclaved, chemically disinfected, or glass bead sterilized scissors or blade, make a transverse cut approximately 2-4 mm from the distal tip of the tail.

v. Place tissue sample into specimen vial.

vi. Apply pressure to the tip of the tail with a sterile gauze pad until bleeding has stopped. Styptic powder or tissue adhesive can also be used to aid hemostasis.

vii. Return pup to its cage once hemostasis has been achieved. It is very important to remove any blood from the paws before returning to dam to discourage cannibalism.

viii. Note: Disinfect scissors or blade between animals. Scissor blades should be sharpened and blades should be replaced regularly to minimize tissue trauma.

□ Toe clipping

i. Cleanse the foot with a dilute betadine solution or betadine swab.

ii. Using autoclaved, chemically disinfected, or glass bead sterilized scissors amputate the first, most distal bone and place in the specimen vial; amputating different digits will provide a unique identification method for each animal, as well as ample tissue for genotyping. Amputate the whole digit! Otherwise mice will regenerate from the last interphalangeal joint outward.

iii. Apply pressure to the paw with a sterile gauze pad until bleeding has stopped. Styptic powder or tissue adhesive can also be used to aid hemostasis.

iv. Return pup to its cage once hemostasis has been achieved. It is very important to remove any blood from the paws before returning to the dam to discourage cannibalism.

v. Note: Disinfect scissors between animals. Scissor blades should be sharpened regularly to minimize tissue trauma.

□ Blood sample

i. Manually restrain mouse.

ii. Moisten site with alcohol.

iii. Using a sterile blade or hypodermic needle, nick the tail vein on the ventral surface.

iv. Gently squeeze a drop of blood from the nick onto filter paper (e.g., Whatman GFrC). A spot greater than 2 mm in diameter provides sufficient DNA for one PCR run.

v. Return pup to its cage. Be sure to remove any blood from the tail before returning to the dam or rub with dirty bedding or nestlet to discourage cannibalism.

vi. Note: Disinfect blade between animals. Blades should be replaced regularly to minimize tissue trauma.

□ Other

i. Buccal Swab:

1. Manually restrain the mouse by the scruff, pulling the neck skin taunt and causing the mouth to open.

2. Swab the inner (buccal) surface of both of the mouse's cheek with a sterile 2mm diameter cotton-tipped swab.

3. Return the animal to its cage.

4. Break the cotton bud of the swab off into a specimen vial.

ii. Hair Collection:

1. Manually restrain mouse by the scruff, and turn over so that mouse is resting in your hand.

2.Using autoclaved, chemically disinfected, or glass bead sterilized forceps, gently pluck a tuft of hair from the ventral abdomen of the mouse and place into a specimen vial.

3. Return animal to its cage.

4.Note: To avoid cross-contamination, use a fresh pair of forceps per animal; otherwise, disinfect forceps between animals, being careful to remove all hair and debris.

References

Ren S., et al. 2001. A simplified method to prepare PCR template DNA for screening of transgenic and knockout mice. Contemp Top Lab Anim Sci 40:27-30.

Campbell D.B., Hess E.J. 1997. Rapid genotyping of mutant mice using dried blood spots for polymerase chain reaction (PCR) analysis. Brain Res Protoc 1:117-123.

Meldgaard M., Bollen P.J., Finsen B. 2004. Noninvasive method of sampling and extraction of mouse DNA for PCR. Lab Anim 38:413-17.

Schmitteckert E.M., Prokop C.M., Hedrich H.J. 1999. DNA detection in hair of transgenic mice – a simple technique minimizing the distress on the animals. Lab Anim 33:385-89.

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