Preclinical Research Center
Ben-Gurion University of the Negev

STANDARD OPERATING PROCEDURE 703
TISSUE COLLECTION FOR GENOTYPING MICE

1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe acceptable methods for collecting tissue samples for genotyping in mice.

2. RESPONSIBILITY

The principal investigator (PI) and their research staff.

3. MATERIALS

3.1. Sharp surgical scissors or sterile, disposable scalpel blades

3.2. Ear punch

3.3. Gauze

3.4. 70% alcohol

3.5. Sterile cotton-tipped swabs

3.6. Collection tubes, correctly identified

3.7. Tissue glue (Vetbond®)

3.8. Glass bead sterilizer

3.9. Anesthetics

3.10. Analgesics

4. CONSIDERATIONS

4.1. The least invasive tissue collection method should be selected, i.e., the process causing the slightest discomfort to the animals. Procedures are listed in this SOP according to the potential pain and distress for the animals, from the least invasive to the most invasive.

4.2. Since animals must be individually identified during tissue collection for genotyping, a method that provides a DNA sample simultaneously as it determines the animal, **e.g., ear punching, should be prioritized.** This minimizes the number of procedures carried out on the animals and hence minimizes pain and distress.

4.3. The tissue collection method selection should consider the animals' age at the time of tissue collection. Biopsies from young mice generally result in more significant amounts of pure DNA than those from adult mice.

5. PROCEDURES

5.1. **Fecal pellet**:

5.1.1. Stools contain sloughed intestinal epithelial cells, which provide a reliable source of DNA for genotyping.

5.1.2. Fresh fecal pellets must be used; genotyping should be performed within 24 hours of collection. More than one fecal pellet per animal is usually required.

5.1.3. Collect fecal pellets from an individual animal using temporary manual restraint or place individual animals in a clean cage without bedding. Care must be taken to prevent cross-contamination during the sample collection process.

5.1.4. Place the fecal pellet in an identified collection tube.

5.2. **Skin swabbing**:

5.2.1. The DNA isolated from skin swabbing can be minimal and difficult to measure by conventional methods.

5.2.2. Care must be taken to prevent cross-contamination during the sampling process.

5.2.3. Restrain the animal.

5.2.4. Using a sterile cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth. Perform a minimum of 3 strokes of 3 cm in length each.

5.2.5. Insert cotton bud into the collection tube and snip off the excess shaft.

5.3. **Buccal epithelial cell**:

5.3.1. Firmly restrain the animal by the scruff to maintain its mouth open.

5.3.2. Using a cotton-tipped swab with a <2mm bud, vigorously rub the inner cheeks while rotating the swab, avoiding the tongue.

5.3.3. Insert cotton bud into the collection tube and snip off the excess shaft.

5.4. **Whole blood**:

5.4.1. Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.

5.5. **Ear punching**:

5.5.1. Do not use this method in rodents under two weeks, as the pinna needs to be fully developed.

5.5.2. A 2 mm ear punch is recommended as this will yield sufficient DNA and ensure the identification is not lost after healing.

5.5.3. Ensure the ear punch apparatus is not dull.

5.5.4. Disinfect the ear punch with 70% alcohol and wipe dry.

5.5.5. Restrain the animal securely with the scruff.

5.5.6. Using the ear punch, punch holes and notches in the ears, following an identification chart.

5.5.7. Use the excised tissue as a sample for genotyping. Place in a well-identified collection tube

5.5.8. Disinfect ear punch between mice.

5.6. **Tail biopsy**:

5.6.1. The tail biopsy is considered invasive since nerves, bones/cartilage, connective tissue, ligaments, and skin are severed.

5.6.2. The tail biopsy is ideally performed on mice before 17 days of age to avoid transection of distal

Mature vertebrae. When collected before 17 days of age, the tail biopsy sample will be less ossified, providing better quality DNA and higher DNA yield.

5.6.3. Minimize the amount of tissue removed. The tail biopsy sample cannot exceed 5mm.

5.6.4. **A tail biopsy should only be performed once over the animal's lifetime**.

5.6.5. Identify animals as per Rodent Identification SOP.

5.6.6. Tail biopsy procedure for mice less than 21 days of age:

5.6.6.1. General anesthesia is recommended but not required.

5.6.6.2. Gently but securely restrain the mouse.

5.6.6.3. Snip 2 mm off the tip of the tail with sharp, sanitized scissors or a disposable scalpel.

5.6.6.4. Remove biological material and sanitize the scissors or scalpel after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you snip several mice tails.

5.6.6.5. Place the tissue sample into an identified collection tube.

5.6.6.6. Check for bleeding before returning the mouse to its cage. If bleeding occurs, apply a drop of tissue glue to the tip of the tail.