Purpose of Policy: The purpose of this policy is to establish standards for genotyping rodents.

Policy Information: Proper genetic identification of genetically engineered animals is essential to facilitate research yet comply with the 3Rs in reducing the numbers of animals used in research. Although all of the following methods are acceptable, investigators should use the least invasive method when possible and consider them in the following order.

**Fecal pellets** are a non-invasive means of obtaining sufficient amounts of DNA for genotyping. The procedure does not require anesthesia and can be used on rodents of any age. Fresh stool pellets may be obtained directly from the animal or from its cage provided it is singly housed.


**Rectal swabs** are a minimally invasive methods that can be performed on animals of any age. Anesthesia is not required.


**Buccal swabs** are a minimally invasive method that can be used on rodents of any age. Anesthesia is not required.

**Hair bulbs** may be obtained by using forceps to pluck a tuft of hair. It is minimally invasive and does not require anesthesia.


**Blood** - Obtaining blood is an invasive procedure that may require anesthesia depending on the method. Investigators are referred to the Institutional Animal Care and Use Committee’s policy on blood collection. However, a 2mm spot of dried blood on Whatman GF/C filter paper provides sufficient DNA for PCR.


**Tail biopsy** is invasive and requires anesthesia for animals over 3 weeks of age, preferably with a short acting gas anesthetic like isoflurane. However, it is recommended that animals be less than 3 weeks of age because the yield of DNA is highest. Moreover, the tail ossifies between 2-4 weeks of age and ossified tissue yields less DNA per gram of tissue.

A maximum of 5 mm of tissue may be removed as larger samples do not yield proportionally larger amounts of DNA due to the presence of cartilage and bone that are not as rich in DNA as the distal end. Animals must be monitored to ensure effective hemostasis which can be accomplished either with pressure, cautery or silver nitrate. However, electrocautery requires anesthesia. Resampling is not permitted without Institutional Animal Care and Use Committee (IACUC) approval.

Regardless of age or species, sampling must be done with a sharp, sterile scalpel blade or scissors and the instruments must be cleaned between animals.

Investigators are cautioned that tail biopsies may affect the results of certain behavioral tests (e.g. tail flick assay and hot plate response) later in life and that this should be taken into consideration when choosing this method.


**Ear punches/notches** are invasive procedures and for rats require anesthesia for animals over 3 weeks of age. Anesthesia is not required for mice regardless of age. A 2 mm ear punch is recommended as a lesser amount of tissue may not be sufficient for genotyping. The procedure may be performed at any age provided that the ears have separated from the head. A sharp punch must be used on the pinna (flap of the ear). Anesthesia or analgesia is not required, except for rats over three weeks of age, but the animal must be securely restrained.

**Ear snipping** may also be done in mice without anesthesia but anesthesia is required for rats older than 3 weeks of age. A 2-3 mm slice of the ear pinna is made using sharp scissors and thus avoiding ear blood vessels

**Considerations:** Animal body temperature and heart rate can increase regardless of the method used.