

# Shock Center Protocol

Protocol: Tail Tip

Date: 2/21/17

Originator: J Neal

## Note:

Following is the SOP for Tail Tipping.

### 1. PURPOSE AND SCOPE

Describes the procedure for obtaining and submitting mouse tail tip samples for genotyping; applies to all Bar Harbor and Sacramento animal room personnel who collect samples for TGS, FML and GQC genotyping

### 2. MATERIALS

- 70% ethanol
- Associated documents:
  - Sacramento only: Materials for shipping samples, per
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- Cap Strips (8-strips), flat top (Eppendorf catalog #0030-124-847, Lawson Item #6926)
- Ear notcher (Fine Science Tools, 24214-02)
- Kimwipes®
- Masking or vinyl tape
- Micro-dissecting scissors and curved iris forceps
- Paper towels
- Plastic bags (8¼"×18" flat-fold "cull" bags or equivalent)
- Pre-barcoded fully-skirted "twin.tech" 96-well PCR plates (Eppendorf catalog number #0030-128-702, Lawson Item #7166)

### 3. DEFINITIONS AND ACRONYMS

- **Ear notch:** A small (1-2mm) punch used to produce a small hole or notch near the edge of the mouse's ear(s). Notches or holes correspond to a predetermined numbering code for individual animals, litters or strains.
- **FML:** Fine Mapping Lab
- **FS:** Foundation Stocks; selecting sibling breeding pairs and pedigreed inbred mice. They are used to produce additional breeding pairs and minimize generation number, thus limiting genetic drift. Foundation Stocks are held separately from other stock to ensure strict genetic and health controls. As part of our Genetic Quality Control program, every Foundation Stock animal is typed using single nucleotide polymorphic (SNP) markers.
- **GQC:** Genetic Quality Control; the program that detects and, if necessary, eliminates potential strain background contaminations in JAX<sup>®</sup> mouse colonies.
- **PED:** Production Stocks; sibling breeding pairs and trios.
- **PES:** Pedigreed Expansion Stock; originate from Foundation Stocks and are propagated to generate sufficient numbers of mice to supply breeders to the production colonies. The number of generations arising from Pedigreed Expansion Stock is kept to a minimum.
- **POOL:** Pooled Production Stocks; non-sibling breeding pairs and trios. One generation of non-sibling mating is allowed for colony expansion. On rare occasions, a second generation of mating is permitted. Offspring from these stocks are distributed to researchers.
- **SNP:** Single Nucleotide Polymorphisms; base change differences at specific DNA loci in mouse genomes and are a tool for testing the genetic stability of a strain, for differentiating between strains, and for detecting contamination with another strain.
- **Tail tipping:** Excision of the tip of the tail, in this case the last 1-2mm of tail tissue (see [4.1](#)).

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- **TGS:** Transgenic Genotyping Service; the laboratory that genotypes individual alleles expected for specific JAX<sup>®</sup> mouse strains.

## 4. ANIMAL WELFARE KEY POINTS

- 4.1 Animal restraint and bio-sampling must be performed only by personnel who have been trained and approved by the LAHS animal use trainer or designee. Refer to [Section 3.1](#).
- 4.2 Tail tip samples must be no larger than 1-2mm. Tail tip each mouse no more than two times.

## 5. SAFETY KEY POINTS

- 5.1 Handle sharp scissors and forceps carefully to avoid cutting yourself.
- 5.2 Ethanol used to disinfect scissors and forceps is toxic if taken internally and flammable.
- 5.3 Restrain mice securely and appropriately to avoid and/or reduce mouse bites. A mouse that appears docile may still bite if given the opportunity.

## 6. QUALITY KEY POINTS

- 6.1 Ear notches (see [Section 3.1](#)) are the preferred bio-sample for all genotyping.
- 6.2 If ear notches cannot be taken, tail tip samples are acceptable. Tail tip samples must be no larger than 1-2mm. Only tail tip each mouse once. If a resample is required, use an ear notch if possible; if it is not possible, contact the scientific director of animal resources for guidance. Refer to [Section 3.1](#) for complete guidelines.
- 6.3 Disinfect the scissors and forceps with 70% ethanol between mice. Before taking the next tail tip, thoroughly blot the scissors and forceps with Kimwipes or a paper towel to remove excess ethanol.
- 6.4 Ethanol severely interferes with testing, therefore, if ethanol is used to sanitize scissors and forceps, blot the scissors and forceps between samples to remove excess ethanol.
- 6.5 Keep all samples at room temperature while awaiting transport; never place samples in a refrigerator or freezer in the work area. Keeping samples at room temperature eliminates the potentially damaging effects of chilling and warming samples.
- 6.6 GQC SNP typing, along with training of animal room personnel in good animal husbandry procedures, helps to ensure the genetic background of all Laboratory mouse strains sold to our customers and used by researchers. Genetic monitoring by SNPs is a key component in maintaining genetic purity.
- 6.7 Consistency in the collection, treatment, and handling of tail tips is important in providing reproducible results.
- 6.8 Send samples to the Transgenic Genotyping Laboratory or Genetic Quality Control the same day they are collected. Do not leave samples in the work area overnight.
- 6.9 Production strains are scheduled for GQC SNP typing for the entire year, usually by December of the preceding year, using the latest strain information available at that time, as follows:
  - 6.9.1 GQC SNP Type every new mating of Foundation Stocks. The number typed depends on the number of new matings.
  - 6.9.2 GQC SNP Type the remaining PES, PED, and POOL colonies twice per year, with six breeders being tail-tipped from each colony for SNPs.

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- 6.9 Repository strains are scheduled for GQC SNP typing on a weekly basis by the Repository. The number of mice being sampled is dependent on colony size. See table below for guidelines.

Colony Size (breeder pairs)	# of mice to sample
<b>1-5 pair</b>	4
<b>6-15 pair</b>	6
<b>16-25 pair</b>	8
<b>26-35 pair</b>	10
<b>36-45 pair</b>	12
<b>46-55 pair</b>	14

- 6.10 At any time, any number of animals can be GQC SNP typed if there is any indication of a genetic problem.
- 6.11 The preferred vessel for tissue collection are pre-bar-coded PCR plates covered with 8-strip strip caps.
- 6.12 In Bar Harbor, plates, strip caps, and instruments are available from the Lab Store.
- 6.13 Storage of tail tip samples:
- 6.13.1 Bar Harbor: Tail tip samples should be kept at room temperature at all times and sent up to the GQC Lab with the 10:00 am or 1:30pm transport pickup during the same day of sampling, or 10:00 am or 2:30pm for the TGS Lab. Plates must be clearly labeled "TGS" or "GQC". If the last pickup is missed for GQC or TGS, contact GQC or TGS and arrange to either walk samples up or coordinate pickup with the GQC or TGS staff.
- 6.13.2 Sacramento: Keep the plates at room temperature until they are ready to ship. Be sure to ship all plates during the same day of sampling at the end of every day. Ship samples per

## 7. PROCEDURE

### 7.1 Scheduling the GQC SNP sampling:

- 7.1.1 GQC: At the beginning of each year, email a schedule to each applicable Bar Harbor and Sacramento Production room regarding that room's scheduled sampling week, including a list of strains to be tail tipped and the number of mice (six or every breeder pair if an FS colony) to be sampled for each strain.
- 7.1.2 Guidelines and exceptions:
- 7.1.2-A Try to tail tip animals that are new matings.
- 7.1.2-B If any unscheduled strain or animal is in question, sample it.
- 7.1.2-C In rooms where make-up is done at the first of the month but the strain isn't scheduled for tail tipping until the end of the month, select new matings.
- 7.1.2-D If you believe tail tipping may compromise the health and welfare of the animal, please contact GQC so other arrangement can be made for typing. In known anemic strains such as CByJ, A-Tic7<fsn>J (JR#001723) and B6;129S4-F3<tm1Kaz>J (JR#004424), ear punch and send the collected sample(s) to GQC.

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7.1.2-E Do not collect a tail tip sample from any animal that has been tail tipped two times previously. If two tail tip samples have previously been collected and there is a request for another sample, contact GQC.

## 7.2 Collecting the samples:

7.2.1 Using green pre-barcoded plates, ensure proper plate orientation (e.g., well A1 is in the top left corner).

7.2.2 Cut the last 1-2mm of tail and place it into a well; refer to For reference, the thickness of a U.S. quarter equals 1.75mm. Be sure that hair is not counted into the 1-2 mm; it must be tissue.



↑ Side View  
(1.75 mm)

↔ Front View

7.2.2-A Submit each strain into a different column, leaving wells empty if necessary. Each strain corresponds to a different requisition in the TGS database (see ). The maximum number of samples that can be submitted is 92 samples (leave 4 wells blank).

7.2.2-B Sanitize scissors and forceps with ethanol between strains, then blot the scissors and forceps with Kimwipes or a paper towel to remove excess ethanol.

7.2.3 When all samples have been placed in the appropriate wells, apply a clear sealing film to the plate per .

7.2.4 Write the sex, date and tail tipped on the cage card

## 7.3 Sending the samples to GQC:

7.3.1 Sacramento:

7.3.1-A Pack the room temperature samples per

7.3.1-B Address the package to:

7.3.1-C Via email, notify the GQC, TGS or FML Labs that samples have been shipped by emailing or

7.3.2 Bar Harbor:

7.3.2-A Place the plates of samples in a plastic bag then secure the bag closed with masking tape or use a bow knot to tie it closed. Label the bag with tape and write either "GQC" or "TGS" on the tape to indicate where plate should be delivered.

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- 7.3.2-B Mop the MEEL with appropriate disinfectant (quaternary ammonia disinfectant), place a brown sheet of paper on the floor, and place the bagged plates onto the paper.
- 7.3.2-C Send the bagged plates of samples to GQC or TGS (depending on where testing will be performed) as soon as possible after sample collection (see [6.13](#)); call Transport for pick-up at ext. 1174).