Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the approved method to ear notch rats using the universal numbering system for identification.

1. Introduction and Definitions: A quick and relatively inexpensive way to identify rats is to create a small punch biopsy of the ear using an ear notch instrument. Ear biopsies can also be used as an alternative to harvesting tissue from the tail for genotyping purposes (see SOP 10.14 “Genotyping Rats”).

Using the universal numbering system described below, animals can be easily identified by anyone trained to read ear notching.

- Rats are usually biopsied between d15-17.
- The ear notch instrument must be cleaned between use, kept sharp and free of debris (tissue or rust).

2. Materials:
- Ear notch instrument
- Fine tipped forceps
- Iris scissors
- Sterile gauze
- 70% isopropyl alcohol or
- Hydrogen peroxide and peracetic acid solution (HP-PA) or
- Bead sterilizer

3. Procedures:
- The procedure is quick, easy, and should not cause bleeding if done properly. If bleeding does occur, gentle pressure with gauze should be applied until hemostasis occurs.
- This technique is best performed at 15-17 days of age but is humane and acceptable at all rodent ages without anesthesia or analgesia.
- Instruments used for ear notching can become dull after use and should be replaced often, as dull instruments can cause trauma to the notch site.
- Ensure the ear notch tool is clean of debris, and disinfected with alcohol or an HP-PA solution between each and every animal.
- Restrain animal using the V-hold technique (as per SOP 10.20 “Manual Restraint of Rats”).
- Place the device on the pinna of the ear (external ear) in the location wanted to mark the animal for identification.
- Press firmly to punch a circular hole through the ear (as indicated on the diagram below).
  - Numbers 1 – 9 are on the right ear
  - Multiples of 10 on the left ear
• As you remove the punch, be careful not to rip the delicate membrane of the pinna. If the punch does not remove all tissue, and you do not need it for genotyping, leave as is and the tissue will shrivel up on its own.
• Place the animal back in cage.
• Record animal’s information on its respective cage card.
• For full circle numeration, ensure the hole is punched ~2mm in from the edge of the ear. This will prevent the hole from tearing, and possible misidentification with a semi-circle.