

Mar 29, 2022

Collecting Spleens for genotyping rats

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dx.doi.org/10.17504/protocols.io.6qpvr665ovmk/v1

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This protocol describes how to dissect and collect soft tissue (spleen) from rats. Collected spleen tissue is intended to be used for DNA extraction and genotyping

DOI

dx.doi.org/10.17504/protocols.io.6qpvr665ovmk/v1

Gabriel J Barrero, Oksana Polesskaya, Abraham Palmer 2022. Collecting Spleens for genotyping rats. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.6qpvr665ovmk/v1>



 protocol ,

Feb 11, 2022

Mar 29, 2022

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1. pre-labeled  **2 mL** microcentrifuge tube (for example,

Microcentrifuge tube
Microcentrifuge tube
Fisher Scientific 05-408-138
cat #05-408-138

-
-). Use “barcode package” for instruction about labels.

 **Sodium Chloride Fisher**

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2. Saline: is  **0.9 % volume** **Scientific Catalog #S271**


in

distilled water

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3. pipette and tips to dispense saline.

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4.  **70 % volume** ethanol to spray dissection site.

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5. surgical instruments for dissection: scissors, forceps.

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6. Container with dry ice to store samples after dissection before transferring them to  **-80 °C**

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7. Labeled cardboard boxes for sample storage

Fisherbrand cryoboxes
Boxes
Fisherscientific 03-395-465

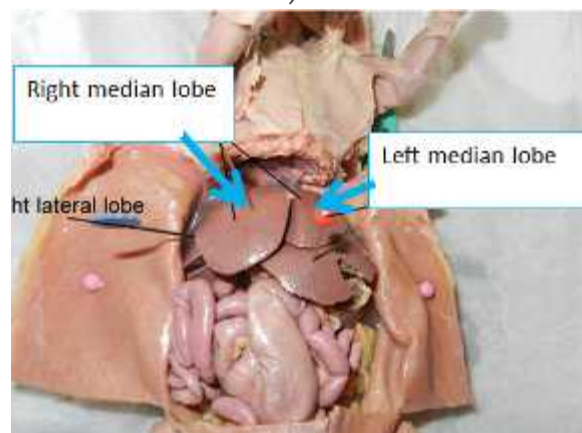
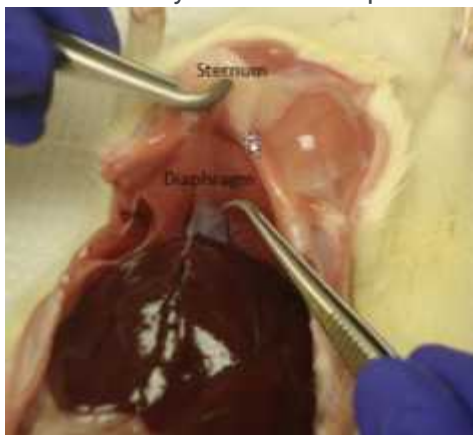
Materials

- 1 Rats are euthanized as per IACUC guidelines
Spray fur with ethanol. Pick up skin above the sternum, and make a cut through skin and muscle layers.
Don't cut the chest cavity (don't go above the diaphragm).



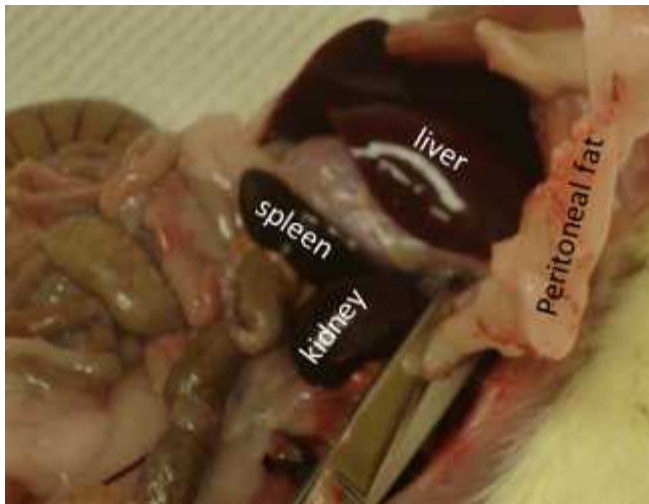
image shows isoflurane cone, but this is not needed for a dead rat)

- 2 Cut skin and muscles from the sternum down to the lower belly. Mostly you will see liver and colon. (Note: After some practice, you will be able to make the small precise cut on the left side of the belly to reach the spleen without extensive dissection)



Fresh Preparation Colors are vivid

- 3 Lift liver lobes and push aside fat tissue and intestines. You will see dark-colored organs – kidney and spleen.
The kidney is bean-shaped. Spleen is elongated. Pull-on the spleen gently to remove from the specimen, trim off fat



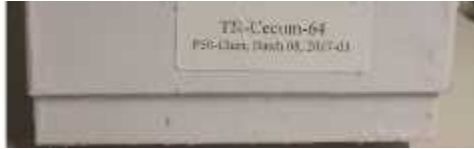
- 4 Put spleen into a pre-labeled **2 mL** microcentrifuge tube (for example, Fisher Scientific cat#05-408-138). Add approximately **0.5 mL** saline solution in the tube with the spleen to prevent dry freezing of tissue, but make sure that tube is not over-filled. If your rats have been implanted with an RFID chip, try to find this chip during dissection and put it directly into the tube with the spleen. We will read both the barcode and RFID chip to confirm sample identity. For labeling use "Cryo Babies" for laser printers (Diversified Biotech cat# LCRY-1700), they withstand long-term storage in **-80 °C** without peeling off the tubes.



- 5 Cut off ~ 5 mm of the tail tip and store it as a backup for genotyping at your lab. Use **2 mL** tube labeled with the barcode and rat ID, add saline to prevent sample desiccation. Store at **-80 °C** long-term

- 6 Store samples in cardboard boxes clearly labeled with the investigator's name, project, tissue,

box number (if out of many), and date. Example: JHOU-HSratsU01-Spleen-Box02_25Feb2019



7 Shipping spleens

- 7.1 Use at least 10 kg (20 lb) dry ice for shipping. If you have a choice, use pellets. If you use slabs, make sure that there is no free space in the shipping box, so slabs cannot move freely and crash things.
- 7.2 For shipping use Styrofoam box that is surrounded by a cardboard box. Choose boxes with thick walls. For example, ULINE S-13392 or ULINE S-11357.
- 7.3 Send by FedEx overnight on Monday or Tuesday, check for holidays. It is best if you send an email to the lab beforehand so they are ready to receive it.
- 7.4 Send a list of samples that you are shipping (RFIDs), we will use it to update the database.