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Mouse Organ Collection (Brain, Bone, Colon, Liver, and Mammary) V.2

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Andrew Davis: Wrote collected protocol. Source for the Brain isolation;
Aaron Havas: Source for Liver isolation;
Sha Li: Source for Bone marrow isolation;
Laurence Haddadin: Source for the colon isolation;
Diana Jurk: PI of the Jurk Lab. Provided direction and improvements on bone marrow collection protocol.
Kenneth Kim: Ken is the director of the histopathology core at LJI. He provided valuable insights and direction on sample preparation for downstream histology, and pointers on the colon collection protocol.
Peter Adams: PI of Adams Lab and NIH grant awardee

Adams Lab

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We use this protocol and it's working

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Abstract

This is a mouse dissection protocol intended to collect the 5 organs outlined in the SBPMDI TMC: Brain, Bone Marrow, Colon, Liver, and Mammary. Liver is collected as whole liver as opposed to hepatocytes or perfused liver. Brain is collected alternatively as hippocampus or a whole hemisphere for OCT or FFPE. Bone marrow is collected as viable cells frozen down in freezing media.

Materials

A	B	C
Name	Catalog number	Source
t-pins	87T	Avantus
iris scissors	501758	World Precision Instruments
forceps	ean code: 0860002672311	amazon In kit purchased on
rounded cotton tipped swabs or metal bar	ean code: 0860002672311	amazon In kit purchased on
aluminum dissection tray	629010	Carolina Biological Supply
wax pad	DTW-5	Braintree Scientific
LN2 Dewar + LN2	116704B	Fisher
bone scissors	ean code: 0860002672311	amazon In kit purchased on
OCT molds	25608-922	Sakura
OCT	4583	Sakura
1.7ml tubes	24-282	Genesee Scientific
RNase-free Ethanol	3916EA	Decon Labs
Paraformaldehyde	P6148-500G	Sigma-Aldrich

	A	B	C
	RNase-free water	BP28191	Fisher Scientific
	Refrigerated centrifuge	5424R	Eppendorf
	Cell strainers (70µm)	22363548	Fisher
	FBS	35-016-CV	VWR
	Mortar and Pestle(s)	B00FGDN8Y6	Amazon
	Metal block	88-860-105	Fisher
	Ice bucket with metal plate for brain	07-210-123	Fisher
	RNase-Away or similar	21402178 (CS)	Fisher
	RBC lysis buffer	555899	BD biosciences
	Histology Cassettes	18000-130	VWR
	Paraformaldehyde (PFA)	P6148-500G	Sigma Aldrich
	Paraformaldehyde (pre-made, optional)	50980494	Fisher Scientific (manufacturer is Electron Microscopy Sciences)

Safety warnings

Always use recommended PPE from your institutional safety department (lab coat, gloves, eye protection, masks, etc.). This can also help to prevent contamination of your samples.

Ethics statement

All experiments using mice should be first approved by the appropriate oversight organization of your locale.

Before start

Make sure to spray down tools and surfaces with RNase-away or similar to avoid RNA degradation of samples.

Pre-collection (at least 1 day before)

1 Re-melt and cool wax pad to ensure stable surface for pinning

2 Prepare labeled plastic cassettes for each PFA tissue and a larger container (250ml+) for depositing the closed cassettes once they have tissues in them. Prepare enough PFA to submerge all cassettes for the collection. Store the made PFA at 4°C, can be made up to a week before dissection date. In a standard 250ml beaker, 150ml should be enough to submerge at least 10 cassettes.

3 Prepare labeled 50ml conical tubes for bone marrow collection. You will also need a 70µm cell strainer for each of these 50ml conical tubes

4 Prepare 60ml of wash buffer for each sample for the bone collection. Store at 4°C overnight

5 Prepare 5ml of RBC lysis buffer for each sample for the bone collection. Can be stored at 4°C over night

6 Prepare 0.5ml tubes for bone centrifugation (1 for each mouse)

6.1 Use a clean pointy metal object (awl, drill, etc), bore a 1-1.5mm hole in the bottom of the 0.5ml tube.

6.2 Cut off the cap of the tube at the base of the tube so the lip is nearly perfectly round.

7 Prepare and label three 2ml freezing tubes for bone marrow collection for each mouse

8 Prepare and label 1.7ml tubes for tissue collection

8.1 Because we are only collecting mammary from female mice, the exact number will also depend on which mice are male or female. (e.g. a male mouse will need 10 labeled tubes, three for bone, three for liver, three for colon, and one for hippocampus, but none for mammary)

9 Prepare and label OCT cassettes for each tissue for each mouse. The exact number will depend on whether brain is collected as OCT/PFA.

Pre-collection (day of collection)

10 Prepare a 10cm dish with 5-10ml dPBS to rinse liver in after excision

11 Dry-ice buckets with pre-labeled freezer boxes for sample storage

11.1 Which go into a -80C freezer after collection is done

12 Liquid nitrogen dewars with Liquid nitrogen in them for flash freezing tubes

12.1 Also need a tool to get the tubes out, we bought a strainer advertised for hot-pot that has a handle canted at roughly 90° to the ladle

13 Ice buckets with ice in them

13.1 at least 1 for the bone team to store bones in 6-well plates in PBS on ice

13.2 at least 1 for the brain team to put a metal plate on to do hippocampus isolation on

13.3 We use metal tube-racks turned upside down for a flat metal surface in an ice bucket full of ice. This keeps the surface cold while we work on the brain. It occasionally needs to be wiped off to remove condensation, but cleaning the surface between brain isolations should be enough during the procedures.

13.4 at least 1 to keep the perfusion media cold

14 Label paper towels or small pieces of paper to stay with the mouse/dissection pad as it changes hands.

14.1 This makes it obvious which mouse is in the tray as it is handed around to different people and reduces the likelihood of mislabeling or forgetting which mouse is where.

15 Prepare weight boats labeled by mouse and by tissue to transfer tissues from the dissection try to the various teams. This is not a necessary step, but we have found it helpful to keep track of tissues during collections with many mice.

16 Spray workspace with RNase-away or similar

17 Wipe down tools with RNase-away or similar

Initial Dissection and Perfusion

18 Set a labeled paper towel or piece of paper down in the tray.

19 Pin euthanized mouse to wax pad with a t-pin through each paw, with the ventral side of the animal facing away from the wax pad. Try to stretch the paws away from each other during pinning to keep the skin taut.

20 Gently lift the head of the mouse and use bone scissors to cut the head off at the base of the shoulders. Secondary cuts along remaining skin and ligaments may be necessary to fully separate the head from the body. Give the head to the Brain Team.

21 Using forceps, pull skin and fur from anterior pelvic region upward away from the body and use scissors to make a ≈1cm cut along the Sagittal plane of the mouse.

22 Using forceps and scissors, expand the Sagittal cut all the way to the neck of the mouse.

23 At the inferior end of the Sagittal cut, make a cut along the transverse plane towards the mouse's right posterior paw (your left), and continue the cut to expose the mammary tissue.

24 Pull the skin taut and pin to the wax pad, exposing the mammary glands.

25 Repeat the last step on the mouse's left side (your right)

26 Using forceps and scissors, pull peritoneum from anterior pelvic region away from the body and use scissors to make a ≈1cm cut along the transverse plane.

27 Expand the cut on the peritoneum on both sides laterally and upward toward the ribcage. Pull the peritoneum flap up and away, exposing the abdominal organs.

28 Whole Body Perfusion

28.1 Make a small cut along the diaphragm to allow air into the chest, and continue the cut along the rib cage to expose the heart and lungs. This will help to avoid accidentally puncturing the heart or lungs which can cause problems for the perfusion later.

28.2 Make two cuts on the left and right ventral chest through the ribcage using bone scissors to better expose the heart.

28.3 Using a 25ga needle, and a 10ml syringe with 10ml of ice-cold dPBS, pierce into the left ventricle of the heart and gently squeeze the plunger to introduce some pressure

28.4 Make a cut in to the right auricle of the heart, which will allow blood and PBS to exit the circulatory system at the opposite end of the circulatory system as the perfusion input

28.5 Continue to squeeze the plunger to deliver the cold dPBS at 1ml/7s

29 Using forceps and scissors, follow the intestine down to the pelvis and cut the colon at the most distal you can reach without damaging the surrounding tissue.

30 Grip the freshly cut end with forceps and pull upward until you can clearly see the caecum. You will feel some pancreas tear away from the colon when you do this.

31 Make a second cut ≈0.5cm below the caecum, isolating the colon.

32 Give colon to Colon team

33 Follow liver Collection steps

34 Give carcass to Mammary Collection team for mammary removal

Colon Collection

35 Receive Colon from Dissection team

36 Clamp the proximal end of the colon with a set of locking forceps to anchor it, and lean the forceps up against the side of an ice bucket or mouse tray so that the colon hangs down from the locking forceps.

37 Using forceps and scissors, put one end of the scissors in the colon and cut along the sagittal plane of the colon, making a single flat sheet.

38 Gently remove any fecal matter still stuck to the now exposed inside of the colon

39 Make 3 cuts along the colon

39.1 From the proximal end (closest to the caecum), cut a 0.5-1cm length and set in an OCT mold into a dry ice + isopropanol bath. Orient the colon vertically in the OCT before adding to the dry ice + isopropanol bath.

39.2 From the proximal end, cut another 0.5-1cm length and set in a tube with 4% PFA for 24 hr, then into 70% EtOH

39.3 The rest (should be >2cm) should be cut into 2-3 pieces. One piece is set in a tube for RNA/Protein, the rest of the pieces should be spread out along the inside of a 2nd tube to allow for easy retrieval after freezing. Both tubes go into LN2 for flash-freezing.

Liver Collection

40 Using a cotton swab or other rounded implement, gently pull the intestinal tract to the side of the mouse, exposing the liver.

41 Using forceps and scissors, make a cut on the most proximal part of the L-lobe to separate it from the rest of the liver. (The L-lobe is the large, singular lobe on the mouse's left side (operator's right).)

42 Orienting the L-lobe from proximal to distal, make 2 cuts through the L-lobe, splitting it into 3 parts.

42.1 Most distal portion is cut into smaller pieces (≈3mm2). One piece is set in a tube for RNA/Protein, the rest of the pieces should be spread out along the inside of a 2nd tube to allow for easy retrieval after freezing. Both tubes go into LN2 for flash-freezing.

42.2 The middle portion is put in OCT and then dry-ice + isopropanol bath. Orient in OCT with one of the two cuts facing the bottom of the mold

42.3 Proximal portion is put in 4% PFA for H&E and RNAScope

43 Collect the other lobes in separate tubes to have more tissue for other analyses

Mammary Collection

44 Remove the lymph node from the mammary glands

45 Using forceps and scissors, pull on the exposed mammary glands and use the scissors to cut the mammary glands away from the skin.

46 Cut the (mouse's) left mammary gland into 2 even pieces

46.1 One piece goes in an OCT mold into a dry ice + isopropanol bath

46.2 One piece goes into a tube with 4% PFA for 24 hr, then into 70% EtOH

47 The (mouse's) right mammary gland should be cut up into smaller pieces (≈3mm2). One piece is set in a tube for RNA/Protein, the rest of the pieces should be spread out along the inside of a 2nd tube to allow for easy retreival after freezing. Both tubes go into LN2 for flash-freezing.

48 Give carcass to Bone collection team to remove the leg bones

Brain Collection

49 Receive the Head from the Dissection team, or follow Mouse Dissection protocol steps 1 and 2

50 Hold head in one hand and pull the skin away from the posterior of the head and towards the anterior of the head to reveal the skull; if necessary, make longitudinal cuts along the skin with scissors to make the skin easier to remove.

50.1 It is recommended to make two cuts under the skin where the ears meet the skull to separate the skin of the ear canal that connects to the skull. This will allow you to pull the skin further back and more easily expose the skull.

51 Once the skull is exposed, take small scissors and cut the skull following the superior midline of the brain so as to avoid damaging the brain tissue as much as possible.

52 The skull may fracture during this process, carefully remove any loose pieces and continue

53 Using fine forceps or your fingers, grip the cut edge of the skull nearest the cut and pull the pieces up until they fracture; remove the fractured pieces and repeat until the brain is exposed (you will need an open area roughly 1-1.5cm2 to get the brain out of the skull).

54 Using a spatula or tweezers: slip a tool underneath the brain between the brain and skull to sever the occular nerves connecting the brain to the eyes.

55 Use a spatula to gently pull the brain out of the skull. There may be remaining neural tissue connections to the spinal cord or eyes that you will need to sever.

56 Split brain along the sagittal plane using a razor blade or scalpel.

57 Continue to Hippocampus isolation or OCT/FFPE preparation

57.1 Options: Because there are 3 desired collection formats and only 2 halves of the brain, you may need to make decisions between taking more FFPE, OCT, or isolated hippocampus, or taking different formats from alternate mice, as suits your downstream plans. For example, for some experiments, we have taken isolated hippocampus from both hemispheres of every other mouse to ensure we have enough hippocampus tissue for snSeq.

Hippocampus isolation for snRNA-seq

58 Take the left half of the brain and use a scalpel or razor blade to remove the olfactory bulb

59 Make a cut along the midbrain to remove the cerebellum, pons and medulla.

60 Using curved forceps and/or small spatulas, gently remove the thalamus and hypothalamus from the cortex and hippocampus, exposing the hippocampus.

61 Use a spatula to isolate the hippocampus from the cortex and put in a tube and set in LN2

Brain for OCT/FFPE preparation (One or the other)

62 Choose to collect OCT or FFPE for each mouse. Ideally your n is large enough to have at least 3 for each.

Bone Marrow Collection

63 Receive carcass from Mammary Collection team

STEP CASE

Collect OCT 32 steps

Take the right half of the brain and set in an OCT mold and set in a dry ice + isopropanol bath

64 Remove legs at the pelvis

65 Using forceps and scissors, remove the bulk of tissue from the leg bones

66 Using gloved hands and a lint-free cloth, remove as much remaining muscle and ligaments as possible.

67 Cut the right femur in the middle and notch the proximal end to allow better diffusion of PFA into the tissue. Set in 4% PFA for 24hr, then into 70% EtOH.

68 Set remaining bones in pre-prepared wash buffer (2% FBS in PBS) on ice

68.1 Setup an ice bucket with a labeled 6-well plate in it with wash buffer in each of the wells ahead of time

69 Repeat until all bones from the day's experiment are collected

69.1 Bone marrow recovery from bones in ice cold PBS gives good yield for at least 5 hours after mouse death (longer intervals have not been tested, sooner is always preferred if possible)

70 After all of the bones are collected:

71 Using a scalpel, make cuts at the metaphyses of the remaining bones (1.5 femurs and 2 tibia)

71.1 This may require some sawing with a scalpel, and doing this on a wax pad has been the most effective for me. Making these cuts will turn the bones into tubes open at either end.

72 Add the bones to a 0.5ml tube with a pre-punched hole in the bottom

72.1 To help avoid more shear forces on the cells, I try to put the bones in with the larger hole facing downward toward the bottom of the tube. For the tibia, it is the proximal end; for the femurs, it usually is the same size on either end

73 insert the 0.5ml tube into a labeled 1.7ml tube

74 Weigh the 1.7ml tubes and make counterbalances if the weights are off from each other more than ≈20%

75 Spin the cells down at 3000xg for 1min at 4°C

76 Open the labeled 50 ml conicals and put 70µl cell strainers on them

77 Use a 1000µl pipet to add 1ml of wash buffer to the tube and pipet up and down to mix

78 Use a 1000µl pipet to take the resuspended cells from the 1.7ml tube, and run them through a cell strainer into a labeled 50ml conical tube.

79 Run 39ml of wash buffer through the top of the cell strainer to help collect any remaining cells in the strainer.

80 Remove strainers and put cap on 50ml conical tube

81 Spin cells down (All spins at 500 x g for 3 min a 4°C unless otherwise noted)

82 Decant supernatant without distrubing the pellet and then add 5ml RBC lysis buffer

83 Allow reaction to proceed for 4 minutes

84 Add 10ml of wash buffer to dilute the reaction and spin for 500xg for 3min at 4°C

85 Decant supernatant without disturbing the pellet

86 Add 10ml of wash buffer

87 Spin down (500xg, 3min, 4°C)

88 Decant supernatant without disturbing the pellet

89 Resuspend the pellet in 1ml freezing media (10% DMSO in FBS)

90 Distribute cells into 3x 2ml cryo tube (≈330µl each) in a slow-freeze container at -80°C