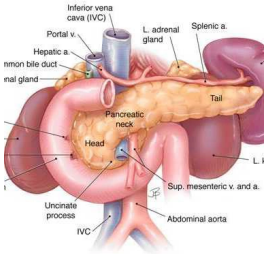
May 31, 2022

Human Pancreas Processing



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Protocol status: Working
We use this protocol and it's working

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Abstract

The pancreas is a combined endocrine and exocrine organ and is uniquely positioned in the upper abdomen between the stomach (posterior and superior margins), duodenum (proximal or medial), spleen (distal or lateral) and bowel (anterior and inferior). The normal pancreas is shaped as for a pistol with the proximal head region attached to the duodenum and comprising the handle while the distal head/neck, body and tail regions form the barrel. The fluid tone of the soft pancreas makes it difficult to maintain its *in vivo* 3D shape once trimmed of attached duodenum, spleen and peripancreatic soft tissues.

The purpose of this protocol is to describe pancreas collection and processing procedures suitable for single cell mapping. We describe the process of dividing the entire pancreas into sequential cross sections that are subsequently photographed, weighed and processed for the wide range of technologies used in HuBMAP and other programs. Steps describe processing samples to fixed samples for paraffin embedding, optical clearing, or fixed frozen blocks as well as unfixed samples for fresh frozen blocks. Methods are based on those established for the Network for Pancreatic Organ donors with Diabetes program as previously published with minor modifications.

Attachments

PDF
Jove Panc Collection...
383KB

PDF
nPOD_program_DMRR_20...
364KB

Materials

Autoclave wraps for trays, boards, and instruments.

PPE (gloves, plastic gown, eye wear, masks)
bsl2 hood

Plastic/metal trays for ice during dissection and for sample holding
Clipboards with printed copies of CaseWorkSheet and pens (2 sets)
Labeling tape and labels
Flexible plastic measuring tape
Plastic metric ruler
Knife, trimming 6", set of 10/box (FisherScientific 22-222-041)
Forceps
Scissors
#6 blade handle and #60 blades (FisherScientific 14-100-250)
ProCUT tissue forceps (Mopex AB079)
Small scale with plastic weigh boats
#5 Whatman filter paper, prelabel orientations and sequential slice numbers
Camera- iPHONE or other
Disposable dissection boards with 1cm grid (Electron Microscopy Sciences 63308-40)
LED lightbox 12" x 17" illumination area with plastic 1 cm grid (Rainbow Resource Center, 077522, 024156)

16% paraformaldehyde (Electron Microscopy Sciences, 15710S), stored room temperature
In fume hood or well ventilated space, make 4% paraformaldehyde (PF) fixative by diluting 16% PF stock and 10x PBS to final 4% PF in 1x PBS with autoclaved water, cool on ice or store 4 °C up to 24:00:00)
1xPBS (autoclave and store at 4 °C) in bottles and frozen as large ice cubes for sample processing
Sucrose (prepare 30% sucrose solution in 1xPBS and store 4 °C up to 1 month, FisherScientific BP220-1)
Nalgene containers (multiple sizes)
Rocker platform in cold room

Paraffin processing cassettes (FisherScientific **B851739WH**)
SupaMega and SupaMega Slim Cassettes (Electron Microscopy Sciences, 70065-W, 62510-W)
#2 pencils for labeling plastic cassettes

Insulated ice pan (FisherScientific **07-210-095**)
Dry ice (10lbs or more)
Isopentane (2-methylbutane), 4L glass bottle (FisherScientific O3551-4)
Carboxymethylcellulose (CMC, prepared per **dx.doi.org/10.17504/protocols.io.br4fm8tn**)
Optimal Cutting Temperature (OCT, FisherScientific 23-730-571)
Cryotray, 4 molds/tray, 25mm x 30mm x 20mmm, case of 50 (FisherScientific NC1877500)
Cryomold, Tissue-Tek, 25mm x 20mm x 5mm (FisherScientific NC9673117)

CryoMarker pens (Electron Microscopy Services, 62050) for labeling cryomolds
Tin foil and cardboard freezer boxes
-80 freezer

Biohazard disposal bags
Bleach (prepare fresh 10% solution on day of use) and 70% ethanol for disinfection and cleanup
Hazardous chemical waste containers for paraformaldehyde and isopentane

Safety warnings

Wear proper PPE for handing fresh human samples and follow biological safety procedures for potentially infectious materials.

Hazardous chemicals in this protocol include paraformaldehyde and isopentane. Work in well-ventilated area. Follow proper handling and disposal procedures.

Before start

Set up dissection area in bsl2 hood and 4 stations for sample weighing and photography, freezing, and fixation. Autoclave cutting boards, metal trays, and dissection instruments and supplies and arrange stations. Prepare several liters of 1xPBS, autoclave and refrigerate to cool. Freeze 1xPBS as ice blocks. Prepare 4% paraformaldehyde in 1xPBS as described in materials and cool on ice right before use.

Pancreas processing

1 Unpack the shipping container and verify the organ container is at an ice-cold receiving temperature. Open the organ container in a BSL2 biosafety cabinet. Verify contents include pancreas with attached fat, duodenum, and spleen. Record any deviations for shipping using the HuBMAP_Pancreas_CaseWorkShee...

1.1 Maintain cold conditions by placing the container on ice.

1.2 Inspect the pancreas and other organs for gross abnormalities and record any findings in the case worksheet. Abnormalities may include hemorrhage/hematoma, mass/cyst, calcifications (fat necrosis), edema and/or stiffness due to fibrosis.

2 Remove spleen and duodenum from the pancreas by blunt dissection using scissors and forceps. Remove peripancreatic tissues containing lymph nodes, fat, and fascia with major vessels. Remove any sutures or staples from the pancreas samples before processing. (Samples for fixation, treat as in steps 10.3-10.5; samples to be frozen, treat as in steps 12-14.)

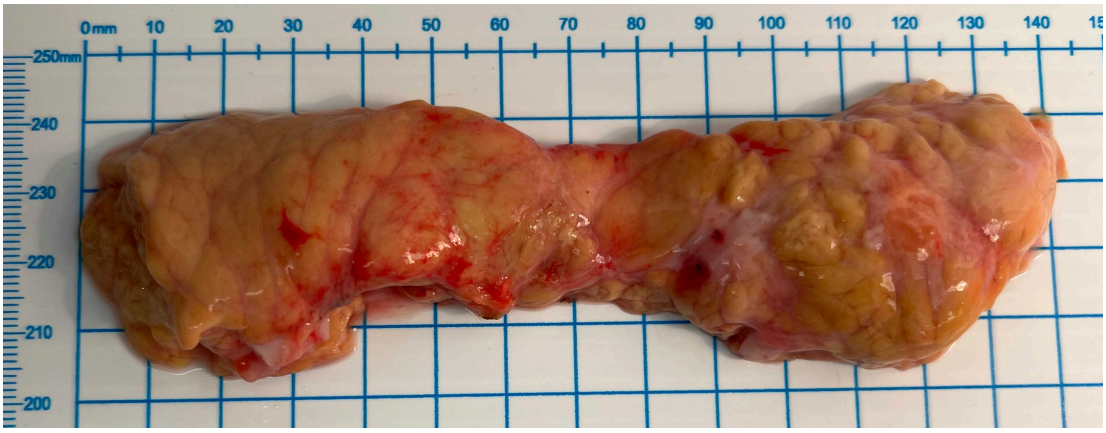
2.1 Collect regional pancreatic lymph nodes (PLN) and process them as fixed and frozen samples in pancreas cassettes or molds, respectively, or as individual samples, as needed.

2.2 Process 0.5 thick spleen samples by inclusion with pancreas cassettes and molds, or individually, as needed.

2.3 Samples from duodenum may be prepared as individual cassettes or molds, as for pancreas samples.

2.4 Place any tissues not to be saved into Nalgene container with fixative for disposal according to UF biological waste regulations. Inactivate the shipping media and/or PBS in contact with tissues with fresh 10% bleach for at least 30 minutes before disposal.

3 Photograph the pancreas in the anterior-posterior, superior-inferior, and head-tail views using 1cm grid.



Anterior view of a trimmed pancreas on grid dissection board.

3.1 Optional: Photograph the whole pancreas, pancreatic regions, and pancreatic slices using a LED light box with 1cm grid. 3D imaging of the pancreas may also be performed (LiDAR, iPhone).

4 Weigh the whole pancreas and record the maximum length and width of each region.

Processing pancreas regions

5 Transect the pancreas into the three major regions (head/neck, body, and tail).
The boundary for the junction between the neck and body is located along the groove from the superior mesenteric artery (SMA) and vein with transection medial to vessels or just distal to the inferior uncinate process.

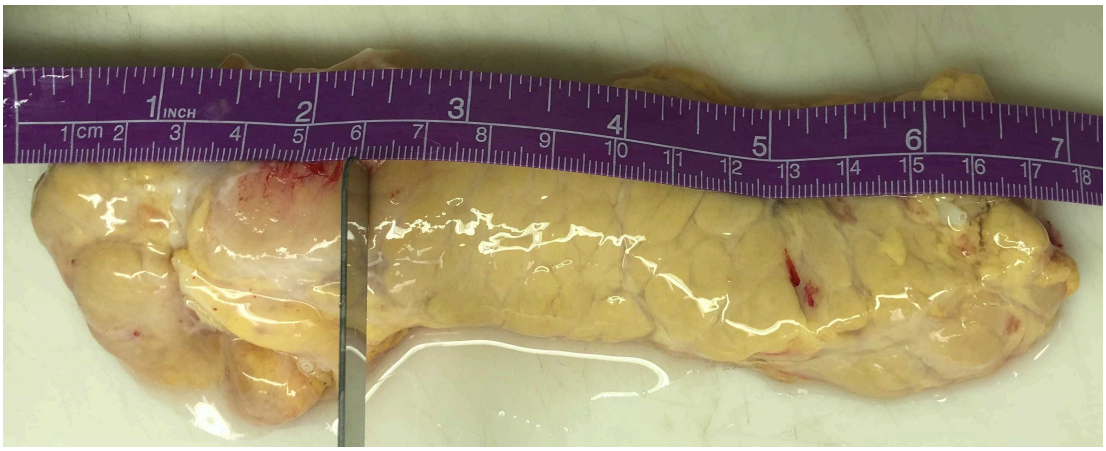


Image showing knife blade denoting the intended neck- body transection site.

Divide remaining pancreas sample in half for body and tail (distal half).

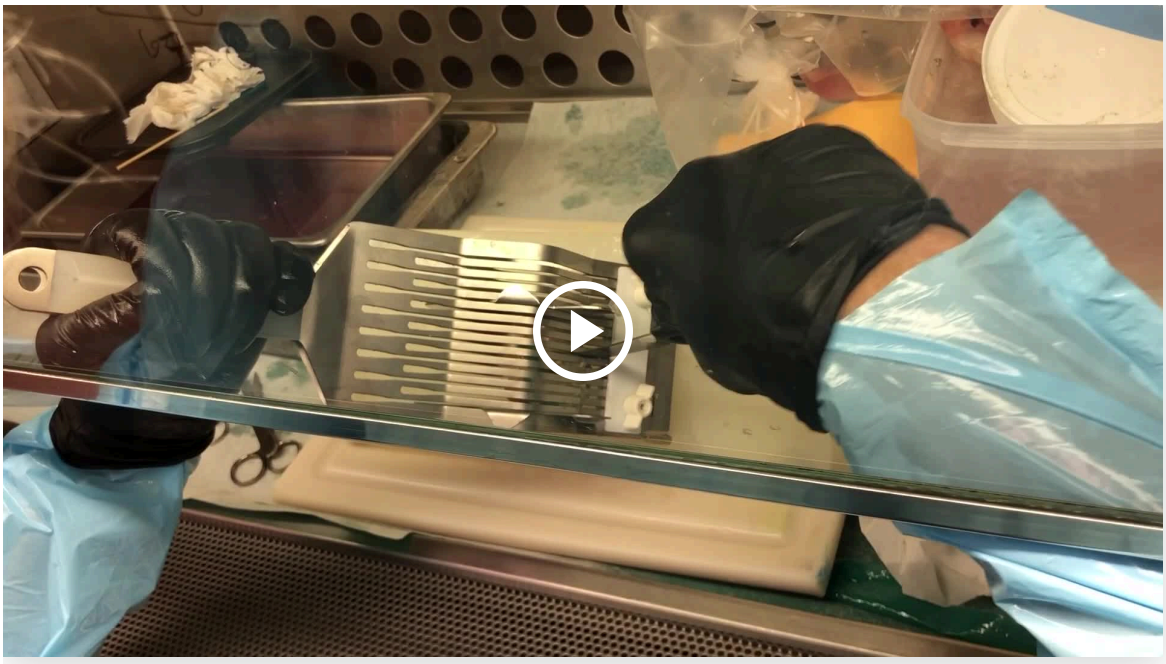
Safety information

Use caution handling knives or scalpel blades to avoid injury.

6 Photograph, weigh, and measure maximum length/widths of each region and record findings.

6.1 Return the body and tail samples to cold shipping media or PBS to hold for later dissection.

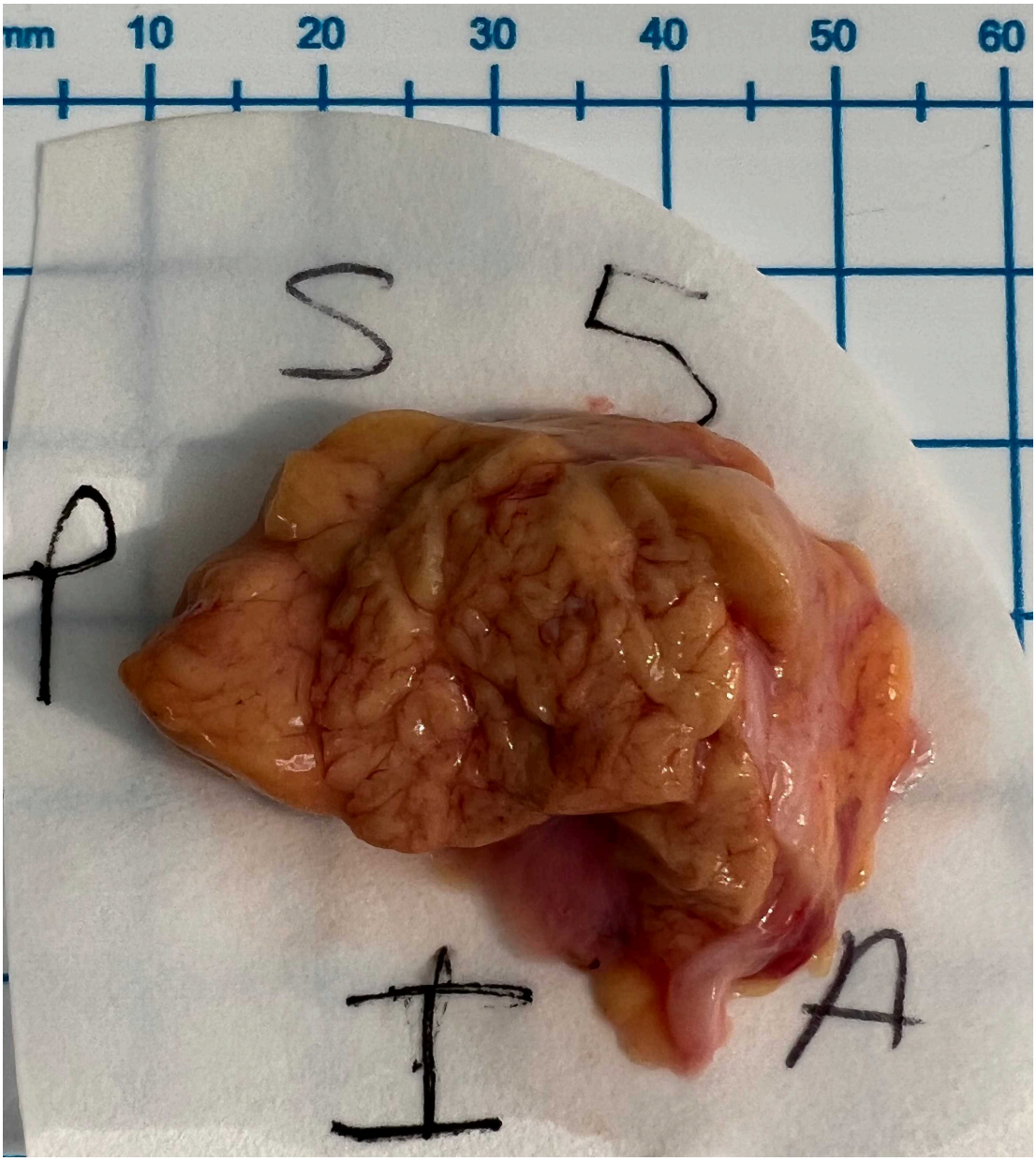
7 Place the subdivided pancreas into the opened ProCUT tissue forceps with the inferior surface toward the hinge and proximal pancreas towards operator. Partially close the forceps then place metal end plates into slots to secure the pancreas. Close the forceps and while holding closed, divide the pancreas into ~ 0.5 cm sections using long-bladed knives and a sawing action. Optional: After one pancreas cut, the knife can be left in the ProCUT forceps to aid in quickly positioning the next knife and to provide additional rigidity to the pancreas for cutting with a second knife (not shown).



Video shows slicing of pancreas tail region with the ProCUT forceps.

8 When the slicing is completed, open the forceps with the hinge to the right such that the pancreas continues to be viewed as if proximal to distal (medial to lateral). Remove the slices sequentially while maintaining the orientation between slices.

9 Transfer a slice to #5 Whatman filter paper, labeled with 4 orientations (A = anterior, P = posterior, S = superior, and I = inferior) and the slice number. Trim the filter paper as needed. Note: slices on filter paper can be held on ice until photographed and weighed before further processing.



Pancreas slice #5 on labeled filter paper and photographed with 1cm grid background.

Fixed paraffin blocks

10 Place the slices in regular or large format plastic cassettes, depending on sample size and intended use, for fixation before paraffin processing and embedding. Slices, including filter paper, can be transferred into the large format cassettes. Photographs of the cassette labels provides quality control for identity and orientation. Another trimmed filter paper can be placed on top of the tissue in the cassette to ensure smooth surfaces during fixation.

10.1 If a slice is needs subdivision, photograph the subdivisions on the filter paper and label starting with A for the superior/anterior quadrant or anterior pieces. Continue lettering in clockwise manner usually up to 4 subdivisions for the largest slices. Samples should not touch the sides of the cassette to prevent compression artefacts. Transfer subdivisions into cassettes.

10.2 Label the cassettes with a #2 pencil for HuBMAP pancreas ID (sequential number 1, 2, etc) followed by slice number with subdivision when used (e.g. P1-3A).

10.3 Place the cassettes into fresh cold 4% paraformaldehyde (PF) in 1xPBS in a 1 liter Nalgene plastic container. The ratio of sample to fixative should be at least 1:20 (vol/vol). The Nalgene container is kept on ice until all the cassettes have been added. Then, move the container to a rocker plate in a cold room to complete fixation.

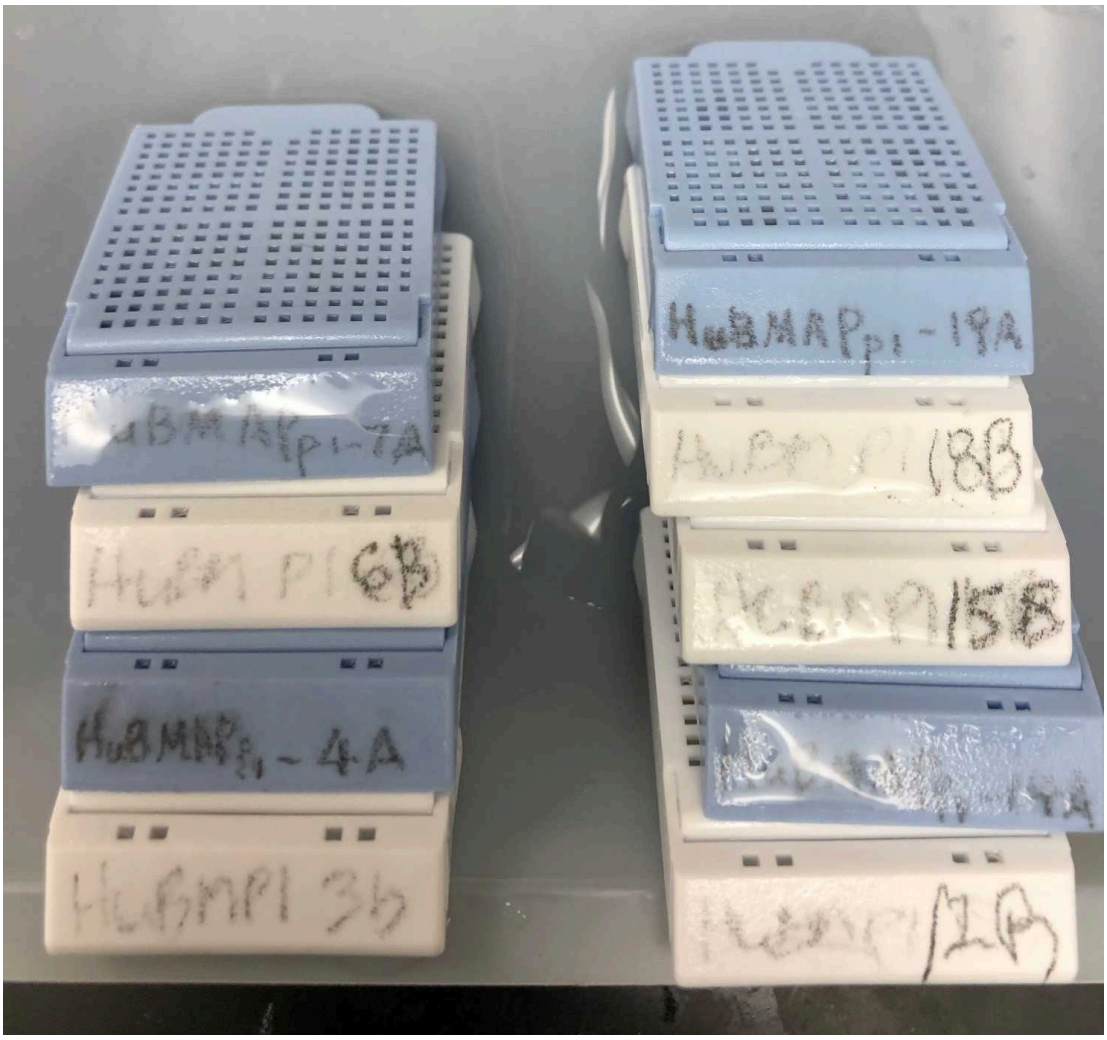
Safety information

Work in well ventilated area and keep fixative container closed when not in use. Avoid contact.

Fixation times are 24 to 48 hours depending on sample thickness and intended use. Document all sample start and finish times on CaseWorkSheet.

10.4 End the fixation by washing the samples with copious cold 1xPBS. Wash samples on a rocker plate in a cold room three times. The first two washes should be >1 hour, followed by a third wash overnight. Discard the first two PBS washes in PF waste container.

10.5 Store washed, fixed samples in 1x PBS at 4 °C prior to paraffin processing.



Regular cassettes following fixation and washing prior to paraffin embedding.

Fixed samples for optical clearing

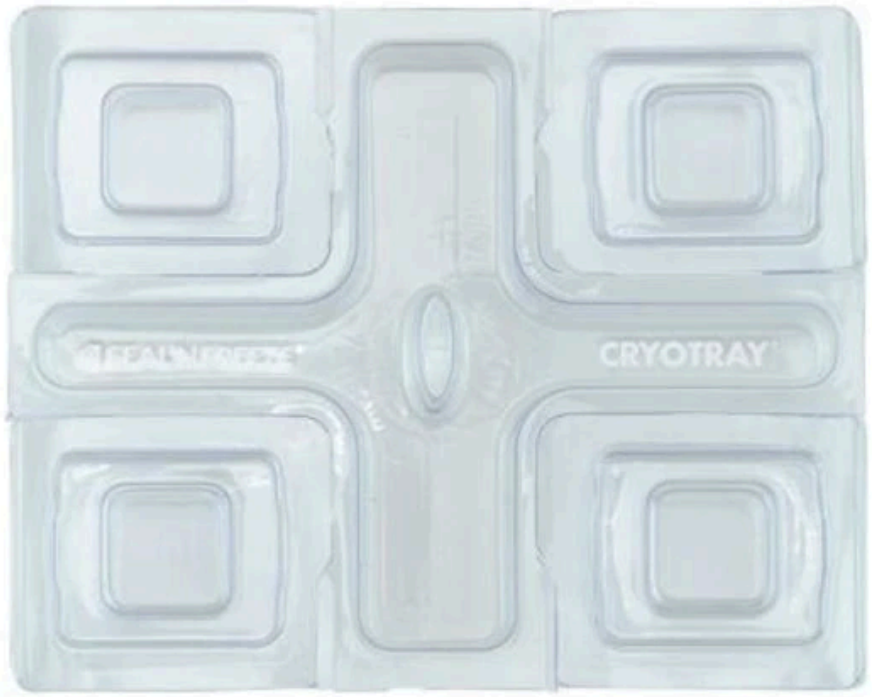
11 Place samples for optical clearing in cassettes and fix with 4% PF for 48 hours at 4 °C on a rocker then, wash as in section 10.

11.1 Stored fixed tissues for optical clearing in 1xPBS with 0.1% PF at 4 °C until use.

Fresh frozen block preparation

12 Samples to be embedded in CMC or OCT media should be rapidly frozen to avoid freezing artefact. Samples for fresh frozen blocks will be subdivided to avoid the sample

touching the walls of the Cryotray mold or regular cryomold. Cryotray molds are generally used for samples with 4 subdivisions.



KLAREX HEALTH LLC Seal' N Freeze Cryotray Large cryomolds (Fisherscientific NC1877500)

12.1 Prepare dry ice/isopentane slurry in an insulated tray or box with lid. Layer dry ice blocks across bottom of container, cover the dry ice with isopentane, and allow isopentane bath to cool ~ 00:10:00 until boiling stops. Close the container when not in use. 10m

Safety information

Work in well ventilated-area.

12.2 Label all cryomolds using a cryo-safe pen with the case, slice, and subdivision identifiers.
Place a small amount of CMC or OCT media in the bottom of each cryomold and pre-chill on regular wet ice.

12.3 Add the sample to the cryomold and gently push it flat to the bottom. Cover sample with additional CMC or OCT media while avoiding the introduction of any air bubbles.

12.4 Immediately freeze by placing the cryomold on top of a dry ice/isopentane bath.

13 After freezing for several minutes, close the Cryotray lid and wrap tray in labeled tin foil before placing in cardboard box on dry ice. Regular cryomolds are also wrapped in labeled tin foil.

13.1 Discard isopentane to chemical waste container after dry ice evaporates.

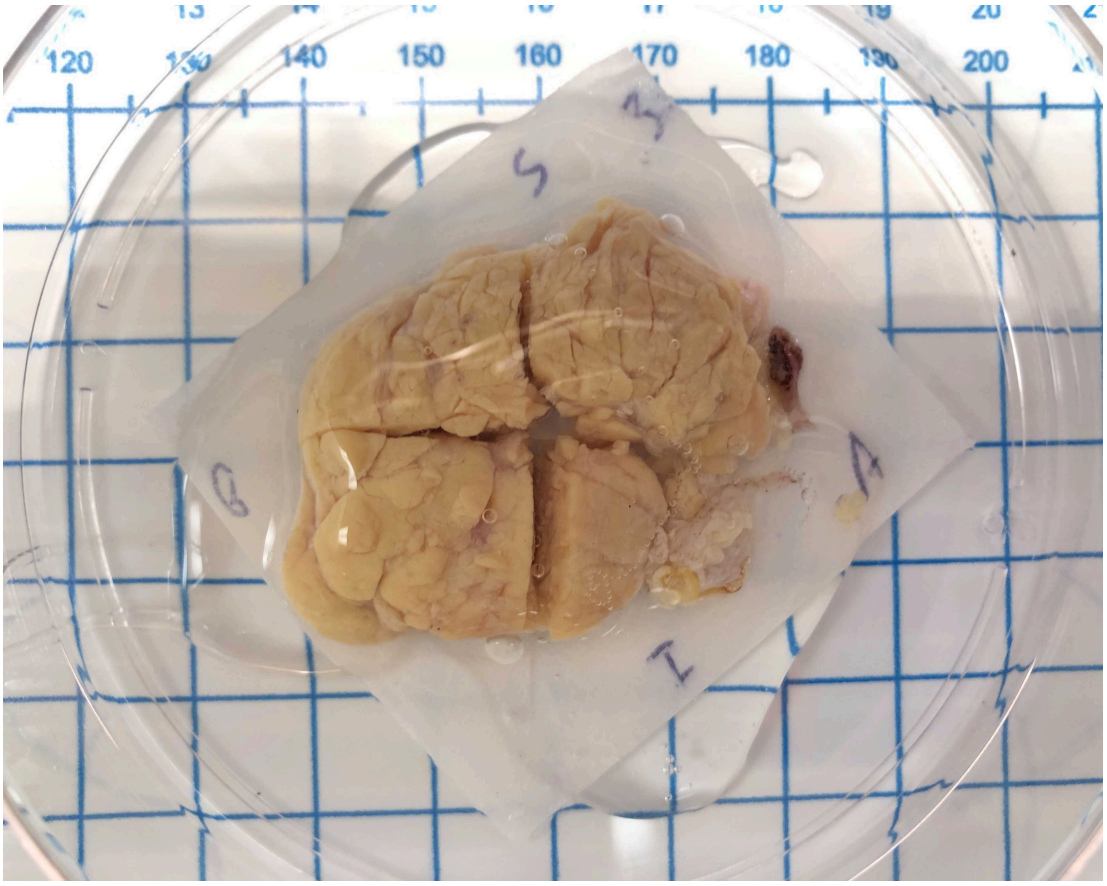
14 Seal frozen blocks in boxes that are sealed in plastic zip-lock bags at -80 °C until use.

Fixed frozen block preparation

15 Transfer washed, fixed samples to 30% sucrose for 48:00:00 at 4 °C to equilibrate. Samples may be in cassettes or in other containers. 2d

16 Optional: Transfer samples from 30% sucrose to 1:1 30% sucrose:OCT and equilibrate 24:00:00 at 4 °C followed by equilibration in 100% OCT for several hours to 16:00:00 at 4 °C. 1d 16h

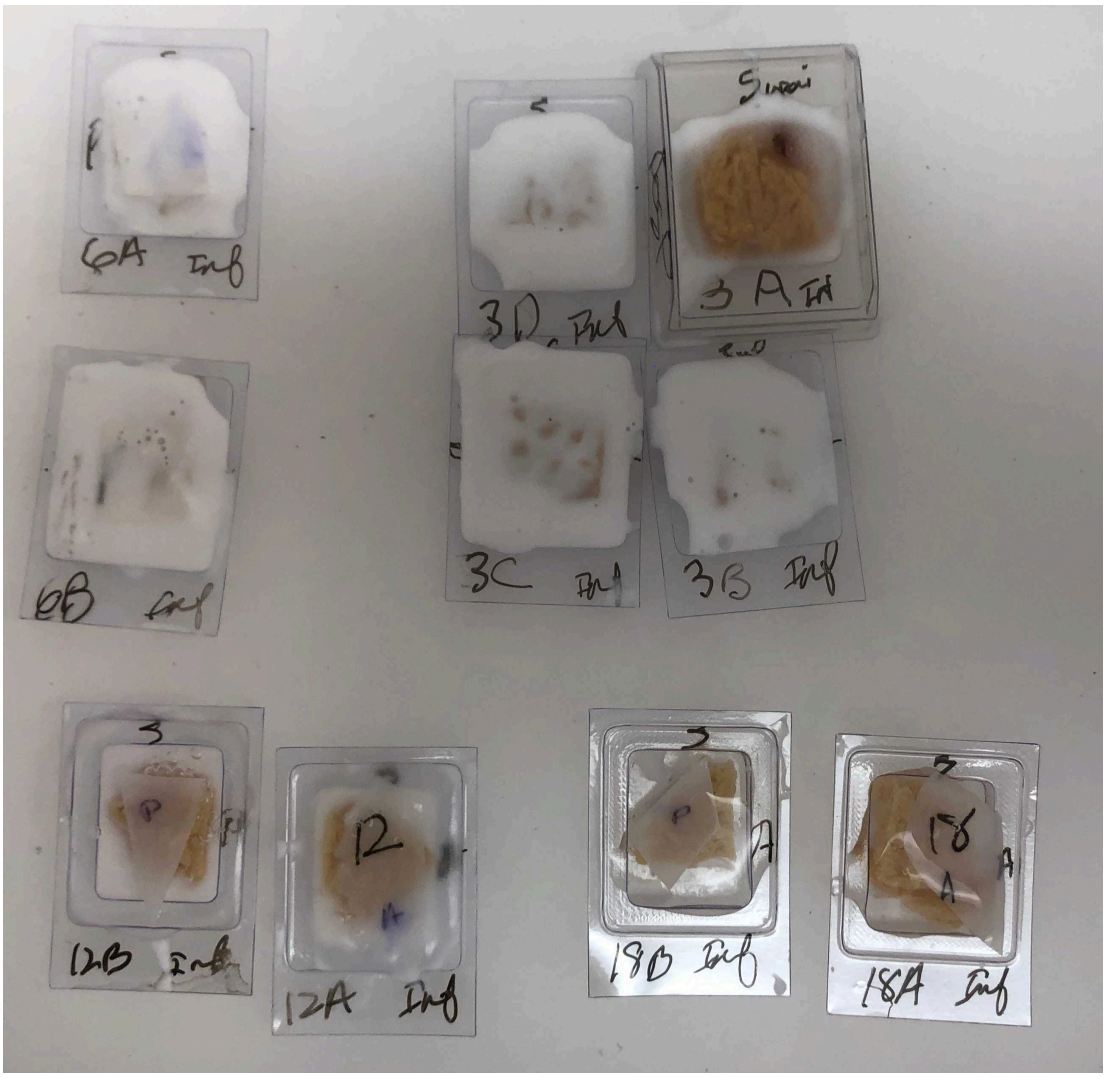
17 Transfer samples to a petri dish or weight boat and cover them with 100% OCT for 00:05:00 if not previously equilibrated in 100% OCT. 5m



Pancreas slice #3 photographed after subdividing into 4 samples. The samples on filter paper are transferred to a petri dish and covered with OCT media to equilibrate for several minutes. 4 subdivisions were labeled A-D (19 photo).

18 Place a small amount of OCT media in the bottom of a cryomold then add the sample, pushing sample flat against the bottom of the cryomold as for fresh frozen samples. Fill the cryomold with OCT media while avoiding introduction of any air bubbles.

19 Freeze samples using dry ice/isopentane bath as in 12.4.



Fixed frozen cryomold undergoing freezing.

20 Wrap the frozen blocks in labelled tin foil and place in a cardboard freezer box sealed with a zip-lock bag for storage at -80 °C until use.