Jul 13, 2021

Human Pancreas Processing for Multiple Applications

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dx.doi.org/10.17504/protocols.io.bwejpbcn

VU Biomolecular Multimodal Imaging Center
Human BioMolecular Atlas Program (HuBMAP) Method Development Community

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ABSTRACT

Procuring and preparing human pancreas for paraffin-embedding, fixed cryosections, flash frozen sections, and RNA isolation.

This protocol was developed in collaboration with Dr. Rita Bottino of Imagine Pharma and Allegheny Health Network and is based on protocols developed by Dr. Marcela Brissova of Vanderbilt University.

DOI

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PROTOCOL CITATION

Diane Saunders, Rita Bottino, Marcela Brissova, Angela Kruse, Jamie Allen, Carrie Romer, Danielle Gutierrez, Jeff Spraggins, Alvin Powers 2021. Human Pancreas Processing for Multiple Applications. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bwejpbcn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Cell Rep. 2018 Mar 6;22(10):2667-2676. doi: 10.1016/j.celrep.2018.02.032

KEYWORDS

HuBMAP, Vanderbilt, Pancreas, Paraffin-embedding, cryosections, Flash frozen sections, RNA isolation, BIOMIC

CREATED

Jul 07, 2021

LAST MODIFIED

Jul 13, 2021

PROTOCOL INTEGER ID

51371

MATERIALS TEXT

Materials

1. Belzer UW® Cold Storage Solution or Custodiol® HTK Solution
2. Dissection tray

3. Wet ice with container
4. Covidien #8044 All Purpose Sponge
5. Plastic weigh boat
6. Dissecting platform
7. Camera
8. Metric Ruler
9. Scalpel
10. Kimwipes
11. Marking dyes: Cancer Diagnostics, Inc. #MD2000- blue, black, green, red
12. 50 mL Falcon tubes Fisher
13. Adjustable Tilt Rocker
14. Cryomold VWR #25608-916
15. OCT Compound, VWR, #25608-930
16. Reusable storage bags
17. 15 mL Falcon tubes Fisher #05-527-90
18. 10-cm petri dish
19. Fine forceps
20. Storage box
21. Dry ice with lidded container
22. Metal weigh boats
23. Ice packs
24. Balance

Solutions

Human Pancreas Processing for Paraffin-embedding (PE):

- **100 mM PBS, 1L:** 12.07 g Na_2HPO_4 (dibasic), 2.04 g KH_2PO_4(monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. Prepare fresh and keep at 4ºC. Based on Electron Microscopy Sciences catalog, 100 mM PBS filtered through a 0.22 µm filter has a shelf life of 1 month at 4ºC.

- **16% Paraformaldehyde (PFA):** Electron Microscopy Sciences, 15710. Right before fixation, prepare 4% PFA solution. Open the vial containing 10 mL 16% PFA stock, transfer contents of vial into a 50-mL Falcon tube, add 30 mL 100 mM PBS, mix and place the tube on ice. *Note: Prepare as many tubes as many tissue slices will be procured considering approximately 1:50 tissue to fixative ratio.*

- **70% Ethanol, 1L:** Mix 700 mL 190 Proof ethanol with 300 mL dH_2O. *Note: Consider approximately 1:50 tissue to ethanol ratio.*

Human Pancreas Processing for Fixed Cryosections (PCMC):

- **1X PBS/10 mM PBS, 1L:**1.44 g Na_2HPO_4 (dibasic), 0.2 g KH_2PO_4(monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. *Note: Prepare from scratch or use 1X PBS without Ca^{2+}/Mg^{2+} (Invitrogen #14190-144).*

- **100 mM PBS, 1L:** 12.07 g Na_2HPO_4 (dibasic), 2.04 g KH_2PO_4(monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. Prepare fresh and keep at 4ºC. Based on Electron Microscopy Sciences catalog, 100 mM PBS filtered through a 0.22 µm filter has a shelf life of 1 month at 4ºC.

- **16% Paraformaldehyde (PFA):** Electron Microscopy Sciences, 15710. Right before fixation, prepare 4% PFA solution. Open the vial containing 10 mL of 16% PFA stock, transfer contents of vial into a 50-mL Falcon tube, add 30 mL 100 mM PBS, mix and place the tube on ice. *Note: Prepare as many tubes as many tissue slices will be procured considering approximately 1:50 tissue to fixative ratio.*

- **Sucrose:** Fisher Scientific, BP220-1. Prepare 30% sucrose solution: 15 g sucrose + 35 mL 10 mM PBS in a 50-mL Falcon tube. Place the tube on a rocker to dissolve sucrose and then keep at 4ºC. Alternatively, 30% sucrose can be prepared in a larger quantity, filtered through a 0.22 µm and stored at 4ºC for 1 month. *Note: Consider approximately 1:50 tissue to sucrose ratio.*

- **2.6% Carboxymethylcellulose (CMC)**: Fisher Scientific, C9481. Prepare 2.6% CMC solution ahead of time: add 500 mL water to 500 mL bottle and microwave for 2 minutes. Place bottle on heated stir plate (~70ºC), and slowly add 13g

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CMC to warmed water. Stir on low hotplate overnight, occasionally tightening lid and shaking bottle. Store at 4ºC for up to 2 months. Before each use, pour solution into 125 mL bottle and sonicate for 10 minutes (smaller bottle is easier to use with transfer pipettes).

Human Pancreas Processing for Flash Frozen Sections (FCMC, FF):

- **Isopentane slurry:** Place a layer of dry ice into a box and add enough isopentane to make a slurry.
- **2.6% Carboxymethylcellulose (CMC)**: Fisher Scientific, C9481. Prepare 2.6% CMC solution ahead of time: add 500 mL water to 500 mL bottle and microwave for 2 minutes. Place bottle on heated stir plate (~70ºC), and slowly add 13g CMC to warmed water. Stir on low hotplate overnight, occasionally tightening lid and shaking bottle. Store at 4ºC for up to 2 months. Before each use, pour solution into 125 mL bottle and sonicate for 10 minutes (smaller bottle is easier to use with transfer pipettes).
- Label **embedding molds** and add a small amount of CMC in each one (barely cover the bottom).
- Label **aluminum foil squares** and storage bags.

Human Pancreas Processing for RNA Isolation (RNA):

- **RNA*later*™ Stabilization Solution:** Fisher Scientific, AM7021. Aqueous, nontoxic tissue storage reagent that rapidly permeates tissue to stabilize and protect the integrity of RNA in unfrozen tissue samples.

SAFETY WARNINGS

Safety glasses, proper gloves, and a lab coat required. The area should be adequately vented.

Preparing Pancreatic Cross-Sections

1 Upon arrival, transfer the pancreas and transport solution (UW or HTK) into a dissection tray placed on ice. Transfer enough of the transport solution to keep the pancreas submerged.

2 Dissect the pancreas by carefully removing duodenum, mesentery, fat, and spleen.

If there are pieces of duodenum and/or spleen, please retain and freeze as tissue blocks *see Human Pancreas Processing for Flash Frozen Sections protocol below

3 Once the pancreas is cleaned, blot with a sterile sponge (Covidien #8044) and quickly take its weight by placing it into a clean plastic weigh boat.

4 Immediately transfer the pancreas on an ice-cooled dissecting platform and take a photograph with a ruler as shown in

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Figure 1.

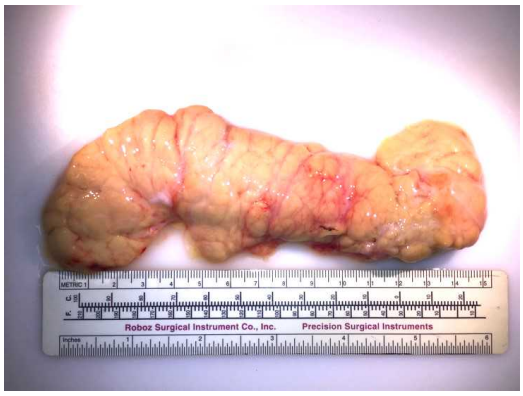
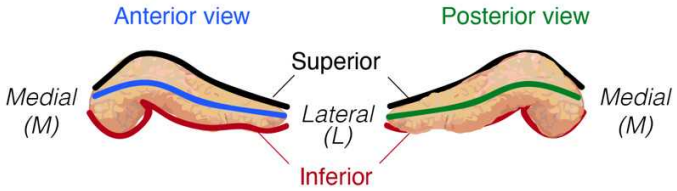


Figure 1. Example photograph of intact human pancreas.

5 Apply marking dyes (Cancer Diagnostics, Inc. #MD2000) to length of organ as follows:
o Anterior: blue
o Superior: black
o Posterior: green
o Inferior: red



Map of Pancreatic Slices

6 Once the pancreas has been marked, start collecting approximately 5-mm thick cross-sectional slices starting from the Head and moving laterally towards the Tail as outlined in **Figure 2**. Each cross-section should contain dye markings on the exterior to provide spatial orientation.

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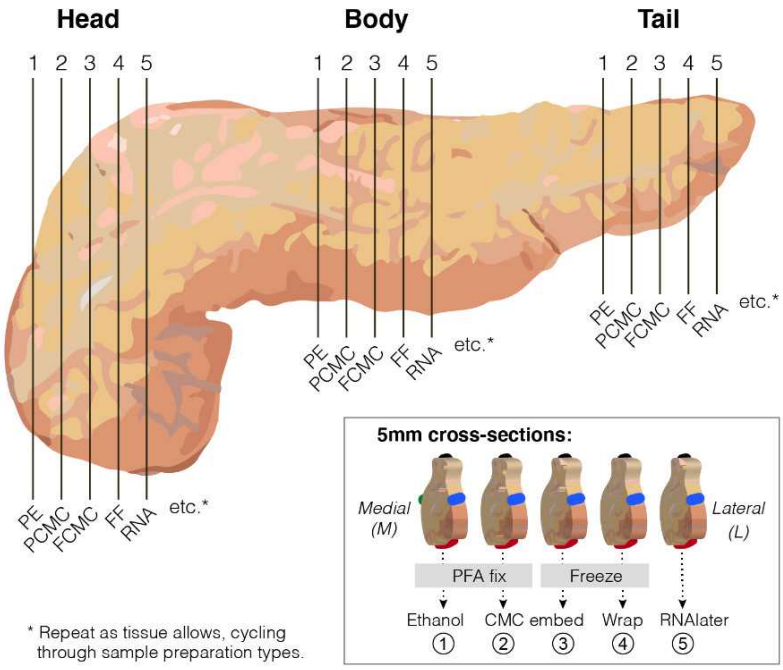


Figure 2. Map depicting serial collection of cross-sections for different modalities/applications

7 Label cross-sections following the naming nomenclature and controlled vocabularies developed for Vanderbilt LIMS, which keeps track of the position of each cross-section in a given anatomical location and processing procedure.

8 When dividing each tissue cross-section into quadrants, orient it medial side down and attempt to bisect dye markings so that each tissue piece contains 2 dye colors (**Figure 3**).

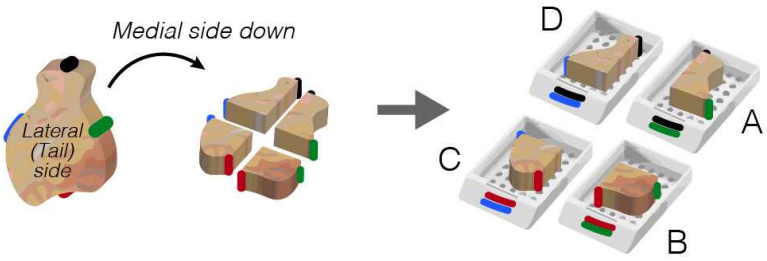
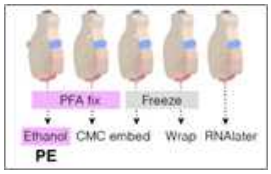


Figure 3. Schematic of tissue cross-section cleaved into four quadrants for fixation/processing

Human Pancreas Processing for Paraffin-embedding (PE) 18h

18h

9 

Cut each tissue cross-section into four quadrants (A, B, C, D; see **Figure 3**) and place all four tissue pieces into the

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same 50-mL Falcon tube containing **40 mL** of freshly prepared fixative (4% PFA/100 mM PBS). Fix **Overnight**, at least **18:00:00**, at 4ºC under mild agitation using an adjustable tilt rocker (LabNet).

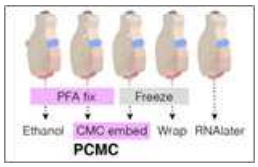
10 Wash tissue four times in **40 mL** of 100 mM PBS at 4ºC for a period of 2-3 hours, rocking. Blot the tube with paper towel before adding the fresh washing solution.

11 After the last wash, add **40 mL** of 70% ethanol and keep at 4ºC.

12 Ship tissue in sealed tubes on ice packs.

Human Pancreas Processing for Fixed Cryosections (PCMC) 3h

3h

13 

Cut each tissue cross-section into four quadrants (A, B, C, D; see **Figure 3**) and place all four tissue pieces into the same 50-mL Falcon tube containing **40 mL** of freshly prepared fixative (4% PFA/100 mM PBS). Fix for **03:00:00** on ice under mild agitation using an adjustable tilt rocker (LabNet).

14 Wash tissue four times in **40 mL** of 100 mM PBS on ice for a period of 2-3 hours, rocking. Blot the tube with paper towel before adding the fresh washing solution.

15 After the last wash, equilibrate the tissue in **40 mL** of 30% sucrose/10 mM PBS at 4ºC overnight. Tissue will settle to bottom of the tube.

If the pancreas is not cleaned sufficiently from fat, it will never drop to the bottom.

16 Prepare a pre-labeled cryomold (VWR #25608-916) and fill it halfway with CMC compound. Pour the pancreas with some sucrose into a 10-cm petri dish. Pick the pancreas with a pair of fine forceps and blot it with Kimwipes to remove an excess of sucrose. Place the tissue into the CMC-containing cryomold. Using forceps, push the tissue lightly to bottom of the cryomold, medial side down. Add more CMC to completely fill the cryomold.

17 Freeze the tissue on a dry ice block. As CMC starts freezing, push the tissue again lightly to bottom of the cryomold.

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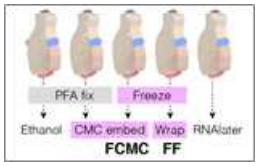
18 When CMC compound is frozen, wrap the cryomold containing the tissue in a pre-labeled aluminum foil, place it a storage bag, seal, and store in a storage box at -80ºC.

Aluminum foil and zip-lock bag prevent the block from drying.

19 Ship tissue blocks on dry ice.

Human Pancreas Processing for Flash Frozen Sections (FCMC, FF)

20 

Cut each tissue cross-section into four quadrants (A, B, C, D; see **Figure 3**) and place into metal weigh boat with sample on top of slurry. Cover box with lid and allow tissue to completely freeze.

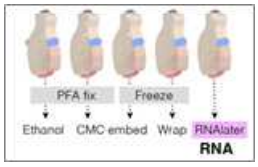
21 For **FF-CMC**, label edges of the molds and place embedding mold with CMC on top of the slurry. Add tissue, medial side down, when the CMC starts to become opaque. Slowly add more CMC on top of the tissue until it is covered, keeping the lid closed over the slurry as much as possible. When completely frozen, wrap mold in foil.

22 **FF-RNA**, label pieces of aluminum foil and corresponding storage bags. Place one tissue piece onto each piece of foil and wrap tightly.

23 Place all samples in labeled storage bags and store at -80ºC.

24 Ship tissue blocks on dry ice.

Human Pancreas Processing for RNA Isolation (RNA)

25 

Cut each tissue cross-section into four quadrants (A, B, C, D; see **Figure 3**) and place all four tissue pieces into a tared plastic weigh boat. Transfer to 15 mL Falcon tube with approximately 5 volumes of RNAlater (e.g., a 1 g sample requires about **5 mL** of solution). Keep at 4ºC.

26 Ship tissue in sealed tubes on ice packs.

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