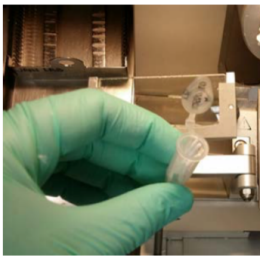
Sep 17, 2019

Preparation of Adult Human Kidney Tissue for Single Nucleus RNA-seq and Other Multiomics Studies



DOI
dx.doi.org/10.17504/protocols.io.568g9hw

Sanjay Jain[1], Masato Hoshi[2], Amanda Knoten[2], Diane Salamon[2]

[1]Washington University School of Medicine, Saint Louis, MO; [2]Washington University

KPMP | Human BioMolecular Atl... | 1 more workspace

Sanjay Jain
Washington University, Saint Louis

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.568g9hw

External link: https://rdcu.be/bPe70

Protocol Citation: Sanjay Jain, Masato Hoshi, Amanda Knoten, Diane Salamon 2019. Preparation of Adult Human Kidney Tissue for Single Nucleus RNA-seq and Other Multiomics Studies. **protocols.io https://dx.doi.org/10.17504/protocols.io.568g9hw**

Manuscript citation:
Blue B. Lake, Song Chen Masato Hoshi, Nongluk Plongthongkum, Diane Salamon, Amanda Knoten, Anitha Vijayan, Ramakrishna Venkatesh, Eric H. Kim, Derek Gao, Joseph Gaut, Kun Zhang, Sanjay Jain. A single-nucleus RNA-sequencing pipeline to decipher the molecular anatomy and pathophysiology of human kidneys Nature Communications 2019 Jun 27;10(1):2832. PMID: 31249312 PMCID: PMC6597610

Protocol status: Working
We use this protocol for use in single nucleus RNA sequencing and ATAC-seq in the HuBMAP and KPMP consortia

Created: August 05, 2019

Last Modified: September 17, 2019

Protocol Integer ID: 26560

Keywords: histology, snRNA-Seq, in situ RNA imaging (DART-FISH), kidney, cryosection, RNA, single nucleus sequencing, embedded tissue cryoblock, preparation of adult human kidney tissue, single nucleus rna, tissue cryoblock, adult human kidney tissue, multiplex in situ rna hybridization, single cell assay, bulk rnaseq, nucleus gene expression data, nucleus gene expression data from adult, tissue gene expression data, situ rna hybridization, thick sections for gene expression study, generating robust single cell, human tissue sample, compatibility with both single cell assay, single cell, generating single cell, interrogation of limited tissue, robust single cell, dna preparation, gene expression study, rna, gene expression, applicable to several solid organ, other multiomics study, limited amount of tissue

Abstract

Critical to generating robust single cell or tissue gene expression data is ensuring that the source samples are procured and processed in a way that minimizes artifacts. This is especially critical for generating single cell/nucleus gene expression data from adult human tissue samples that are difficult to dissociate and might require prolonged manipulations that will introduce stress response signatures. We describe methods to: minimize these unwanted processing effects to enable interrogation of limited tissues, such as biopsies; allow histological validation and quality assurance and control of the samples; ensure compatibility with both single cell assays and orthogonal technologies. This involves preparing fresh frozen O.C.T. embedded tissue cryoblocks to collect thick sections for gene expression studies and adjacent sections for QA/QC, bulk RNAseq, DNA preparation or multiplex in situ RNA hybridization. The protocol for embedding limited amount of tissue for cryosections and susequent OMICS studies should be applicable to several solid organs and biopsies.

Attachments

PDF

UCSD_WU_Protocols_V1.

..

160KB

Guidelines

General Note: Perform procedures in a RNase-free working area (e.g. clean and sterile environment).

Specimen preservation in RNAlater protocol modified from USER GUIDE from Ambion:

Protocol

NAME

Specimen preservation in RNAlater

CREATED BY

Jeff Spraggins

Preview

Materials

MATERIALS

RNAlater **Thermo Fisher Scientific** **Catalog #**AM7020

RNase Zap **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**R2020-250ML

Tissue-Tek Cryomold Intermediate **Sakura Finetek** **Catalog #**4566

O.C.T. compound: Tissue-Tek **Sakura Finetek** **Catalog #**4583

Materials:

1. Two Cryomold (cassettes): Tissue-Tek Cryomold Intermediate (#4566); for larger tissue Standard size can be used. One is used for bathing the tissue and the other for embedding.
2. O.C.T. compound: Tissue-Tek (#4583)
3. RNaseZAP: Sigma (R2020-250ml)
4. Cryostat: set at -20 °C to -25 °C
5. Powdered dry ice
6. Small ziplock bags
7. 2 ml cryovials (bigger tubes if you plan to harvest large chunks of tissue)
8. Pipet tips with aerosol barrier
9. Sterile PBS (phosphate-buffered saline)
10. Forceps
11. Scalpel blade
12. RNAlater® (AM7021). If the solution appears cloudy, warm it at 37 °C for 00:30:00 or refer to the Ambion protocol. Specimen labels to be provided by the Central Hub or printed locally from a centrally managed specimen software if available.

Troubleshooting

Safety warnings

See SDS (Safety Data Sheet) for hazards and safety guidelines.

Before start

General Note: Perform procedures in a RNase-free working area (e.g. clean and sterile environment).

FrozenTissue embedding and OCT Block Preparation

1 Label one cryomold with specimen number and place it on powdered dry ice. This will be the cassette in which tissue will be embedded.

2 Take the second cryomold and fill it with O.C.T.

Note

Tissue will be bathed in it before transferring to the cryomold in step 1.

3 Rapidly dissect the tissue, dab away excess blood using a pad or gauze. Cut desired size section that will fit easily in the cryomold chosen and briefly bathe the tissue in O.C.T. in the cassette in step 2, gently swishing around with a clean instrument 00:02:00 (Figure 1A).

Note

Keep separate forceps to handle tissue during dissection from the one used for bathing as the O.C.T. will stick to the forceps and can make dissection difficult. Handle the tissue gently, for a long biopsy one could also use a pipette tip held in hand to bathe the tissue and lift to transfer without squeezing it.

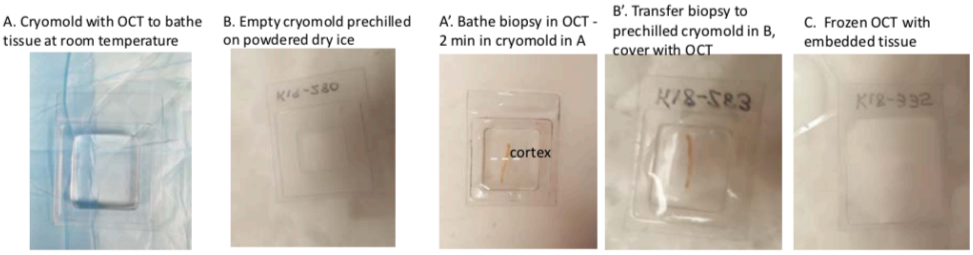


Figure 1. Preparation of cryomolds for fresh frozen OCT embedding of research core.

4 **QA/QC**: Using a smartphone camera, an image of the tissue can be captured while the tissue is bathing that can be used for gross identification of cortex and documentation.

5 Transfer the tissue into the pre-labeled and chilled cryomold (step 1), orient the tissue as desired and pour O.C.T. till it completely covers the tissue (Figure 1B, C).

Note

Avoid bubbles; can move the bubbles out of the way with forceps or pipette tip.

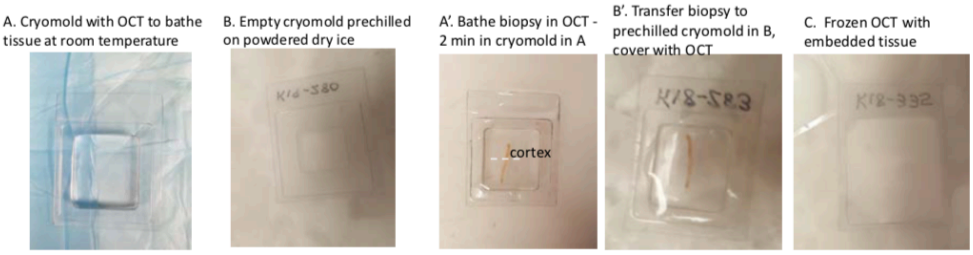


Figure 1. Preparation of cryomolds for fresh frozen OCT embedding of research core.

6 Keep the cassette on powdered dry ice for freezing. Keep the dry ice bucket covered to keep a cold environment.

Avoid flash freezing as that could result in tissue fracturing and thus loss of integrity.

7 Transfer the frozen block into a pre-chilled ziplock (on dry ice), seal it taking as much air out as possible and keeping the block chilled, and store it in a freezer box at -80 °C until ready to section or ship overnight packed in dry ice shipping container.

To avoid damaging the block during transit, place it securely in a pre-chilled freezer box and pack in a Styrofoam container with ample dry ice.

Sectioning

8 Acclimate O.C.T. blocks in cryostat for about 00:10:00 (check cryostat temperature, usually kept at -20 °C to -25 °C) .

9 Prepare tubes, slides, and containers before cutting and as needed.

10 Pre-chill the tubes for RNAlater in wet ice. Slides on which sections will be mounted for histology can be at room temperature.

11 At Room temperature spray all instruments (forceps/paintbrushes) with RNaseZAP.

12 Add O.C.T. to a pre-chilled metal chuck kept in the cryostat and while freezing place the O.C.T. block with the tissue (tissue facing up) on the chuck.

In a few minutes the O.C.T. block with the tissue will be attached to the metal chuck.

13 Mount block with the tissue on the cryostat stage (tissue facing the user).

Safety information

Caution: Be careful when inserting or changing or cutting with cryostat blade and use gloves while cutting. All tissue should be handled keeping universal precautions in mind and consider them biohazardous. In the event of injury contact your local safety office

14 Sections are collected as described below for various processing conditions (see table as an example, the thickness of sections for histology can be at 3um. The preservative solution can be different depending on reproducibility and number of orthagonal technologies to be tested.

Slides 1-5 (for histology -5-10µm)	Thick sections RNALater/Dry Ice (for snRNA-seq; RNAseq)	Slides 6-10 (for histology-5-10µm)	Thick sections RNALater/Dry Ice (for snRNA-seq; RNAseq)	Slides 11-15 (for histology-5-10µm)	Sections on Coverslips (For DART-FISH- 10-20µm)

15 Sections **on slides** are collected in 3-5 slide intervals at 5-10 µm, 1 section per slide, for histology/immunofluorescence to **validate** the tissue being used.

Slowly bring slide to the cut section and as sections transfer to the slides, briefly touch the back surface of the slide (opposite to the section) with your finger to facilitate adherence of the tissue section.

One slide from each interval will be H&E stained for adequacy, quality, composition and integrity (**QA/QC)**. Keep the remainder in the cryostat and transfer to -80 °C .

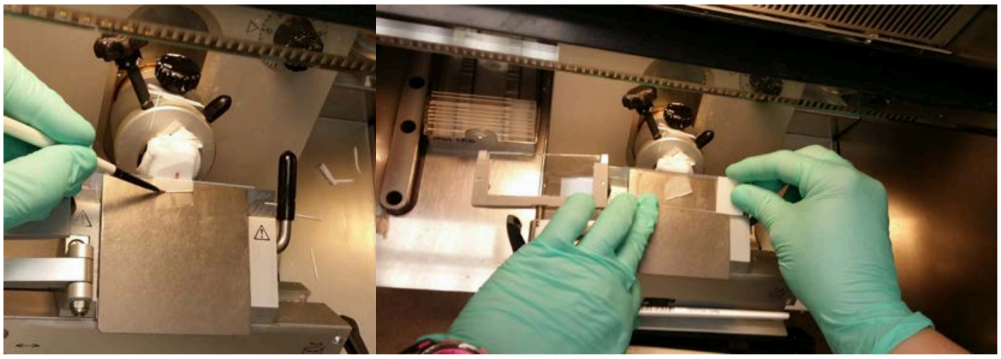


Figure 2. Collecting cryosections on a slide.

16 **Thick section rolls** (35-40 µm) (use for **RNA** or **protein** studies) (**series of tubes with tissue curls allows for reproducibility testing and comparing different processing methods – QA/QC)** are collected in microfuge tubes (**Figure 3**).

Note

Examples:

- Dry Ice, no solution: 7 rolled sections (total 280µm) collected in chilled 1.7ml centrifuge tube. Keep chilled at all times so OCT doesn't melt. Process immediately. Used for: **RNA or protein** assays - e.g. snRNA-seq or single cell chromatin accessibility.
- RNALater: 7 rolled sections are collected in centrifuge tube containing 1mL RNAlater and kept chilled on ice (**RNA** - snRNA-seq or total RNA). At this stage, the RNAlater containing tubes can be shipped overnight (packed with ice packs) to another site or stored at -20°C for a few weeks (RNAlater will not freeze). Used for: **RNA** assays – e.g. snRNA-seq or total RNA isolation.
- Collected in Other media: as needed

Figure 3. Cryosection rolls.

Note

Be careful when handling the tubes, avoid touching the surface with the tissue as it could cause local thawing, better to hold at the lip of the tube or anywhere that is away from the tissue if need be.

17 Sections for **coverslips** are collected as follows (for **DART-FISH):**

17.1 Keep the prepared coverslips at Room temperature .

17.2 Section tissue at desired thickness (we cut at 10 µM and are in the process of testing 20 µM) and place the coverslip so tissue is in the center. Use forceps to pick up and flip the coverslip so the tissue is now facing upwards. **Keep chilled.**

Note

Be gentle and avoid pressing too firmly as this will smash the tissue. Gently tilting the coverslip (or slides in case of histology) usually will cause the tissue to lift and adhere due to static generated.

Note

Adjacent tissue sections allows for reproducibility testing and comparing different processing conditions – **QA/QC**

17.3 Place a slide in the cryostat and, once chilled, put a drop of water (~ 25 µL) towards the center of the slide and place coverslip (tissue facing upwards) over the water.

Keep the slide flat until the water freezes, attaching the coverslip to the slide. This makes it easier to ship the coverslips.

17.4 Place slides with the attached coverslip in pre-chilled slide container for shipment or store at 80 °C .