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Substitution of the sequencing (snRNA-Seq) V.2 Substitution of the sequencing (snRNA-Seq) V.2

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Cellular Senescence Net...



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We use this protocol and it's working

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Abstract

These samples are part of the JAX-Sen project in the SenNet Consortium. Here we provide details on specimen collection and shipment to the Robson laboratory at The Jackson Laboratory for Genomic Medicine (JAX-GM) in Farmington, CT for its processing for single-nuclei RNA-sequencing (snRNA-seq).

Troubleshooting



Reagents and Materials

- 2mL Cryovials/ screw-cap tubes
 - ice cold 1X PBS
 - Kimwipes
 - Petri dishes
 - Liquid Nitrogen
 - Dry ice
 - Tweezers (clean, sterile)

Quality Key Points:

- 2 The tissue specimen should be kept at 4 degrees Celsius and RNase-free until snapfreezing and maintained at -80 degrees C or on dry ice thereafter, until it is processed at JAX.
 - It is crucial to not store the tissue specimen at RT to avoid freeze-thaw cycles, any cell death, and tissue and/or RNA degradation.

Procedure:

- 3 **Timeline:** The daily shipping deadline at Jax BH is 12:00 noon. So, harvest the tissues before and closer to noon.
- 4 **Collection/Harvest:**
- 4.1 Animal was euthanized via cervical dislocation.
- 4.2 Animal was pinned to a necropsy tray which remained on wet ice throughout the harvest.
- 4.3 Animal was perfused with 20ml cold PBS.
- 4.4 Heart, pancreas, and kidney was collected and the specimen was collected and the specimen was rinsed with ice-cold 1x PBS in a Petri dish to remove blood and other debris.
- 4.5 Pat the specimen dry using clean Kimwipes and freeze as quickly as possible after harvest.



- 4.6 To flash freeze (snap freeze), place the tissue in a cryo-tube and submerge the tube in liquid nitrogen or immerse deeply in dry ice.
- 4.7 Wait at least 2-3 minutes for the tissue to completely freeze, and transfer the tube containing the tissue to -80 degrees C.
- 4.8 Keep at -80 degrees Celsius thereafter, until shipping.
- 5 **Shipment:**
- 5.1 Place sample tubes in a plastic box (cardboard boxes insulate the samples from the cold ice) or in double Ziplock bags after checking that they are completely sealed.
- 5.2 Ship the sample box on dry ice (O/N shipping) to: The Jackson Laboratory for Genomic Medicine, Farmington, CT, 06032