Aug 17, 2022

O Human Knee Meniscus Collection Protocol for scRNA-seq

DOI

dx.doi.org/10.17504/protocols.io.6qpvr614zvmk/v1

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Protocol Citation: molmer, Martin Lotz 2022. Human Knee Meniscus Collection Protocol for scRNA-seq. protocols.io <u>https://dx.doi.org/10.17504/protocols.io.6qpvr614zvmk/v1</u>



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

Created: August 15, 2022

Last Modified: August 17, 2022

Protocol Integer ID: 68682

Keywords: Meniscus, knee, human knee meniscus collection protocol, meniscal tissue, seq meniscus, medial knee compartment, scrna

Abstract

Meniscus is taken from the medial knee compartment is collected for scRNA-seq processing. The attached image indicates where the meniscal tissue is collected from.

Materials

Sterile drapes
Sterile gauze
Autoclaved tools
Disposable #21 scalpels
DPBS
DMEM
Antibiotic-Antimycotic
50mL conical tubes

Protocol materials

X DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate Fisher Scientific Catalog #MT-10-013-CV

X Antibiotic-Antimycotic (100X) Thermo Fisher Scientific Catalog #15240062

X PAXgene Tissue FIX Qiagen Catalog #765312

X PAXgene Tissue STABILIZER Qiagen Catalog #765512

Before start

Knee blocks are shipped on wet ice and received within 48 hours post-mortem from certified tissue banks. The average time from receiving the sample to harvesting cartilage from both knees is about 2 hours. Pictures are taken of knee tissues and macroscopic scoring of the cartilage is completed.

- 1 Prepare harvesting area with sterile drapes, tools and gauze. All harvesting is completed within an aseptic environment.
- 2 Wipe down the knee blocks with 95% ethanol prior to opening the joint capsule.
- 3 Once the joint capsule is opened, the femur and tibia are disarticulated and menisci are resected.
- A full-thickness (sagittal) piece of medial meniscus is cut out using a #21 scalpel; the piece measures about 1.60cmX15mmx8mm and weighs around 1g. This is collected into a 50mL tube that is filled with 40mLs of

MEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate Fisher Scientific Catalog #MT-10-013-CV

+ 1% Antibiotic-Antimycotic (100X) Thermo Fisher Scientific Catalog #15240062, then placed in wet ice until the cell isolation protocol for scRNAseq begins.

Note

See Description Section for image for the area of interest.