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Single nuclei RNA sequencing (snRNA-seq) of frozen human lung tissue and hPCLS

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TriState SenNet

Cellular Senescence Net...

1 more workspace



Nayra Cardenes

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We use this collection and it's working

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Abstract

Single-cell RNA sequencing (scRNA-seq) has become an essential tool for delineating cellular diversity in normal tissues and alterations in disease states. This technique requires the dissociation of tissue specimens into cell suspensions. However, the isolation of intact cells can be challenging due to factors such as fragility, large size and tight interconnections. Additionally, single-nuclei isolation can be performed on frozen tissue, enabling the analysis of biobanked samples in a single batch. This protocol for single-nuclei RNA sequencing (snRNA-seq) provides an alternative approach to scRNA-seq, overcoming these limitations to generate high-quality transcriptomic data.

The analysis of gene expression at the cellular level has proven to be a powerful tool for understanding various aspects of lung biology and disease, particularly the process of lung aging. Aging affects different cell types within the human lung heterogeneously, leading to a range of associated changes in their function. Examining the patterns of gene expression in the numerous lung cell types provides insights into the aging and disease processes that contribute to cellular dysfunction. By analyzing these changes at the single-cell level, we can delineate the complex cellular diversity in the human lung and track alterations in molecular pathways involved in the dynamic process of lung aging. Understanding these gene expression patterns will offer opportunities for timely interventions and the identification of biomarker for early prognosis and personalized treatment therapies.

This protocol collection describes the process of single nuclei RNA sequencing from nuclei isolation from frozen tissue (whole, or from PCLS), to barcoding, library construction and sequencing.

Attachments



snRNAseq_ProtocolsIO..

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Image Attribution


Nayra Cardenes, PhD



Materials

-  Chromium Nuclei Isolation Kit with RNase Inhibitor **10x Genomics Catalog #PN-1000494**
- Chromium Next GEM Single Cell 3' GEM Kit v3.1 16 rxns PN-1000123
-  Library Construction Kit, 16 rxns **10x Genomics Catalog #PN-1000190**
- Chromium Next GEM Single Cell 3' Gel Bead Kit v3.1, 16 rxns PN-1000122
-  Dual Index Kit TT Set A, 96 rxns **10x Genomics Catalog #PN-1000215**
-  Dynabeads MyOne Silane **10x Genomics Catalog #2000048**

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Attachments



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Files

 SEARCH

Protocol



NAME

Nuclei Isolation from Frozen Tissue or Frozen hPCLS

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GEM Generation and Barcoding

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Post GEM-RT Cleanup and cDNA Amplification

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Gene Expression Dual Index Library Construction

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Protocol references

https://cdn.10xgenomics.com/image/upload/v1660261285/support-documents/CG000505_Chromium_Nuclei_Isolation_Kit_UG_RevA.pdf