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🌐 Hybrid protocol for Nuclei Isolation and 10X Genomics Single Cell 5' Gene Expression for Human Ovary Explants

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Protocol status: Working

We use this protocol and it's working



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Keywords: gene expression hybrid protocol, 10x genomics single cell, gene expression for human ovary explant, hybrid protocol for nuclei isolation, gene expression libraries from cdna input, 10x genomic, gene expression, preparing gene expression library, human ovary explant, nuclei isolation, frozen human ovary explant, using nuclei suspension, nuclei suspension, hybrid protocol, nuclei, cdna input, chromium connect, cell, protocol for nuclei isolation, fresh frozen human ovary explant

Disclaimer

This protocol needs prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Abstract

This is the 10X Genomics Single Cell 5' Gene Expression hybrid protocol using nuclei suspension isolated from fresh, frozen human ovary explants. The Chromium X (PN-1000331) was used for Gem Generation, and the Chromium Connect (PN-1000171) was used for preparing Gene expression libraries from cDNA inputs.

Guidelines

This protocol needs prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Materials

Refers to the various protocol documents for a complete list of the material required.

Nuclei Isolation Protocol for Human Ovary Explants

- 1 Chapter 1—Single Cell Gene Expression & Chromium Fixed RNA Profiling of the protocol CG000505 REV A was used to isolate nuclei from frozen human ovary explants with the following modifications: 1) a cordless motor pestle (VWR, Catalog number 47747-370) was used for Step f, Page 30 and 2) the samples were incubated for 15 min on ice for Step h, Page 30.

<https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/chromium-nuclei-isolation-kit-sample-prep-user-guide>

Chromium Next GEM Single Cell 5'Reagent Kits v2 (Dual Index). GEM Generation, Barcoding, Post GEM-RT Cleanup and cDNA Amplification.

- 2 Step 1 and step 2 of protocol CG000331 REV E were used for GEM Generation, Barcoding, Post GEM-RT Cleanup, and cDNA Amplification. The Chromium X was used for Gem Generation.

<https://www.10xgenomics.com/support/single-cell-immune-profiling/documentation/steps/library-prep/chromium-single-cell-5-reagent-kits-user-guide-v-2-chemistry-dual-index>

Chromium Next GEMSingle Cell 5'Reagent Kits v2 (Dual Index). Gene expression library construction.

- 3 The protocol CG000474 Rev B was used for Automated Gene expression dual index library construction from cDNA inputs.

<https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/library-prep/automated-gene-expression-library-construction>



Protocol references

The following protocols from 10X Genomics were used for the different steps:

Nuclei Isolation: CG000505 REV A

Chromium Next GEM Single Cell 5'Reagent Kits v2 (Dual Index). GEM Generation, Barcoding, Post GEM-RT Cleanup and cDNA Amplification using manual workflow: CG000331 Rev E.

Chromium Next GEMSingle Cell 5'Reagent Kits v2 (Dual Index). Automated Gene expression library construction: CG000474 Rev B.