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Protocol for Nuclei/ Cell Isolation and 10X Genomics Fixed RNA profiling for Human Ovary V.3

Version 1 is forked from Protocol for Nuclei/ Cell Isolation and 10X Genomics Fixed RNA profiling for Human Skeletal Muscle

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

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Disclaimer

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

Abstract

This is the 10X Genomics protocol to fix, dissociate, and profile RNA from human ovary tissue.

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 materials required.

Cell/Nuclei Isolation Protocol for Human Ovary

1 The protocol CG000553 REV B was used to fix, dissociate, and isolate cells/nuclei from frozen human ovaries with the following modifications:

1) 0.2 mg / mL of Liberase TH was used for dissociation at Step 2b, Page 6.
2) Two extra "spin only" (i.e., steps 3 and 4) of the octodissociator protocol were run for each sample due to the presence of intact/ large tissue pieces at the end of the run at Step 2c, Page 6.
3) Counts were performed using the Cellaca PLX Automated Cell counter (PN: PLX-SYS1) and ViaStain AOPI staining solution (PN: CS2-0106-5mL) at Step 2i on page 6.

https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/sample-prep/tissue-fixation-and-dissociation-for-chromium-single-cell-gene-expression-flex

Chromium Fixed RNA Profiling Reagent Kits

2 The protocol CG000527 Rev E was used to generate gene expression libraries from fixed cell/nuclei suspension inputs.

https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/library-prep/chromium-single-cell-gene-expression-flex-reagent-kits-for-multiplexed-samples

Samples were multiplexed in batches of 16 using the user guide CG000565.

https://www.10xgenomics.com/support/single-cell-gene-expression-flex/documentation/steps/library-prep/chromium-single-cell-gene-expression-flex-reagent-kits-for-multiplexed-samples

Protocol references

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

Tissue Fixation & Dissociation for Chromium Fixed RNA Profiling: *CG000553 Rev B*

Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples: CG000527 Rev E

Chromium Fixed RNA Profiling Multiplexed Samples Pooling Workbook: CG000565 RevB