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Protocol for Nuclei Isolation and Automated 10X Genomics Single Cell 5' Gene Expression for Human Ovary Explants

Forked from 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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Disclaimer

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

Abstract

This is the 10X Genomics Automated Single Cell 5' Gene Expression protocol using nuclei suspension isolated from fresh frozen human ovary explants. The Chromium Connect (PN-1000171) was used from Gem generation to final gene expression libraries.

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.

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 Automated Single Cell 5' Reagent Kits v2

2 The Protocol CG000384 Rev D was used to generate gene expression libraries from nuclei suspension inputs.

https://www.10xgenomics.com/support/instruments/chromium-connect/chromium-next-gem-automated-single-cell-5-reagent-kits-user-guide-v-2-chemistry

Protocol references

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

Nuclei Isolation: CG000505 REV A

Chromium Next GEM Automated Single Cell 5' Reagent Kits v2: CG000384 Rev D