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Multiome sequencing murine ovaries

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

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Abstract

Protocol for single nuclei multiome sequencing - mouse ovaries.

Ovary collection and nuclei isolation

1 Female mice were staged for estrous cycles, and both ovaries were collected at diestrus.

2 Ovaries were immediately flash frozen in liquid nitrogen and stored at −80 °C until use.

3 For single-nuclei isolation, frozen ovaries were processed using the Chromium Nuclei Isolation Kit (10x Genomics, Pleasanton, CA) following the manufacturer's protocol for frozen tissue.

4 Nuclei were washed, counted using an automatic hemocytometer, and resuspended in Diluted Nuclei Buffer (10x Genomics) to the concentration recommended for Multiome library preparation.

Single-nuclei Multiome library preparation and sequencing

5 Nuclei were loaded on the Chromium Next GEM Chip J using the Chromium Single Cell Multiome ATAC + Gene Expression kit (10x Genomics, Pleasanton, CA), following the manufacturer's instructions.

6 The targeted recovery was 10000 nuclei per sample.

7 Libraries were sequenced on a NovaSeq X platform (Illumina, San Diego, CA). Gene expression (GEX) and ATAC libraries were run across NovaSeqX-25B lanes.