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Single-cell transcriptome library preparation

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Maria Jose Perez J.¹

¹Institut Imagine

Team Deleidi



Federico Bertoli

ASAP

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Protocol status: Working

We use this protocol and it's working

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Abstract

Single-cell transcriptome library preparation



Single-cell transcriptome library preparation

- 1 Dissociate iPSC-derived cells in the triculture system into single-cell suspensions by incubating with Accutase for 15 minutes at 37°C.
- 2 Filter the dissociated cell suspensions through a 40 μ m filter.
- 3 Resuspend the filtered cells in PBS containing 0.04% BSA at a final concentration of 1000 cells/ μ L. Ensure that cell viability exceeds 95%.
- 4 Generate single-cell RNA-Seq (scRNA-Seq) libraries using the 10X Chromium Next GEM Single Cell 3' Reagent Kit v3.1 (Cat. number 1000128, 10x Genomics, Pleasanton, CA, USA) following the manufacturer's instructions.
- 5 Pool the libraries for sequencing.
- 6 Paired-end sequencing on the Illumina NovaSeq 6000 platform (SP Flow Cell, Illumina) with a sequencing depth of 300 million reads per library was performed by GENEWIZ GmbH (Leipzig, Germany).
- 7 scRNA-Seq data were deposited into GEO under accession number GSE274289