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JAX_DPC: scRNA sequencing using 10X Chromium Fixed RNA Profiling



Forked from [JAX_DPC: Library preparation using KAPA RNA HyperPrep Kit for Bulk RNAseq](#)

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We use this protocol and it's working

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Abstract

This protocol outlines the use of the Chromium Fixed RNA Profiling Reagent Kits (10x Genomics) for single-cell gene expression analysis of multiplexed samples derived from cortical brain organoids, across a three-day consecutive time course.

Procedure

1 Fixation of Cells

- 1.1 Dissociate differentiated iPSCs into single cells
- 1.2 Centrifuge sample at 350 rcf for 5 min at 4°C.
- 1.3 Remove the supernatant without disturbing the pellet.
- 1.4 Add 1 ml Fixation Buffer to the sample pellet and pipette mix 5x.
- 1.5 Incubate for 1 h at room temperature (20°C) or for 16-24 h at 4°C for long term storage.
- 1.6 Centrifuge at 850 rcf for 5 min at room temperature.
- 1.7 Remove the supernatant without disturbing the pellet.
- 1.8 Add 1 ml chilled Quenching Buffer to the sample pellet and pipette mix 5x and keep on ice.
- 1.9 Determine cell concentration of the fixed sample using AO/PI (acridine orange/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.
- 1.10 Thaw Enhancer (10x Genomics PN-2000482) for 10 min at 65°C. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.
- 1.11 Add 1 volume pre-warmed Enhancer to fixed sample in Quenching Buffer. Pipette mix.
- 1.12 Store sample at 4°C for up to 1 week.



For long term storage (up to 6 months) also add 50% glycerol for a final concentration of 10% with Quenching buffer and store at -80°C.

2 **Chromium Fixed RNA Profiling for Multiplexed Samples**

2.1 The reagents and process workflow are described below:

https://cdn.10xgenomics.com/image/upload/v1694503892/support-documents/CG000527_ChromiumFixedRNAProfiling_MultiplexedSamples_UserGuide_Rev_E.pdf