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Single-cell RNA-seq for mDA neurons

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We use this protocol and it's working

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Abstract

Harvesting and performing single-cell RNA-seq on hiPSC-derived mDA neurons.

Troubleshooting

Harvesting cells for RNA-seq

5m

1 At the desired age of mDA neurons, they are harvested for single-cell RNA-seq:

1.1 mDA neurons are washed 1x in PBS.

1.2 They are incubated with Accutase (Thermo Fisher Scientific) for 00:05:00 at 37 °C . 5m

1.3 mDA neurons are collected as a single cell suspension and diluted 1:3.

Quality and concentration of cells

2 The quality and concentration of each single-cell suspension was measured using Trypan blue and the Eve automatic cell counter.

3 Each sample presented a concentration between a 1200-1700 cell/µl and viability ranged between 55-68%, samples with a viability above 57% were used for sequencing.

single-cell RNA-seq

4 Approximately 10000 cells were loaded for each sample into a separate channel of a Chromium Chip G for use in the 10X Chromium Controller (cat: PN-1000120).

5 The cells were partitioned into nanoliter scale Gel Beads in emulsions (GEMs) and lysed using the 10x Genomics Single Cell 3′ Chip V3.1 GEM, Library and Gel Bead Kit (cat: PN-1000121).

6 cDNA synthesis and library construction were performed as per the manufacturer's instructions.

7 The RNA was reversed transcribed and amplified using 12 cycles of PCR.

8 Libraries were prepared from 10µl of the cDNA and 13 cycles of amplification. Each library was prepared using Single Index Kit T Set A (cat: PN-1000213) and sequenced on the HiSeq4000 system (Illumina) using 100 bp paired-end run at a depth of 65-100 million reads. Libraries were generated in independent runs for the different samples.