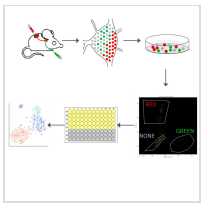

Jul 06, 2022

Single-cell sequencing and analysis

In 1 collection

Daniele Neri[1], Lori Zeltser[1]

[1]Columbia University Irving Medical Center

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.bp2l61drdvqe/v1

SPARC
Tech. support email: **info@neuinfo.org**

Daniele Neri

ABSTRACT

This protocol describes single-cell sequencing and analysis.

DOI

dx.doi.org/10.17504/protocols.io.bp2l61drdvqe/v1

PROTOCOL CITATION

Daniele Neri, Lori Zeltser 2022. Single-cell sequencing and analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l61drdvqe/v1

COLLECTIONS

Single cell RNA sequencing of retrogradely labeled mouse stellate ganglion neuron

CREATED

Apr 01, 2022

LAST MODIFIED

Jul 06, 2022

PROTOCOL INTEGER ID

60200

PARENT PROTOCOLS

Part of collection

Single cell RNA sequencing of retrogradely labeled mouse stellate ganglion neuron

MATERIALS TEXT

References:

Plate-based single-cell sequencing:

- Snyder, M. E., Finlayson, M. O., Connors, T. J., Dogra, P., Senda, T., Bush, E., Carpenter, D., Marboe, C., Benvenuto, L., Shah, L., Robbins, H., Hook, J. L., Sykes, M., D'Ovidio, F., Bacchetta, M., Sonett, J. R., Lederer, D. J., Arcasoy, S., Sims, P. A., & Farber, D. L. (2019). Generation and persistence of human tissue-resident memory T cells in lung transplantation. *Science Immunology*,*4*(33), eaav5581. https://doi.org/10.1126/sciimmunol.aav5581

STAR:

- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics (Oxford, England)*,*29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635
- http://code.google.com/p/rna-star/
- https://github.com/alexdobin/STAR/releases

featureCounts:

- Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general-purpose program for assigning sequence reads to genomic features. *Bioinformatics (Oxford, England)*,*30*(7), 923–930. https://doi.org/10.1093/bioinformatics/btt656
- *featureCounts* is available under GNU General Public License as part of the Subread (http://subread.sourceforge.net)

SAMtools:

- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics (Oxford, England)*,*25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352
- http://samtools.sourceforge.net/

UMI-tools:

- Smith, T., Heger, A., & Sudbery, I. (2017). UMI-tools: modeling sequencing errors in Unique Molecular Identifiers to improve quantification accuracy. *Genome* Research,*27*(3), 491–499. https://doi.org/10.1101/gr.209601.116

R toolkit Seurat:

- Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*,*36*(5), 411–420. https://doi.org/10.1038/nbt.4096
- http://satijalab.org/seurat/

Citation: Daniele Neri, Lori Zeltser Single-cell sequencing and analysis https://dx.doi.org/10.17504/protocols.io.bp2l61drdvqe/v1

Sequencing

1 We performed plate-based single-cell sequencing as described in Snyder et al., 2019 (https://doi.org/10.1126/sciimmunol.aav5581)

2 Pooled 3'-end sequencing libraries were sequenced on an Illumina NextSeq 500/550 platform

Alignment and count matrix generation

3 The reads were aligned to mouse genome, GRCm38, gencode version M13 (Ensembl 88) using STAR v2.5.3a

4 The aligned reads were assigned to genes using featureCounts v1.5.3.

5 bam files were sorted and indexed using Samtools v1.4.1, duplicated reads were removed and a UMI count matrix was generated using umi tools

Analysis

6 We performed analysis of the count matrices using Seurat v2.3.4 in R v3.4.3 (R Core Team, 2013).

Citation: Daniele Neri, Lori Zeltser Single-cell sequencing and analysis https://dx.doi.org/10.17504/protocols.io.bp2l61drdvqe/v1