May 28, 2019 Version 1

‘Frankenstein’ protocol for nuclei isolation from fresh and frozen tissue for snRNAseq V.1

DOI
dx.doi.org/10.17504/protocols.io.3eqgjdw

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Human Cell Atlas Metho... MMG MoPED

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.3eqgjdw

External link: https://research.unimelb.edu.au/centre-for-cancer-research/our-research/single-cell-innovation-lab

Protocol Citation: Luciano G Martelotto 2019. ‘Frankenstein’ protocol for nuclei isolation from fresh and frozen tissue for snRNAseq. **protocols.io https://dx.doi.org/10.17504/protocols.io.3eqgjdw**

Manuscript citation:

Protocol status: Working
We use this protocol in our group and it is working very well. This version can also be found in 10x User developed Protocols (https://community.10xgenomics.com/t5/Customer-Developed-Protocols/ct-p/customer-protocols). If you have any queries please do not hesitate in contacting me via email: lucianomartelotto@gmail.com or luciano.martelotto@unimelb.edu.au

Created: May 28, 2019

Last Modified: May 28, 2019

Protocol Integer ID: 23728

Keywords: snRNAseq

Abstract

This protocol is the result of the combination of various nuclei isolation protocols for single cell RNA-seq experiments using droplet-based methods, hence the name Frankenstein. Developed to prepare nuclei isolates from small sample sizes (as little as grain of rice), this protocol uses FACS to identify cell subpopulations based on ploidy (e.g. tumor versus stroma), to ensure that nuclei suspensions are not clumped, and to remove any debris, especially ambient RNA, to help reduce background. The reference protocols can be found in the following papers: Hu, et al., Habib, et al. (2016), Habib, et al. (2017), Lake, et al., and Lacar, et al.

This protocol is routinely used in the single-cell innovation lab for single nuclei experiments using 10x Genomics technologies.

The protocol has been demonstrated to work successfully with fresh, snap/flash frozen, cryopreserved cells, and cell lines, as well as various solid cancers: pancreas, pheochromocytomas, paragangliomas, breast cancer, lymphoma, xenografts and tumors.

Attachments

PDF

Frankenstein___p...

379KB