Jun 23, 2021

Modifications to the "HTAPP_TST- Nuclei isolation from frozen tissue V.2" Protocol

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ABSTRACT

A few modifications

DOCUMENT CITATION

Xian Adiconis 2021. Modifications to the "HTAPP_TST- Nuclei isolation from frozen tissue V.2" Protocol. **protocols.io** https://protocols.io/view/modifications-to-the-34-htapp-tst-nuclei-isolation-bv2sn8ee

CREATED

Jun 23, 2021

LAST MODIFIED

Jun 23, 2021

DOCUMENT INTEGER ID

50994

ABSTRACT

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When working with larger amount tissues (i.e. combined kidney tissue from 3 mice), the following modifications are made to the original "HTAPP_TST- Nuclei isolation from frozen tissue V.2" protocol.

1) In step 2, used a razor blade (RNaseZapped and rinsed) instead of spring scissor to chop the tissues in a petri dish with 1 ml TST buffer. This is to increase the homogenization efficiency for the larger tissue quantity.

2) In step 5, the 3 ml TST buffer was first added to the petri dish to give the additional rinse then pass through the 40 µm filter.

3) After step 8, do another 2 washes by adding 3 ml 1x ST buffer and spinning at same condition each time.

4) Given there is certain buffer condition requirement for compatibility with the downstream applications (i.e. 10x SC RNA-seq assay), https://kb.10xgenomics.com/hc/en-us/articles/115001937123-What-buffers-can-be-used-for-washing-and-cell-resuspension-, the final loading volume (if re-suspended in ST buffer) should be less than 6.17 µl, as this would give the final Mg concentration at max allowed 3mM (ST buffer contains 21 mM Mg and the H2O and sample volume for 10x V3.1 assay is 43.2 µl). If lower nuclei concentration (therefore higher volume is needed) is anticipated, the final nuclei suspension should be made with 1x PBS (containing 1% BSA, 0.2U/µl RNaseIn).