Dissociation of Nuclear Suspensions from Human Breast Tissues

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Working

ABSTRACT

This protocol can be used to prepare nuclear suspensions from fresh or frozen tissue for single cell DNA or RNA sequencing experiments. For RNA analysis it is recommended to use fresh tissue samples, due to RNA degradation that occurs during freeze-thaw cycles, however for DNA analysis either frozen or fresh tissue can be used. The protocol has been tested on breast tissues, and many frozen tumor tissues including breast, colon prostate and pancreatic samples.

TAGS

breast cancer

NL_Breast tissue nuclei dissociation_protocol_NN.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This protocol is for fresh or frozen tissue.

MATERIALS

NAME	CATALOG #	VENDOR
Flowmi™ Cell Strainer 40 µm	H13680-0040	Bel-Art
DNA LoBind Tube 1.5ml	022431021	Eppendorf
Ultrapure BSA	AM2616	Ambion
RNase Inhibitor (40U/µl)	N2615	Promega

MATERIALS TEXT

Consumables:

- Scalpels, 1.5ml tubes, 50ml conical, syringe, PBS.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

BEFORE STARTING

- NST-DAPI: Mix 800 ml of NST solution (146 mM NaCl, 10 mM Tris base (pH 7.8), 1 mM $CaCl_2$, 21 mM $MgCl_2$, 0.05% (wt/vol) BSA and 0.2% (vol/vol) Nonidet P-40) with 200 ml of DAPI solution (106 mM $MgCl_2$ and 10 mg of DAPI). Filter-sterilize the

solution and store it at 4°C in the dark for up to 1 year.

- Prepare chilled (4°C) Nuclei Wash and Resuspension Buffer: 1X PBS with 1.0% BSA and 0.2U/µl RNase Inhibitor.

Tissue preparation

1 Cut fresh or frozen tissue (about 1x1x1cm) and mince it with a scalpel in a 10-cm Petri dish with **2 ml NST-DAPI buffer** for **00:10:00** (10-15 min) until tissue chunks are no longer visible.

2 Filter the mixture through a 40 ul flowmi into 1.5 ml DNA LoBind tubes. Place the tube on ice.

Centrifugation

3 Centrifuge nuclei at **4 °C** 300-500 xg for **00:05:00** .

Wash

4 Remove the supernatant and resuspend with **1 ml cold Nuclei Wash and Resuspension Buffer** . (1/3)

5 Wash again with **1 ml cold Nuclei Wash and Resuspension Buffer** . (2/3)

6 Wash again with **1 ml cold Nuclei Wash and Resuspension Buffer** . (3/3)

Counting nuclei

7 Resuspend pellet in cold Nuclei Wash and Resuspension Buffer. Count nuclei (Make a 1:1 solution of Hoechst and PI, pipette **2 µl** of this mix to **10 µl filtered nuclei** and count in the Countess II FL).

8 Adjust concentration to 700-1200 nuclei/µl.

9 Proceed immediately with 10X genomics RNA experiments or single cell DNA sequencing experiments.